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Emerging Infectious Diseases

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

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

Emerging infections are new or newly identified pathogens or syndromes that have been recognized in the past two decades. Reemerging infections are known pathogens or syndromes that are increasing in incidence, expanding into new geographic areas, affecting new populations, or threatening to increase in the near future.

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. Inquiries about the suitability of proposed articles may be directed to the editor at 404-639-3967 (telephone), 404-727-8737 (fax), or eideditor@cidod1.em.cdc.gov (e-mail).

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

Instructions to Authors

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).

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

Documents sent in hardcopy should also be sent on diskette, or by e-mail. Acceptable electronic formats for text are ASCII, WordPerfect, AmiPro, DisplayWrite, MS Word, MultiMate, Office Writer, WordStar, or Xywrite. Send graphics documents in Corel Draw, Harvard Graphics, Freelance, .TIF (TIFF), .GIF (CompuServe), .WMF (Windows Metafile), .EPS (Encapsulated Postscript), or .CGM (Computer Graphics Metafile). The preferred font for graphics files is Helvetica. If possible, convert Macintosh files into one of the suggested formats. Submit photographs as glossy,camera-ready photographic prints.

Send all manuscripts and correspondence to the Editor, *Emerging Infectious Diseases*, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop C-12, Atlanta, GA 30333, USA, or by e-mail to eideditor@cidod1.em.cdc.gov.

Perspectives: Contributions to the Perspectives section should provide insightful analysis and commentary about new and reemerging infectious diseases or related issues. Perspectives may also address factors known to influence the emergence of infectious diseases, including microbial 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. 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 emerging and reemerging infectious diseases; however, timely updates of other diseases or topics are also welcome. Synopses should be approximately 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are 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. Include descriptions of new methods for detecting, characterizing, or subtyping emerging or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome. Dispatches (1,000 to 1,500 words of text) should not be divided into sections. Provide references, not to exceed 10, and figures or illustrations, not to exceed two.

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Globalization, International Law, and Emerging Infectious Diseases

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The global nature of the threat posed by new and reemerging infectious diseases will require international cooperation in identifying, controlling, and preventing these diseases. Because of this need for international cooperation, international law will certainly play a role in the global strategy for the control of emerging diseases. Recognizing this fact, the World Health Organization has already proposed revising the International Health Regulations. This article examines some basic problems that the global campaign against emerging infectious diseases might face in applying international law to facilitate international cooperation. The international legal component of the global control strategy for these diseases needs careful attention because of problems inherent in international law, especially as it applies to emerging infections issues.

The growing literature on new and reemerging infectious diseases often emphasizes the global nature of their threat; the U.S. Centers for Disease Control and Prevention (CDC) defines these diseases as "diseases of infectious origin whose incidence in humans has increased within the past two decades or threatens to increase in the near future" (1). The World Health Organization has asserted that emerging infections "represent a global threat that will require a coordinated, global response" (2). The threat is global because a disease can emerge anywhere on the planet and spread quickly to other regions through trade and travel. The global challenge of emerging infections has serious consequences for national and international law; a state's ability to deal with them is eroded because microbes do not respect internationally recognized borders (3). Experts grappling with these diseases no longer consider that the pursuit of a strictly national public health policy is adequate. The need for global cooperation increases the importance of international law in the public health arena. Part of the effort to create a global response to emerging infections should be an understanding of the problems that may arise from relying on international law in dealing with these diseases. This article outlines issues that will have to be confronted in using international law to combat emerging infections.

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Globalization

The assertion that emerging infections are a global problem requiring a global strategy echoes observations made in other spheres of public policy: the traditional distinctions between national and international political, social, and economic activities are losing their importance (4). Globalization is eroding traditional distinctions between domestic and foreign affairs. Globalization has been defined as the "process of denationalization of markets, laws, and politics in the sense of interlacing peoples and individuals for the sake of the common good" (5). Globalization is distinguished from internationalization, which is defined "as a means to enable nation-states to satisfy the national interest in areas where they are incapable of doing so on their own" (5). Internationalization involves cooperation between sovereign states, whereas globalization refers to a process that is undermining or eroding sovereignty.

Globalization arises from the confluence of something old and something new in international relations. It involves the very old process of political and economic intercourse among sovereign states. The new element is the intensification and expansion of such intercourse made possible by technological advances in travel, communications, and computers. Encouraging such intensification and expansion is liberal economic thinking, which posits that economic interdependence makes all states economically better off and builds order and peace in the international system (6).

The changes wrought by new technologies unleashed in the receptive international milieu

created by liberal trade and economic policies have led to the belief that these developments are undermining sovereignty. Observers of international relations frequently note that governments no longer have control over economic forces at work within their countries. The speed and volume of international capital flows illustrate the denationalization of economics occurring through the process of globalization (7). Another example is the development of the global company—an enterprise that can no longer be considered national because of the global reach of its operations, financing options, markets, and strategies (7). The globalization of finance and business has ramifications for politics and law as leaders and legal systems adapt to the global era (8).

In public health, a similar combination of old and new factors can be seen. States have historically cooperated on infectious disease control, first through international sanitary treaties and later through the World Health Organization (WHO) (9). While international cooperation is not new, current global circumstances confronting the control of infectious disease are. Globalization is also at work in public health. The assertion that a country cannot tackle emerging infectious diseases by itself demonstrates that public health policy has been denationalized.

Globalization has affected public health in three ways. First, the shrinking of the world by technology and economic interdependence allows diseases to spread globally at rapid speed. Two factors contributing to the global threat from emerging infections stem directly from globalization: the increase in international travel (2, 10) and the increasingly global nature of food handling, processing, and sales (2, 10). HIV/AIDS, tuberculosis, cholera, and malaria represent a few infections that have spread to new regions through global travel and trade (10). The beneficial economic and political consequences of economic interdependence may have negative ramifications for disease control. In the European Union, for example, the free movement of goods, capital, and labor makes it more difficult for member states to protect domestic populations from diseases acquired in other countries (11).

Second, the development of the global market has intensified economic competition and increased pressure on governments to reduce expenditures, including the funding of public health programs, leaving states increasingly unprepared to deal with emerging disease problems. Industrialized as well as developing countries confront deteriorating public health infrastructures (12). Referring to the United States, one author described this deterioration as the "thirdworldization" of the American health care system (13).

Third, public health programs have also "gone global" through WHO and health-related nongovernmental organizations. Medical advances have spread across the planet, improving health worldwide. The worldwide eradication of smallpox in 1977 is a famous example. The global reach of health care advances has, however, a darker side. The globalization of disease control has contributed to the population crisis because people are living longer. Overpopulation creates fertile conditions for the spread of disease: overcrowding, lack of adequate sanitation, and overstretched public health infrastructures (2). Further, the widespread use and misuse of antibiotic treatments has contributed to the development of drug-resistant pathogens (1, 2). Finally, the success of control efforts in previous decades caused interest in infectious diseases to wane in the international medical and scientific communities and is now hampering emerging infectious disease control efforts (14).

International Solutions to Emerging Infections

International efforts are under way to respond to the threat of emerging infectious diseases. WHO and CDC have drafted action plans that stress the need to strengthen global surveillance of these diseases and to allow the international community to anticipate, recognize, control, and prevent them (1, 14, 15). WHO has also established a new unit to control and prevent emerging infections by mobilizing resources rapidly at the first signs of outbreaks (16). The Pan American Health Organization has also adopted a regional plan for controlling emerging infections in the Americas (17). Health authorities from Central American countries have adopted an emergency plan to control the epidemics of dengue and dengue hemorrhagic fever that recently swept through Central and South America (18). Physicians in the European Union recognize the need for better surveillance of infectious diseases (11). A U.S. government interagency working group has underlined the importance of international cooperation in dealing with the emerging infections threat (19). The U.S. Senate Labor and Human Resources Committee held hearings in October 1995 on "Emerging Infections: A Significant Threat to the Health of the

Nation" (20). At the Halifax Summit in 1995, the major industrialized countries adopted a pilot project called "Toward a Global Health Network" designed to help governments deal with emerging infections and other health problems (19) (Table 1). Clearly, the emerging infections threat and the need for action are on the international diplomatic and public health agendas.

Although international control plans would involve private organizations like universities and nongovernmental organizations, the primary actors on the emerging infections stage are sovereign states. The action plans are predominantly blueprints for cooperation among states and represent a call for the internationalization of responses to a problem caused by globalization. Put another way, the proposed solutions to the emerging infections threat rely on the sovereign state, while the threat feeds off the impotence of the state in addressing global disease problems. When it comes to public health activities, globalization erodes sovereignty, but the proposed solution makes sovereignty and its exercise critical to dealing with the threat of emerging infections.

The consequences of the unavoidable emphasis on international cooperation in the proposed action plans for emerging infections are troubling. To achieve the desired objectives (Table 1), states will have to agree on many issues and translate such agreement into guidelines or rules. International law becomes important to the effort for emerging infections control. Political leaders, diplomats, and scholars have long recognized the weakness of international law in regulating state behavior. At first glance, the prospect of having to rely on a notoriously weak institution of international relations as part of the global effort to combat emerging infections is unsettling.

Table 1. Some common elements of global emerging-disease control plans

Strengthen international surveillance networks to detect, control, and reduce emerging diseases.
Improve the international public health infrastructure (e.g., laboratories, research facilities, technology, and communications links).
Develop better international standards, guidelines, and recommendations.
Improve international capabilities to respond to disease outbreaks with adequate medical and scientific resources and expertise.
Strengthen international research efforts on emerging diseases, particularly with regards to antibiotic-resistant strains of diseases.
Focus attention and resources on training and supporting medical and scientific expertise.
Encourage national governments to improve their public health care systems, devote resources to eliminating or controlling causes of emerging diseases and coordinate their public health activities with WHO and the international community.

Sources: refs. 1, 14, 15, 19.

International Law and Infectious Disease Control

We might have been less unsettled if our experience with international law in controlling infectious diseases had been more positive. The success of WHO in globalizing disease control programs might suggest that the defects of international law have not hobbled its effectiveness in improving health care worldwide. However, despite having the authority to do so, WHO has been reluctant to use international law (21, 22). The International Health Regulations administered by WHO represent the most important set of international legal rules relating to infectious disease control, but the regulations only apply to plague, yellow fever, and cholera (23). The importance of health is mentioned in international declarations (for example, see the Universal Declaration of Human Rights, art. 25 [1]) and treaties (for example, see the International Covenant on Economic, Social and Cultural Rights, art. 12), leading some legal scholars to argue that international law creates a "right to health" (24); but this "right" does not directly address the control of infectious diseases. WHO has refrained from adopting rules on trade in human blood and organs, which does raise issues of infectious disease control as illustrated by the sale of HIV-contaminated blood in international commerce (25). Issues of disease control also appear in specialized treaty regimes outside WHO, such as treaties controlling marine pollution from ships (26). Other areas of international public health law, for example, rules about infant formula and guidelines on pharmaceutical safety, do not deal with the control of infectious diseases (25).

The effectiveness of existing international law on infectious disease control has been questioned. A 1975 WHO publication stated that the International Health Regulations have not functioned satisfactorily at times of serious disease outbreaks (27). More recently, WHO's efforts with the International Health Regulations have been called a failure, and noncompliance with these regulations

has increased in connection with reporting disease outbreaks (25). The HIV/AIDs crisis dramatically illustrated the weaknesses of the health regulations. Since AIDs was not originally (or subsequently) made subject to the regulations, states had, and continue to have, no notification requirements in connection with this new disease. Further, as HIV/AIDs spread globally, many states adopted exclusionary policies that, according to experts, violated provisions of the health regulations (25). In relation to one of the biggest disease crises of this century, parts of the International Health Regulations were irrelevant, and other parts were openly violated.

WHO's reluctance to apply international law has been attributed to its organizational culture, which is dominated by scientists, doctors, and medical experts. Perhaps the current weakness of international law on infectious disease control reflects WHO's nonlegal strategy rather than the inherent problems in international law itself. In connection with emerging infections, however, WHO is advocating an international legal strategy by recommending revision of the International Health Regulations (28). This recommendation suggests that WHO acknowledges the need for international legal agreement in dealing with emerging infections. The global threat posed by these infections represents in many ways a test case for international public health law.

The Challenge to International Law

The threat of emerging infectious diseases poses two challenges to international law: first, the emerging infections problem exacerbates basic weaknesses in the law. Second, these infections pose specific difficulties in the law, which are related to the nature of disease and its prevention.

Basic Weaknesses

The effectiveness of international law depends on the consent of states, which means that sovereignty and its exercise determine the fate of international legal rules (29). In adopting a legal strategy for its emerging infectious disease action plan, WHO has to convince its member states to take certain actions in response to disease emergence. The sovereignty of states looms large in formulating a global response to emerging infections, despite the fact that the process of globalization undermines the sovereignty of the state to deal nationally with these infections. In other words, the problem by-passes the state, but the solution has to rely on the state through the medium of international law. The central importance of the state and its sovereignty constitutes a basic weakness in international law because international legal rules tend to reflect the compromises necessary to achieve agreement and the unwillingness of states to restrict their freedom of action through international law. Part of the reason that the existing International Health Regulations cover only a few diseases might be the unwillingness of WHO member states to commit to more serious infectious disease control measures. The vagueness and lack of specificity in the so-called "right to health" also illustrate this problem. What is scientifically and medically necessary to combat emerging diseases may not be what states are willing to agree to undertake.

A second basic weakness follows from the "sovereignty problem"—the lack of effective enforcement of international law. States often agree to an international legal obligation without any serious intent of fulfilling it. The alleged failure of the International Health Regulations may be due to the failure of WHO member states to fulfill the duties they accepted. Neither the regulations nor WHO has any power to enforce compliance (25). An international legal regime on emerging diseases would also face this enforcement problem.

Specific Difficulties

The very nature of the emerging disease threat poses special difficulties for international law. The global scope of the problem necessitates agreement by most states to control emerging diseases. If any major country or group of countries does not participate, a gap in the global surveillance and control network threatens the efficacy of the entire effort. The negotiation of agreements involving many states is usually difficult, because each state knows that its nonparticipation threatens the success of the entire venture. This problem has occurred in international environmental law, where global regimes have been needed to deal adequately with environmental threats, such as ozone depletion.

A second specific difficulty arises from the extent of medical and scientific resources needed to establish an effective global surveillance and control network for emerging diseases. Fundamental aspects of the proposed action plans involve improving surveillance networks, public health infrastructures, scientific research, and

medical and scientific training (Table 1). Some states, particularly in the developing world, do not have the medical, scientific, and financial resources to undertake such measures. Unless more affluent countries provide the resources, developing states may use the inequity of wealth in the international system as an argument to complicate negotiating a global agreement. The so-called "North-South problem" has made the negotiation of international environmental agreements more difficult, as developing countries have bargained for more lenient treatment or a transfer of resources from affluent countries to help them improve environmental protection. A similar dynamic may appear in any negotiations for a global emerging disease effort. The U.S. interagency working group on emerging diseases has observed that major U.S. contributions to developing countries for emerging disease control purposes "is not a likely prospect during this period of deficit reduction and downsizing" (19), which suggests that resource availability will probably complicate international efforts in this area.

The problems associated with using international law in a global strategy to combat emerging diseases raise the question whether international law can provide an adequate foundation for the control of these diseases. The uncomfortable position of having no choice but to rely on international law when its weaknesses are substantial highlights the importance of thinking through the international legal aspects of a global emerging disease plan carefully.

WHO's Proposed Legal Strategy

WHO wants to revise the International Health Regulations as part of its global emerging disease strategy (28). WHO's proposal deserves some critical attention. It is not clear that the organization has adequate authority to incorporate comprehensive emerging disease control measures within the international regulations. Under Article 21 of the WHO Constitution, the World Health Assembly can adopt binding regulations in sanitary and quarantine requirements and other procedures to prevent the international spread of disease (22). The World Health Assembly adopted the International Health Regulations under Article 21. While Article 21 and the regulations are relevant to emerging disease control efforts, it is doubtful whether the regulations can serve as a foundation for a comprehensive emerging disease control plan. The disease-outbreak notification requirements in the regulations could be expanded to include more diseases, but nothing in Article 21 gives the World Health Assembly the authority to require WHO member states to strengthen public health infrastructures, which is considered critical in the emerging disease actions plans proposed to date (Table 1). It has been argued that attempting to address such infrastructure problems "is a solution which cannot be obtained by an international instrument but only by the improvement of the health conditions of the peoples of WHO's member states" (30). But, as the history of administering the International Health Regulations has shown, notification requirements have not worked satisfactorily and are weakened by the absence of adequate public health resources. Further, Article 22 of the WHO Constitution makes regulations promulgated under Article 21 automatically binding on WHO member states, except for member states that reject such regulations or make reservations thereto (31). Article 22 relates to the sovereignty problem and may deter WHO member states from agreeing to serious revisions of the regulations. Analysis of the regulations may question the wisdom of using the regulations as the legal basis for dealing with emerging diseases.

The World Health Assembly has the power to adopt conventions or agreements within WHO's competence (21). The Assembly could use this authority to address aspects of the global emerging disease control strategy that cannot be handled with a revision of the regulations. However, parceling up emerging disease control measures between the International Health Regulations and separate agreements would be legally complicated. Further, WHO has not used this power to adopt conventions or agreements, which explains its unwillingness to explore all legal options open to it.

Possible Alternative Legal Strategies

Alternative legal strategies to revising the International Health Regulations range from reliance on the development of customary international law to the adoption of multilateral treaties specifically on emerging-disease control (Table 2). An issue related to these alternative approaches is the substantive nature of the obligations contained in legal documents. We have to ask not only how states might agree on control rules but also what these states might agree to do. The proposed revision of the regulations

Table 2. Alternative international legal strategies to revising the International Health Regulations

Alternative legal strategies	Possible advantages	Possible disadvantages
1. WHA incorporates emerging disease control as part of the proposed World Health Charter scheduled for initial negotiations in 1997	Integrates emerging disease control measures into the overall WHO approach to international health issues	a. Emerging disease control would not be primary focus b. World Health Charter is likely to be more aspirational than obligatory
2. WHA adopts an emerging disease–specific convention under Article 19 of the WHO Constitution	a. Avoids IHR model b. Has potential to set out comprehensive global approach to emerging diseases	a. WHA has no experience with using Article 19 b. Large multinational treaties tend to contain general obligations rather than specific duties
3. States negotiate a framework multilateral treaty on general emerging disease obligations, accompanied by disease-specific or region-specific protocols containing detailed and specific commitments on emerging disease control	a. Takes emerging disease control out of WHO, eliminating problem of WHO's reluctance to use international law b. Allows for new protocols to be adopted for new diseases c. Framework-protocol approach has been used with some success in international environmental law on ozone depletion	a. WHO has to play central role in any emerging disease plan b. Framework-protocol approach might not be appropriate model for emerging disease control because the emerging disease problem differs from ozone depletion
4. Encourage regional arrangements and integrate them into global regime over time	a. Builds on strong regional systems of cooperation and coordination b. Offers "legal laboratories" to try various approaches to emerging disease control c. Avoids diplomatic headaches involved in trying to negotiate truly global legal regimes	a. Emerging diseases require a global approach not just a regional approach b. Amounts to emerging disease control for rich regions, leaving many developing countries outside legal regime c. Risks inconsistencies in how emerging diseases are handled by different regions
5. Encourage a bilateral approach in which individual countries negotiate detailed and specific commitments on emerging diseases and perhaps condition trade benefits and aid on emerging disease performance	a. Gives states flexibility in constructing legal obligations b. Permits possibility for sanctions for failure to live up to emerging disease obligations	a. Does not address global nature of emerging disease problem b. Sanctions element is unrealistic and might be unfair to developing countries lacking the resources necessary to implement adequate emerging disease control measures
6. Incorporate emerging disease control as part of international "right to health," making emerging diseases a human rights issue	a. Links emerging disease control with larger, powerful concepts of human welfare b. Builds on existing international law on the "right to health"	a. International "right to health" has no definitive meaning or scope and thus is a bad foundation for emerging disease control b. Human rights are inherently divisive in the international system; linkage with such a controversial area would hurt emerging disease control prospects
7. Rely on customary international law to develop emerging disease-control norms	Customary international norms on emerging disease control would be binding on all states except persistent objectors	a. It will be nearly impossible to develop general and uniform state practice recognized by states as legally binding in the emerging disease-control area b. Any customary norms that might form will probably be vague and hard to identify definitively c. Customary norms can take a very long time to develop

WHA = World Health Assembly; WHO = World Health Organization; IHR = International Health Regulations.

apparently would only apply the notification duties (currently found in the regulations) to more diseases. As indicated earlier, WHO cannot address in its revision of the regulations any of the improvements in public health infrastructures, surveillance networks, scientific research, or medical and scientific training at the heart of proposed emerging disease action plans. Further, it is not clear whether WHO intends to supplement expanded notification duties with any mechanism to monitor or enforce such duties.

International environmental law had to overcome some of the same obstacles encountered by WHO's international legal effort for emerging disease control. States realized that they could not handle global environmental problems without international cooperation and rules (32). Further, states knew that addressing environmental concerns would require changes for governments and companies within states and that developing states might have financial and technological difficulties implementing international agreements (32). In developing international environmental law, states, international organizations, and nongovernmental organizations did not rely on old approaches but instead crafted new international legal rules to deal with the global nature of the threats posed, the resource issue, and compliance and enforcement problems (33). Whether international environmental law has been successful is controversial; but it is important that states have not been willing to admit that improving environmental conditions within states is a solution that cannot be obtained by international agreements. Models and precedents from international environmental law are not in all respects helpful to the challenge of emerging-disease control; but, at the very least, those grappling with an international strategy for the emerging-disease threat could analyze international environmental law and other innovative legal responses to globalization to look for ways of making WHO's international legal strategy on emerging diseases as effective as possible.

Those currently designing global emerging-disease control strategies will eventually have to translate what is scientifically and medically needed to combat these diseases into international agreement and cooperation through international law. The movement from science and medicine into the realm of international law will not be easy. Relying on the International Health Regulations as the centerpiece of international law on emerging-disease control may not be the most effective international legal strategy. Whatever international legal approach is eventually taken will have to confront somehow a fundamental paradox: globalization jeopardizes disease control nationally by eroding sovereignty, while the need for international solutions allows sovereignty to frustrate disease control internationally. The combination of the process of globalization and the unavoidable need to rely on international law produces a most unattractive medium in which to wage potentially one of the most important medical and scientific endeavors in history.

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On Epidemiology and Geographic Information Systems: A Review and Discussion of Future Directions

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Geographic information systems are powerful automated systems for the capture, storage, retrieval, analysis, and display of spatial data. While the systems have been in development for more than 20 years, recent software has made them substantially easier to use for those outside the field. The systems offer new and expanding opportunities for epidemiology because they allow an informed user to choose between options when geographic distributions are part of the problem. Even when used minimally, these systems allow a spatial perspective on disease. Used to their optimum level, as tools for analysis and decision making, they are indeed a new information management vehicle with a rich potential for public health and epidemiology.

Geographic information systems (GIS) are "automated systems for the capture, storage, retrieval, analysis, and display of spatial data" (1). Common to all GIS is a realization that spatial data are unique because their records can be linked to a geographic map. The component parts of a GIS include not just a database, but also spatial or map information and some mechanism to link them together. GIS has also been described as the technology side of a new discipline, geographic information science (2), which in turn is defined as "research on the generic issues that surround the use of GIS technology, impede its successful implementation, or emerge from an understanding of its potential capabilities." Recently, GIS has emerged as an innovative and important component of many projects in public health and epidemiology, and this disciplinary crossover is the focus of this review.

Few would argue that GIS has little to offer the health sciences. On the other hand, like other new technologies, GIS involves concepts and analytic techniques that can appear confusing and can lead to misunderstanding or even overselling of the technology. In this article, we attempt to bridge the gaps between the principles of geographic information science, the technology of GIS, the discipline of geography, and the health sciences. Our intent is to introduce to the epidemiologist a set of methods that challenge the "visual" half of the scientist's brain.

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Computers were first applied to geography as analytical and display tools during the 1960s (3). GIS emerged as a multidisciplinary field during the 1970s. The discipline's heritage lies in cartography's mathematical roots: in urban planning's map overlay methods for selecting regions and locations based on multiple factors (4); in the impact of the quantitative revolution on the discipline of geography; and in database management developments in computer science.

Several factors combined in the 1970s to reinforce GIS development. First, computers became more accessible and less costly. Second, mainframe computers gave way to minicomputers and then workstations, which gave great power to the user and included the access to networks that has led to its own revolution in technology. Third, the types of user interface required to operate technical software changed from batch, command-line, and remote access to windowing systems and "point and click" graphic interaction. What had been expensive, slow, and difficult has rapidly become inexpensive, fast, and easy to use. A final but essential precondition to GIS development was the broad availability of public domain digital map data, in the form of maps of the landscape from the U.S. Geologic Survey and for census areas from the U.S. Census Bureau. The current GIS World Sourcebook (5) lists hundreds of system suppliers and sources of information and catalogs system capabilities. In short, GIS has now come of age, to the extent that the contributions of a growing number of parallel disciplines have both influenced and been influenced by GIS. Other disciplines now affecting GIS include forestry, transportation planning, emergency services delivery, natural

hazards planning, marketing, archeology, surveying, and criminal justice. A wide array of capabilities and information awaits the health scientist ready to pursue an interest in GIS.

In this article, we consider the functional capabilities of GIS and how they can relate to epidemiology. We then review studies in epidemiology and health science where GIS has already made a contribution and introduce the technologic and analytic background. We review spatial analytic methods and concepts of use in epidemiology and conclude by examining what the near future holds for technologic changes and what these changes mean for the study of emerging infectious diseases and other health applications.

GIS Functional Capabilities

GIS definitions usually focus on what tasks a GIS can do rather that what it is. GIS functional capabilities follow the standard GIS definitions; therefore, GIS can bring together the elements necessary for problem solving and analysis.

Data capture implies that 1) data can be input into the GIS from existing external digital sources; this is particularly the case when no data exist for a project, and the base data must be assembled from other studies, public domain datasets, and images. This usually means that GIS must be able to import the most common data formats both for image-type (raster) and line-type (vector) maps. 2) GIS can capture new map data directly; this means either that the user can scan the map and input it into the GIS or trace over a map's features using a digitizing tablet and enter them into the GIS map database. 3) The GIS can accomplish everything that a regular database system can, such as enter and edit data and update information in the existing database.

Data storage implies storage of both map and attribute data. Attribute data are usually stored in a relational database management system contained within the GIS and accessed by a spreadsheet or query-driven user interface. For storage, map data must be encoded into a set of numbers so that the geometry of the map is available for query, but also so that the map is stored digitally in one or more files. Image maps are usually stored as gridded arrays. Line maps are encoded by any one of several systems, but usually by using both the coordinate information and encoded topology, so that the relationships between points, lines, and areas, such as the adjacency of regions or the connectedness of lines, are known in advance. The more efficient and flexible these data formats or structures, the more operations can be performed on the map data without further processing.

Data records in GIS can be retrieved in one of two ways. The relational database manager allows searching, reordering, and selecting on the basis of a feature's attributes and their values. For example, the user may wish to select out and order alphabetically the names of all health clinics that had positive results in more than 10% of their tests. GIS also allows spatial retrieval. The user could select all clinics by region, by their latitude, or by their distance from the capital. The user could also select all clinics that are more than 10 km from a major road and within 100 m of a river or lake. In addition, combining searches is possible. There could be several data "layers," for example vegetation, rivers, transportation, and population of villages. A single retrieval could combine data from each of these layers in a single query. Layers can also be weighted, so that rivers, for example, are twice as important as roads in selecting villages with a population under 500 surrounded by forest.

Display functions include predominantly the making of maps. Tools must exist for constructing many types of maps, such as contours, symbols, shading or choropleth, and sized symbols. Formal map display often follows a series of more temporary map images, usually without a strict map composition, and the result of a test, an analysis, or a query. In addition, the GIS must be able to output finished format of maps to a medium, such as PostScript, on a plotter or printer, or onto photographic film.

Many tools exist to support field data collection. Tasks in which ancillary demographic information needs to be input and coregistered are simple. Habitat associated with a vector (e.g., a snail or a mosquito) may need remotely sensed data, such as vegetation cover or weather data. If these data are georegistered, integration is possible. One of the most useful functions is called address matching, in which street addresses with house numbers and street names are automatically placed into an administrative unit or placed as a dot on the map. Thus a digital phone list or mailing list of patients can be merged with the remainder of the data. In the United States, the Census Bureau's TIGER files can usually match 70% to 80% of unedited address records, and higher percentages if the address files are proofed and/or the more detailed

and up-to-date commercial street files are used. In some field projects, the GIS's ability to make maps became the mainstay of the effort, allowing planning of truck and jeep routes, sequencing field clinics for optimal routes for visits, and even for local navigation. The ability to display maps often goes far beyond their final or use in the laboratory. Often a GIS image map is more accurate and up to date than anything available locally.

Existing Applications of GIS in Epidemiology

Epidemiologists have traditionally used maps when analyzing associations between location, environment, and disease (6). GIS is particularly well suited for studying these associations because of its spatial analysis and display capabilities. Recently GIS has been used in the surveillance and monitoring of vector-borne diseases (7-9) water borne diseases (10), in environmental health (11-13), modeling exposure to electromagnetic fields (14), quantifying lead hazards in a neighborhood (15), predicting child pedestrian injuries (12), and the analysis of disease policy and planning (16).

In a recent study in Baltimore County, Maryland, GIS and epidemiologic methods were combined to identify and locate environmental risk factors associated with Lyme disease (7). Ecologic data such as watershed, land use, soil type, geology, and forest distribution were collected at the residences of Lyme disease patients and compared with data collected at a randomly selected set of addresses. A risk model was generated combining both GIS and logistic regression analysis to locate areas where Lyme disease is most likely to occur.

GIS allows analysis of data generated by global positioning systems (GPS). Combined with data from surveillance and management activities, GIS and GPS provide a powerful tool for the analysis and display of areas of high disease prevalence and the monitoring of ongoing control efforts. The marrying of GIS and GPS enhances the quality of spatial and nonspatial data for analysis and decision making by providing an integrated approach to disease control and surveillance at the local, regional, and/or national level.

GIS is being used to identify locations of high prevalence and monitor intervention and control programs in areas of Guatemala for onchocerciasis (9) and in Africa for trypanosomiasis (17). Spatial and ecologic data are combined with epidemiologic data to enable analysis of variables that play important roles in disease transmission. This integration of data is essential for health policy planning, decision making, and ongoing surveillance efforts. For example, as part of the guinea worm eradication effort, the United Nation's Children's Emergency Fund placed pumps in villages most infected with the disease to ensure access to a safe water supply (18). GIS enabled researchers to locate high prevalence areas and populations at risk, identify areas in need of resources, and make decisions on resource allocation (16). Epidemiologic data showed a marked reduction in prevalence in villages where pumps were introduced.

GIS was used in designing a national surveillance system for the monitoring and control of malaria in Israel (19). The system included data on the locations of breeding sites of *Anopheles* mosquitoes, imported malaria cases, and population centers. The GIS-based surveillance system provided means for administrative collaboration and a network to mobilize localities in the case of outbreaks.

In 1985, the National Aeronautics and Space Administration (NASA) established the Global Monitoring and Disease Prediction Program at Ames Research Center in response to the World Health Organization's call for the development of innovative solutions to malaria surveillance and control (20). A major aspect of the program was to identify environmental factors that affect the patterns of disease risk and transmission. The overall goal of the program was to develop predictive models of vector population dynamics and disease transmission risk using remotely sensed data and GIS technologies.

Remotely sensed data have been used in many vector disease studies (8,17,21-24). Remote sensing and GIS were used to identify villages at high risk for malaria transmission in the southern area of Chiapas, Mexico (8). An earth environmental analysis system for responding to fascioliasis on Red River Basin farms in Louisiana was developed by integrating LANDSAT MSS imagery with GIS (22). In Kwara State, Nigeria, a temporal analysis of Landsat Thematic Mapper (TM) satellite data was used to test the significance of the guinea worm eradication program based on changes in agricultural production (21).

Spatial Analysis and GIS

GIS applications show the power and potential of such systems for addressing important health issues at the international, national, and local levels. Much of that power stems from the systems' spatial analysis capabilities, which allow users to

examine and display health data in new and highly effective ways. Spatial analysis refers to the "ability to manipulate spatial data into different forms and extract additional meaning as a result" (25). It encompasses the many methods and procedures, developed in geography, statistics, and other disciplines, for analyzing and relating spatial information. Spatial relationships, those based on proximity and relative location, form the core of spatial analysis.

Gatrell and Bailey (26) describe three general types of spatial analysis tasks: visualization, exploratory data analysis, and model building. These range in complexity from simple map overlay operations to statistical models such as spatial interaction and diffusion models. The value of maps for public health analysis has long been recognized; John Snow's now classic maps of cholera cases in relation to the Broad Street pump are a good example. However, with its extensive data management and display capabilities, GIS offers much more than simple mapping. Map overlay operations allow the analyst to compute new values for locations based on multiple attributes or data "layers" and to identify and display locations that meet specific criteria (27). For example, in targeting locations for mosquito vector control, one might want to identify areas that have low elevation, specific types of vegetation favored by mosquitoes, and are within 100 m of ponds or other water bodies. Each of these attributes comprises a distinct data layer. With GIS, one can create 100-m buffers around water bodies and then select areas meeting all three criteria. Display of these areas on a GIS-generated map has obvious benefits for planning vector control strategies.

As indicated previously, this general class of procedures for weighing and overlaying maps, also known as "suitability analysis," has been used in diverse health applications. Typically the criteria and weights attached to them are specified by the analyst based on expert knowledge or prior research. Using the computational and visual display capabilities of GIS, one can then explore the sensitivity of results to the weights and cutoff values used. Another approach is to employ regression analysis to generate the linear combination of factors that best explain spatial variation in disease prevalence. The weights from the regression model are used to create a composite index of risk which can then be mapped (7).

Visualization is also an important tool for showing the change in disease patterns over time. Animation, embedded within a GIS, is highly effective in depicting the spread or retreat of disease over space and time. A series of animated maps were created to show the advance of the AIDS epidemic in the United States as it moved from and within major cities (28). One could imagine a similar animated map sequence showing the retreat and eventual eradication of a disease like smallpox. Clearly much more research is needed in this area, especially research that links animation to theoretical models of disease diffusion, within a GIS environment.

Visualization can be used in novel ways to explore the results of traditional statistical analysis. Displaying the locations of outlier and influential values on maps and showing variation in values over space can add a great deal to epidemiologic research. Although such tools are being developed and explored, they would benefit greatly from a closer and more seamless link between statistical packages and GIS (25).

The second general class of GIS methods addresses exploratory spatial analysis. These methods allow the analyst to sift meaningfully through spatial data, identify "unusual" spatial patterns, and formulate hypotheses to guide future research (26). The quantity and diversity of spatial data in GIS can be overwhelming: exploratory methods help the analyst make sense of data and address "what if" questions. Advances in computing and graphics technology have made this one of the most active areas in GIS/spatial analysis research.

Among the most important exploratory methods for epidemiology and public health are methods for identifying space-time clusters or "hot spots" of disease. Openshaw's geographic analysis machine (GAM) was an early method that worked completely within a hybrid GIS. The GAM's many applications included an attempt to determine if spatial clusters of childhood leukemia were located near nuclear facilities in Britain (29). The GAM works with point data on disease cases and searches at regular intervals for statistically significant clusters of disease prevalence. Maps display the locations of significant clusters, showing the proximity of clusters to hypothesized environmental threats such as nuclear facilities. Although Openshaw's work was widely criticized on statistical grounds, it opened the door for an active body of research on exploratory spatial analysis of disease. Some of the new methods that have been developed as outgrowths of Openshaw's approach have been published (30).

Exploratory methods are also valuable in searching for zones or districts of high disease prevalence. Because areas may differ greatly in population size, prevalence rates have different levels of variability and thus reliability (31). Researchers have long used probability mapping to show the statistical significance of prevalence rates (32); however, probability mapping does not give a sense of the actual rates or the populations on which they are based. An alternative method is to smooth rates towards a regional or local mean value using empirical Bayes methods (33). Although GIS and empirical Bayes methods have developed separately, there is much scope for interaction. For example, GIS can be used to generate geographically based regional or local means to which actual rates are smoothed. These might be based on averaging rates for contiguous areas (33,34); or they might rely on more complex, multivariate, spatial clustering procedures that incorporate proximity as well as population attributes.

Many methods for exploratory analysis of disease patterns are not appropriate for infectious diseases because the methods are essentially static and assume independence. For infectious diseases, cases clearly are not independent and the diseases move through time and space. In these situations, one can use spatial autocorrelation methods and space-time correlograms to explore the spatial and temporal patterns of infectious disease spread (35).

These methods provide a general sense of the speed and geographic pattern of disease transmission. Although the methods have not typically been incorporated in GIS, there is great potential for doing so, especially with recent advances in computer animation.

Modeling, the final class of spatial analysis methods, includes procedures for testing hypotheses about the causes of disease and the nature and processes of disease transmission. In general, modeling involves the integration of GIS with standard statistical and epidemiologic methods. GIS can assist in generating data for input to epidemiologic models, displaying the results of statistical analysis, and modeling processes that occur over space. The first two points are evident in recent, regression-based analyses of disease risk, such as the study of Lyme disease (7). There GIS was used not only to integrate diverse datasets and calculate new variables, such as slope and distance from forest, but also to map geographic variation in disease risk, as predicted from a logistic regression model.

Other GIS models are more explicitly spatial, expressing relationships or flows between people and places. Spatial interaction and spatial diffusion models are of particular relevance to the study of emerging diseases. Spatial interaction models analyze and predict the movements of people, information, and goods from place to place (36). The flows of people between rural areas, villages, cities, and countries are all forms of spatial interaction that are central to disease transmission. By accurately modeling these flows, it is possible to identify areas most at risk for disease transmission and thus target intervention efforts. Spatial interaction models reflect two general principles: that interaction decreases with distance and increases with population size or "attractiveness." Given actual flow data, one can estimate values that show the effects of distance and population size (or other "attractiveness" factors) on interaction. The models can then be used to predict spatial interaction patterns elsewhere. Although spatial interaction models and GIS developed separately, some GIS now have spatial interaction modeling capabilities (37).

Spatial diffusion models analyze and predict the spread of phenomena over space and time and have been widely used in understanding spatial diffusion of disease (38). Such models are quite similar to spatial interaction models except that they have an explicit temporal dimension. By incorporating time and space, along with basic epidemiologic concepts, the models can predict how diseases spread, spatially and temporally, from infected to susceptible people in an area (39) and aid in understanding the emergence of infectious disease (40).

Data

Important technical and logistic innovations in data and data access for GIS are under way and will come to fruition before the end of the century. First, and by far the most important, have been increased access to the Defense Department's global positioning systems (GPS), the availability of inexpensive hand-held devices for using the system, and the addition of direct-to-GIS data links to these systems. For a relatively modest investment, field users can add geographic coordinates to their data collection from anywhere in the world, at any time, and in any weather. These systems are so flexible that their antennas can be

placed on top of a car, and the logger can be connected to a portable computer on the dashboard, so that as the user drives along, the path of the vehicle is permanently recorded in the GIS's own data format and displayed on screen with a 1-s update. As these systems have become more common, they have also gained in precision and accuracy. It is not uncommon for fixes to be corrected using a process known as differential GPS, either after the fact by computer software or in real time, so that each point is recorded to the nearest meter on the ground. GPS and GIS together have permanently altered the relationship between field data collection and data analysis. Data collected in real time can be analyzed the same day and acted upon immediately.

Similarly, various devices used for capturing overhead images and photographs have undergone a similar revolution. First, technology has improved, allowing images in the infrared, thermal, radar and other wavelengths to be collected at higher and higher spatial resolutions. Second, massive changes in policy have resulted from the end of the Cold War. Formerly secret satellite data, such as the CORONA and Russian spy imagery, are now broadly available, even searchable on the Internet. In the United States, the National Air Photo program intends to remap the country every 5 years at a scale of 1:12,000 with 1-m resolution and publish the images as CD-ROMs. In addition, NASA's largest ever Mission to Planet Earth and its Earth Observation System will begin to return unimaginable amounts of information about the whole earth's geography and atmosphere well before the end of the century. The data will be available to any Internet user and distributed by a set of active archive centers.

Third, technical issues related to data transfer have been partially eliminated. This has come about by the convergence toward sets of industry standard formats such as GIF and TIF for images and new national and international digital map data standards. In addition, efforts are now under way to standardize reference information about datasets, termed metadata, so that the equivalent of a Library of Congress cataloging will be possible.

Finally, many datasets have become available that can form at least the skeleton of a new GIS project almost anywhere in the world. By combining public domain datasets, such as the Digital Chart of the World and satellite imagery, with GPS and field data, the claim that data collection and changes in format constitute 80% of the effort in a GIS project is rapidly being eroded and replaced by a mere morning spent surfing the Internet. Nevertheless, many of the world's nations are still poorly mapped at the more detailed spatial scales required for local analysis.

Hardware

GIS hardware has continued to improve. On the high end, workstations have both increased in power and dropped in price, making this platform the choice for large, laboratory-based GIS projects. As the GIS software packages have been modified for the workstation operating systems, most commonly UNIX with X-Windows, operations that were impossible because of computational complexity have now become commonplace. This trend will continue to the extent that few technical constraints like memory and central processing unit (CPU) power will exist for GIS. Some tasks, such as skilled visual image identification and interpretation, have been partly or wholly automated. On the low end, microcomputers have become immensely powerful and fast, easily capable of performing basic GIS operations even on portable computers. The theme of GIS mobility, added to satellite and cellular telephone communications, has permanently transformed the ability to operate with GIS in the field, and will lead to a new "data rich" era for epidemiologic study.

In addition, the next generation of systems will depend on network computing. Networks have allowed de facto parallel computing within a local area network. By supporting personal multitasking, they have allowed data to be held in a distributed way and retrieved for use on demand, and the network has built an immensely powerful support structure for information sharing. The World-Wide Web, for example, can deliver to a workstation-user free GIS software, data, and information on how to install and use the system, support for technical problems, and even an outlet to publish scientific results.

Software

GIS software has improved remarkably in the latest generation and will undergo still more changes. The basic tools of the computer programmer have undergone a transition from first generation to object-oriented database and programming languages, offering some benefits in program module reusability, improved data

handling, and ease of use as more and more packages are rewritten to take advantage of these tools. The WIMP (windows, icons, menus, and pointers) interfaces so common today owe their origins to this technology. Today, the GIS research community suggests that as the "desktop metaphor" becomes more commonly accepted, increasingly sophisticated metaphors will take over for organizing computing, including perhaps using maps themselves to manage the computer rather than vice versa.

Some changes are far more practical but still of great value. Most software systems now support context-sensitive help, electronic manuals, and automatic installation and update procedures. Each of these could benefit from intelligent software that uses an expert system base and continues to tailor the system around the GIS operator's revealed use. Such software, used over a network, has been termed an intelligent agent. Most GIS of the future will use these methods to seek out new data over the network that relate to your problem, alert you to mistakes in your data management and analysis, and perhaps automatically compose maps and reports at the completion of a project.

Multimedia and hypermedia are also rapidly becoming a component of GIS software. Multimedia allow simultaneous use of text, sound, animation, and graphics. GIS software has also developed the ability to interact in many spoken languages, under different operating systems, and on many different computers. The independence of the software and the tasks from particular computer platforms, or even vendors, are a highly desirable element in a distributed system.

GIS and Public Health

While it holds distinct promise as a tool in the fight against emerging infectious diseases and other public health problems; it is not simply the next widget to come into play. GIS can be seen as a new approach to science, one with a history and heritage, a finite and well researched suite of methods and techniques, and a research agenda of its own. It does not fit neatly into the health scientist's toolbox. It requires rethinking and reorganizing the way that data are collected, used, and displayed. It requires expense, training, and a climb up a learning curve. It needs maintenance and support and can be both overwhelming and threatening to the uninitiated.

On the other hand, the base of research and scholarship using GIS in the health sciences cannot be ignored. A first step would be to integrate instruction on GIS into college curricula in public health. An admirable body of experience in GIS education already exists, even a thoroughly tested national curriculum that can be easily adapted to a new set of demands (41). A second step would be to seek out more formal links between the research communities working with GIS. There are astonishing similarities for example in the field requirements for using GIS between forestry, ecology, archeology and epidemiology that could provide substantial benefits by the sharing of experiences and the pooling of resources.

Above all, GIS should be seen as improving the set of tools to promote public health. Good epidemiologic science and good geographic information science go hand in hand. The future of GIS has already retained a role for the geographically literate public health expert. Epidemiologists should seize the opportunity to set their own agenda and influence the technology and science toward the goal of public health.

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The Evolution and Maintenance of Virulence in Microparasites

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In recent years, population and evolutionary biologists have questioned the traditional view that parasite-mediated morbidity and mortality—virulence—is a primitive character and an artifact of recent associations between parasites and their hosts. A number of hypotheses have been proposed that favor virulence and suggest that it will be maintained by natural selection. According to some of these hypotheses, the pathogenicity of HIV, *Vibrio cholerae*, *Mycobacterium tuberculosis*, the *Shigella*, as well as *Plasmodium falciparum*, and many other microparasites, are not only maintained by natural selection, but their virulence increases or decreases as an evolutionary response to changes in environmental conditions or the density and/or behavior of the human population. Other hypotheses propose that the virulence of microparasites is not directly favored by natural selection; rather, microparasite-mediated morbidity and mortality are either coincidental to parasite-expressed characters (virulence determinants that evolved for other functions) or the product of short-sighted evolution in infected hosts. These hypotheses for the evolution and maintenance of microparasite virulence are critically reviewed, and suggestions are made for testing them experimentally.

How much of the emergence and reemergence of infectious diseases is due to evolution, rather than ecological, technical, and social change (1)? Under what conditions will attenuated vaccine organisms become virulent? Are hospitalized and immunocompromised hosts reservoirs for the evolution of virulent pathogens (2)? The answers to these and related questions require an understanding of the ecological conditions and genetic processes responsible for the evolution and maintenance of parasite-mediated morbidity and mortality in infected hosts—virulence, as we shall define it here.

At least since Darwin's time (3), evolutionary biologists have been interested in infectious diseases, but primarily with respect to the role of these diseases in the adaptation and evolution of humans and other species (4). A bit more than 15 years ago, this interest in infectious disease took a new turn, a focus on the microbes responsible for these diseases and the evolution and maintenance of their virulence. Here I offer a relatively brief and personal review of current theories of the evolution and maintenance of virulence in the bacteria, viruses, protozoa, and single cell fungi, "microparasites" (to use the term employed by population biologists), responsible for infectious diseases. I consider how these theories fit, what is known about the epidemiology of microparasite infections and the mechanisms of pathogenesis, and discuss procedures to test hypotheses derived from these theoretical considerations of the population biology and evolution of microparasites. For other recent reviews of this subject, see (5-8).

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The Conventional Wisdom

At one time, virulence was almost universally considered an artifact of recent associations between parasites and their hosts (9, 10), and to a fair extent, it still is (11). In accord with this view, which Bob May and Roy Anderson called "conventional wisdom" (12), parasite-host coevolution is necessarily in the direction of commensalism or, nicer yet, mutualism. The logic behind this view is pleasing to human sensibilities. A fully evolved parasite would not harm the host it needs for its survival, proliferation, and transmission. Indeed, the appeal of this view of nature of parasite-host coevolution was sufficient for its corollary to also be assumed valid. That is, pathogenesis is often taken as evidence of recent associations between parasites and their hosts.

Many observations are consistent with conventional wisdom about parasite-host coevolution. This is particularly so for most of the so-called

emerging diseases. For example, Legionnaires' disease, Lyme disease, and pneumonia caused by hantavirus are consequences of human infection by parasites and/or commensals of other species, rather than by organisms that have had a long association with humans. In fact, for these emerging diseases and some older microparasitic diseases, like Rocky Mountain spotted fever, anthrax, and rabies, humans play no (or at best a negligible) role in the transmission of the parasite and, in that sense, are an evolutionary dead end. While HIV is transmitted between humans, its association with our species is almost universally considered recent (13, 14).

Other observations can be interpreted as inconsistent with conventional wisdom. For some virulent pathogens, like *Shigella* and *Neisseria gonorrhoeae*, humans appear to be either the unique or the dominant host and vector for infectious transmission (15). For other lethal microparasitic diseases like malaria and tuberculosis (TB), there is evidence that these microparasites have had a long history in human populations and that humans play a major if not unique role in their infectious transmission. However, for the pathogens involved in both these diseases, animal origins have been implicated, and it is difficult to find clear evidence of their existence (or that of other extant pathogens) before the origins of agriculture (16-18).[1] One can always rescue conventional wisdom from these inconsistent observations by assuming that "long" is not long enough for these microparasites to evolve or coevolve with humans to a more amenable relationship. Then again, it may well be that some microparasites responsible for new infections in human hosts will evolve to become increasingly virulent human pathogens and be readily transmitted between human hosts.

Conventional wisdom is not based on hypotheses that can be readily tested and rejected. Microparasites that lead to the extinction of their only host face the same fate as the host. On the other hand, evolving and becoming gentle and prudent in treating their hosts (when natural selection operating at the level of individual microparasites favors profligate behavior like virulence) require some form of group-level or kin selection (8), and/or a host evolutionary response that unilaterally converts an otherwise virulent microparasite into a commensal. Conventional wisdom does not account for the actual mechanisms responsible for the evolution of benign associations between microparasites and their hosts.

Epidemiologic Models and the "Enlightenment"

In the early 1980s, at least among evolutionary biologists, conventional wisdom gave way to what, in an earlier consideration of this subject Catharina Svanborg and I satirically (but sympathetically) referred to as the "enlightenment" (24). In accord with this new view, natural selection could favor the evolution and maintenance of virulence as well as commensal and symbiotic associations between microparasites and their hosts. In other words, virulence could be the evolved as well as the primitive stage of these associations. The direction of natural selection in any given situation depends on the epidemiology and ecology of the microparasite and, in particular, the relationship between its virulence and its rate of infectious transmission in the host population.[2] This can be seen in the equation for the finite rate of increase of a directly transmitted microparasite in a wholly susceptible host population (12, 25, 26)

$$R_0 = \frac{ßN}{\alpha + b + \nu}$$

[1] The existence of genetic polymorphisms, like sickle cell, thalassemia, and glucose 6-phosphate dehydrogenase (G6PH) deficiency (19, 20), Duffy-negative blood groups (21), and specific HLA alleles (22) maintained by *Plasmodium*-mediated selection can also be interpreted as evidence for malaria's long association with humans. There is evidence for inherited resistance to TB among mammals (23), and arguments that TB epidemics have selected for inherited resistance in humans (16). However, that evidence is not as compelling as that for malaria.

[2] In at least the mathematical theory, the morbidity component of microparasite virulence is not treated explicitly (25). The symptoms and pain resulting from infection are implicitly incorporated in the rates of disease-associated mortality, recovery, and transmission. Moreover, while acknowledging the existence of microparasite-mediated selection and evolution in the host population, for the most part, the enlightened view of microparasite-host coevolution has concentrated on the changes in the microparasite population. The idea is that because of the relatively longer generation times, the rate of evolution in the host population is going to be low.

where ß is the rate constant of infectious transfer of the microparasite, N the density of the susceptible host population, α the rate of microparasite-induced mortality (virulence), b the rate of microparasite-independent mortality, and ν the rate of recovery. R_0 is the number of secondary infections caused by a single primary infection and serves as a measure of the fitness (here and elsewhere in a Darwinian sense) of the parasite in this naive host population. At any given host density, N, this measure of fitness of the parasite is directly proportional to its transmissibility, ß, and the term of its persistence in an infected host, the reciprocal of $\alpha + b + \nu$.

If the parameters of the R_0 equation were independent of each other, the predictions derived from this equation would be consistent with conventional wisdom: benign parasites would evolve. That is, natural selection would favor highly transmissible ($b \rightarrow \infty$), incurable ($\nu \rightarrow 0$), commensals ($\alpha \rightarrow 0$), or symbionts ($\alpha \rightarrow -\infty$). On the other hand, if transmission and virulence, the parameters ß and α in the R_0 equation, were positively coupled, natural selection could favor the evolution and maintenance of some level of virulence, $\alpha \rightarrow 0$, in the microparasite population.

In accord with the epidemiologic perspective implicit in the R_0 equation, an understanding of the evolution of virulence in microparasites comes down to elucidating the relationship between the rate at which the microparasite is transmitted between hosts and the rate of parasite-mediated mortality in individual infected hosts. If that relationship is positive, then some level of virulence may be favored. And, since the first statements of this new view of parasite-host coevolution (12, 26, 27), much of the research on the evolution of virulence has focused on the association between these two components of parasite fitness.

The most cited, and to me the single most compelling, evidence in support of this new interpretation of microparasite-host coevolution comes from the "experiments" using myxoma virus to control European rabbit populations in Australia and Europe (26, 28, 29). Within a relatively short time after the release of highly virulent myxoma, the viruses recovered from the then decimated and sometimes more resistant wild rabbit populations were less virulent and had lower rates of disease-induced mortality on control laboratory rabbits than those initially released. However, the extent to which myxoma virus from the wild became attenuated was substantially less than that which could be achieved experimentally (29). This was interpreted as evidence for a positive coupling between the rates of infectious transmission and rates of virus-induced mortality, a trade-off between virulence and transmission. Highly virulent forms of the virus had a disadvantage because they killed the rabbits too quickly and thus reduced the time available for them to be picked up by the insect (mosquito or flea) vectors required for their infectious transmission. Viruses that were too attenuated had a disadvantage because they generated fewer skin lesions and had lower densities of circulating virions, which presumably would reduce the rate at which they would be bitten by these insect vectors, the likelihood of biting vectors picking up myxoma, and the number of virions picked up at any given bite. Thus, in contrast to conventional wisdom and in accord with the enlightened interpretation, natural selection could favor and maintain the virulence of microparasites. This results when there is a positive coupling between a parasite's virulence and its capacity for infectious transmission.

The myxoma story is particularly compelling because the quantitative relationship between virulence and transmissibility inferred from the epidemiologic data and models was independently tested and demonstrated experimentally (30, 31). The myxoma story remains the only one for the microparasites of eukaryotic hosts where the predictions about transmission and virulence made from an interpretation of epidemiologic observations were tested experimentally. With few exceptions (32), inferences about the relationship between transmission and virulence and the trade-offs between these two attributes of a microparasite's association with its host have been derived from comparative evolution studies or retrospective interpretations of epidemiologic data. In some cases, these inferences are reasonably strong, e.g., in the study by Alan Herre (33) on fig wasps and a nematode parasite and by Deiter Ebert (34) on a planktonic crustacean with a protozoan parasite. The latter study is particularly convincing because it includes independent, experimental evidence of a positive correlation between the density of spores in infected hosts and the virulence and transmissibility of this protozoan parasite.

The enlightened view on the virulence of microparasites sometimes takes the positive association between the virulence of a microparasite and its transmissibility as axiomatic; therefore, it

assumes that a microparasite's virulence is constrained solely by the need to keep the host alive to facilitate its transmission to new hosts.[3] This is implicit in much of Paul Ewald's writing on this subject (6, 35) and is the basis of his main thesis that changes in rates of infectious transmission will select for microparasite strains or species with different levels of virulence.

By assuming a necessarily positive relationship between a microparasite's capacity for infectious transmission and the extent of morbidity and rate of mortality it causes in infected hosts, a positive "trade-off" (relationship) between transmissibility and virulence, Paul Ewald has generated scenarios for the evolution of virulence and changes in virulence for a diverse array of microparasites, including those responsible for cholera, influenza, dysentery, and AIDS (6, 35). While the details of Ewald's stories may differ, the plot is almost always the same: increases in the rates of transmission favor increases in virulence, and the reverse. For example, Ewald has postulated that the virulence of HIV observed in contemporary human populations, AIDS, is in large part due to evolution in this retrovirus responding to the increases in human-human transmission rate resulting from more promiscuous sexual behavior.[4] However, even when a direct relationship between the virulence and transmission rate of HIV is assumed, a deeper consideration of the epidemiology and course of this sexually transmitted disease shows that this simplistic conclusion about evolution and the virulence of HIV is chock full of caveats (36, 37). The relative contributions of transmission and virulence (as measured by the time before the onset of AIDS) to the fitness of HIV in the population of hosts depends on whether the disease is in an epidemic or endemic phase. Moreover, as I consider later, there are other, very different, hypotheses for the evolution of the virulence of this retrovirus and other pathogenic microbes that do not require the necessarily positive association between infectious transmission and virulence upon which Ewald has based his arguments for the evolution and maintenance of virulence in microparasites.

A corollary of the hypothesis of a positive trade-off between transmissibility and virulence is that if all else were equal, increases in the degree of vertical (e.g., from a mother to a fetus) transmission of a parasite, relative to its horizontal (infectious) transmission would favor reductions in its virulence (38). There is compelling, experimental evidence to support this corollary. However, the evidence is restricted to experiments with *E. coli* and its phage, *f1*, which can be transmitted vertically, in the course of cell division, or horizontally, by infecting susceptible, uninfected bacteria (39). While some, like me most of the time, may believe in the adage "what is true for *E. coli* is true for elephants, but only more so," other, less *coli*-centric souls, may want to see more experiments of this type with microparasites and vertebrate hosts. I certainly do.

Within-Host Population Dynamics and Virulence of Microparasites

There is a dearth of experimental investigations of the quantitative relationship between the transmission and virulence of microparasites. During the past few years, however, there has been a flurry of theoretical studies of the within-host population dynamics of microparasites that have specifically considered the relationship between the virulence and transmission rates of microparasites and their densities and/or rates of replication in infected hosts (40-44). In the simplest models developed in these theoretical studies of the within-host population dynamics of

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[3] One way to experimentally augment the virulence of a microparasite, as, for example, measured by declines in its LD_{50}, is to artificially pass that microbe between hosts (15). From one perspective, this result is consistent with the trade-off hypothesis, as the effect of passage is to make the parasite's transmission independent of the host's survival, thereby allowing it to become more virulent without compromising its need to be transmitted to other hosts. However, increased virulence in a passage experiment is not sufficient evidence for that trade-off. (It may well be that the parasite's capacity for infectious transmission is impaired as a consequence of whatever increased its capacity for infectious transmission.) I know of no experiments that demonstrate that the increase in virulence generated during a passage experiment is also reflected as increased—transmissibility, as is necessary for the trade-off interpretation. Indeed, it may well be that an increase in the case-mortality rate or a reduction in the LD_{50} of a microparasite will be reflected as a reduction in its natural transmissibility.

[4] I quote: "Severe immunodeficiency could develop in an old association [between a sexually transmitted virus or SIV and its host] as a result of increases in sexual partner rates causing evolution of increased virulence" (p. 143, reference 6). "If rates of unprotected sexual contact decline, so should the virulence of HIV" (p. 144, reference 6).

microparasites, the virulence of the microparasite, as measured by either the rate at which it kills its host or its LD_{50}, is assumed to be directly proportional to its rate of proliferation in that host, and its rate of infectious transmission is directly proportional to its within-host density (41). Under these conditions, in the absence of superinfection or mutation, selection favors microparasites with intermediate rates of within-host replication, i.e., intermediate levels of virulence. More complex situations, like the coexistence of microparasite lineages with different levels of virulence, result when virulence is proportional to the within-host growth rate of the parasite and single hosts can be infected with parasites of different growth rates (43) or when there are high rates of mutation to different levels of virulence within a host (45). Moreover, with superinfection and mutation, the theory developed in these two reports predicts that the average level of virulence of a parasite in an infected host can exceed that anticipated from models that do not allow for superinfection and/or assume that the parasite's level of virulence in an infected host remains invariant.

The Convergence of Theories

The predictions that can be made on the basis of the current view of the evolution of virulence differ from predictions that might follow conventional wisdom because the new view allows for natural selection in the parasite population to favor the evolution and maintenance of some level of virulence. Moreover, even when there is a positive association between a parasite's virulence and its transmissibility, under the conditions described in the following paragraph, the predictions of new methods can still converge with those of conventional wisdom.

If the density of the sensitive host population is regulated by the parasite, an extension of the enlightened theory predicts that natural selection in the microparasite population can lead to continuous declines in the level of virulence, possibly to immeasurable values (46). Although not stated in this general way, the same conclusion about declining virulence can be drawn from models of the epidemiology of HIV/AIDS (36, 37). During the epidemic phase of a microparasitic infection, when the host population is composed primarily of susceptible hosts, selection favors parasites with high transmission rates and thus high virulence. As the epidemic spreads, the proportion of infected and immune hosts increases and the density of susceptible hosts declines. As a result, the capacity for infectious transmission becomes progressively less important to the parasite's Darwinian fitness and persistence in the host population. Selection now favors less virulent parasites that take longer to kill their host and, for that reason, are maintained in the host population for more extensive periods. Analogous arguments have been made for the latent period of a bacteriophage infection (47), the evolution of lysogeny (48), the tradeoff between vertical and horizontal transmission (49, 50), and the advantages of microparasite latency in general (40).

Alternative Models for the Evolution of Microparasite Virulence

For any microparasite, the rate of transmission between hosts will always be a significant component of fitness, and, if all else is equal, parasites transmitted at higher rates in the host population have a selective advantage over less transmissible forms. On the other hand, there is no reason to assume that in general a microparasite's rate of infectious transmission will be positively associated with its virulence. Moreover, even when there is no relationship or a negative relationship between transmission and virulence, there are at least two ways by which natural selection can lead to the evolution and maintenance of virulence, coincidental evolution (24) and short-sighted within-host selection (51).

Coincidental Evolution

According to the coincidental evolution hypothesis, parasite-mediated morbidity and mortality are what Gould and Lewontin (52) likened to the spandrels of gothic churches. While these structural necessities may frame the frescos and paintings within, that is not the reason for their existence. They are architectural constraints. Analogously, the factors responsible for the virulence of a microparasite in an infected host may have evolved for some purpose other than to provide the parasite an advantage within a host or its transmission to other hosts.

It would be difficult to account for the evolution of botulism toxin by selection favoring *Clostridium botulinum* that kill people who eat improperly canned food. The same argument could be made for the toxins of *C. tetanae* and possibly for those produced by other free-living *Clostridia*. Although these organisms may proliferate in humans, they

are soil bacteria, and the effects of the toxin may not contribute to their capacity to colonize, proliferate, and be maintained in humans or to their capacity to be transmitted between human hosts. How many other microparasite-induced symptoms, and the resulting host morbidity and mortality, provide no advantage to that microbe in (or on) a host or its transmission between hosts? Did the lipopolysaccharides and other components of bacterial cell walls and cell membranes evolve because the fitness of bacteria expressing them is enhanced by "endotoxin"—induced overresponse of the immune system responsible for the morbidity and mortality of sepsis (53)? Do the toxins confer an advantage on *E. coli* O157 or *Staphylococcus aureus* (or the plasmids and phages that code for these toxins) because they produce, sometimes lethal, symptoms in infected hosts, hemolytic uremic and toxic shock syndromes, respectively? An earlier paper on this subject (24) argued that the adhesins produced by the *E. coli* responsible for the morbidity of symptomatic urinary tract infections evolved and are maintained to facilitate colonization of the gut. The painful symptoms of urinary tract infections generated by an inflammatory response to these adhesins may confer no advantage for the *E. coli* expressing them in the urinary tract and may in fact lead to the clearance of those bacteria (24).

Each of the symptom-inducing toxins and adhesins described above, as well as many other so-called "virulence determinants" (54) may indeed facilitate the microparasite's ability to colonize, proliferate, or be maintained in infected hosts, and/or be transmitted between hosts. This certainly sounds reasonable for many virulence determinants, e.g., the somatic cell invasiveness mechanisms of *Shigella*, the capsules of *Streptococcus*, the diarrhea-inducing toxins produced by *Vibrio cholerae*, and the sneezing and coughing induced by rhinoviruses. On the other hand, it is necessary to formally test this hypothesis that these symptoms have that effect and reject the alternative, that the morbidity and mortality generated by the expression of a specific virulence determinant provides neither a within- or between-host (infectious transmission) advantage to the parasite.

Short-Sighted Evolution

Natural selection is a local phenomenon. Characters that confer a survival or replication advantage on the individual organisms that express them at a given time or in a given habitat will be favored and evolve at that time and in that habitat. Whether the expression of those temporally or locally favored characters will increase or reduce the fitness of that organism at other times or in other habitats is irrelevant. Also irrelevant is whether a locally favored character makes the population better or less adapted to its environment at large or augments the likelihood of its survival in the future. This myopia is a fundamental premise of the theory of evolution by natural selection and the basis of the short-sighted evolution hypothesis for microparasite virulence (51).

Within an infected vertebrate host, microparasite populations go through many replication cycles and may achieve very high densities. They may also reside and proliferate in many different subhabitats (tissues and cells) and confront a variety of different and ever-changing constitutive and inducible host defenses which may, sequester, kill, or in other ways inhibit their proliferation. As a consequence of classic mutation, transposition, and recombination, genetic variability will be continually generated in the populations of infecting microbes. Mutant or recombinant microparasites that are better able to 1) avoid being done in or inhibited by the host's defenses; 2) proliferate in the host; or 3) invade and replicate in novel habitats, tissues, and cells where there is less competition from members of its species would have an advantage in that host. This would occur even when the expression of the characters responsible for that local advantage reduces likelihood of the transmission to other hosts. Stated another way, the morbidity or mortality caused by a microparasite infection could be the result of the within-host evolution that is short-sighted because that virulence actually reduces the rate at which that parasite is transmitted to other hosts.

Three examples of microparasite virulence that could be products of this mode of evolution can be considered (51). For two of these examples, bacterial meningitis and poliomyelitis, many human hosts are infected by the responsible microparasites, primarily *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* for meningitis and poliovirus for poliomyelitis, but very few manifest the symptoms of these infections. In the case of meningitis, the neurologically debilitating and sometimes fatal symptoms of the infection are a consequence of an inflammatory response against the bacteria entering and

proliferating in the cerebral spinal fluid. These meningitis-causing bacteria normally reside in the nasopharyngeal passages and are transmitted by droplet infection. The cerebrospinal fluid is, at least with respect to their infectious transmission, a dead end. On the other hand, bacteria capable of invading and proliferating in that habitat could have a local advantage as there are no other competing populations and only modest defenses. An analogous argument can be put forth for poliovirus. Symptomatic infections with this virus are caused by their invasion of and proliferation in the neurologic tissue of the central nervous system. Poliovirus normally replicates in the mucosal cells of the mouth, throat, and intestines and is transmitted by the oral-fecal route. Poliovirus virions proliferating in the central nervous system would almost certainly not be transmitted. The evidence in support of short-sighted evolution for the virulence of these specific microparasites is mostly circumstantial (51). On the other hand, short-sighted evolution for the virulence of specific microparasites is a hypothesis that can be tested. If the hypothesis is valid, the microparasites responsible for the symptoms would be genetically different from their ancestors that infected the host and better adapted for proliferation in the site of the symptoms than the ancestors themselves.

The third example of short-sighted evolution of virulence considered, HIV, is different from the other two in that virtually every human infected with this retrovirus that does not die of other causes, eventually manifests and succumbs to AIDS. However, although the case mortality of HIV infection may approach unity, as measured by the rate of mortality (deaths per unit of time), from an epidemiologic perspective, HIV is not a very virulent virus. There is substantial variation in the time between infection and the onset of AIDS. On average in industrialized countries, the term of this infection is 8 to 10 years (55). During the early phase of an HIV epidemic, most transmission of the virus occurs during the initial viremia, probably before seroconversion and certainly before the onset of AIDS (37, 56). It is not at all clear how the transmissibility of HIV virions during this early phase of the infection is related to the time of onset of AIDS. HIVs that are more transmissible early in the infection may lead to an earlier onset of AIDS. If this is the case and all else were equal, increasing opportunities for transmission during the epidemic phase would favor increases in HIV virulence (36, 37). However, there may be no association between HIV's capacity to be transmitted early and the time of onset of AIDS, or the time until the onset of AIDS may increase with the transmissibility of the virus during the early phase of the infection. Under either of these conditions, selection during the epidemic phase of the disease would favor more transmissible but less virulent HIVs.

In the course of HIV infection, the HIV population undergoes continuous genetic changes. In fact, in a number of hypotheses of HIV pathogenesis, AIDS is a consequence of mutation and selection in the HIV population that occurs during the course of the infection in individual hosts (57-60), i.e., short-sighted, within-host evolution. Albeit different in their details, all of these hypotheses are consistent with what is known about HIV infection, and all can account for the course of these infections and variable time of onset of AIDS.

Experimental Evolution Meets Experimental Epidemiology

Results of recent studies by population and evolutionary biologists predict at least three ways by which the virulence of microparasites can be favored and will be maintained by natural selection. 1) Direct selection: there is a positive relationship between the parasite's virulence and its rate of infectious transmission; 2) coincidental evolution: the parasite's virulence is due to character(s) favored and maintained by selection for some other function and the expression of those virulence determinants in an infected host does not confer a net advantage or disadvantage in the parasite population at large; and 3) short-sighted, within-host, evolution: the parasites responsible for the morbidity and mortality of an infection are selected for within the host because of a local advantage, and that evolution reduces the rate at which that locally adapted parasite is transmitted between hosts.

At this time, these predictions are based almost entirely on general theory and retrospective interpretations of epidemiologic and other observations about specific microparasites. Although this theory and these interpretations may be appealing, in a formal Popperian sense (61), almost all the mechanisms postulated for the evolution of virulence of specific microparasites are no more than untested hypotheses. However, unlike most evolutionary hypotheses, those about the evolution of microparasite virulence can be tested and rejected with prospective, experimental studies with

laboratory animal and plant hosts. These tests could be at two levels; first, tests of the validity of the assumptions behind these models of the evolution of virulence and second by tests of the predictions made from the consideration and analysis of these models.

For the direct selection hypothesis, it is essential to demonstrate a positive relationship between a microparasite's virulence and its rate of (or capacity for) infectious transmission. For mammalian hosts, protocols exist for determining this relationship (30-32). The object would be to estimate the densities of microbes at the sites of transmission (e.g., feces, nasal passages) during the entire course of the infection. Moreover, it would be useful to separately test the colonization ability and virulence of the microbes from these sites. According to the coincidental evolution hypothesis, it is possible that the virulence determinant responsible for morbidity and mortality in the host provides a local advantage to the parasite expressing it; whether it does or not could be tested with competition experiments between strains of that microparasite that are isogenic save for that virulence determinant. The genetic basis of many virulence determinants are known, and it should be possible to construct these strains. However, unlike in the direct selection hypothesis, in coincidental evolution, microbes expressing the virulence determinants should not be over represented at the sites of infectious transmission. Under the short-sighted evolution hypothesis, microbes isolated from the tissues and organs responsible for the symptoms of the infection (e.g., in the cerebrospinal fluid) should be better adapted for proliferation in those organs and tissues than the originally infecting strain from which they were derived. This could be tested with pairwise competition experiments between the original and potentially evolved strains injected at the site of the symptoms with a common, genetically marked competitor of that parasite. Here, too, it is necessary to demonstrate that the strain responsible for the symptoms is not overrepresented at the site of infectious transmission.

To test the prediction of the direct selection hypothesis and to exclude that mechanism in tests of the coincidental and short-sighted alternatives, it is necessary to study the epidemiology of the microparasites as well as their within-host properties. For bacteriophages and bacteria this is a relatively easy task, e.g., testing Abedon's hypothesis (47) about the direct relationship between the density of sensitive bacteria and selection for latent period length (a measure of virulence) and burst size (a measure of transmissibility). For eukaryotic hosts, this kind of study is going to be more difficult and, at this time, may not be possible. The basic protocols for experimental studies of the epidemiology of bacterial and viral infections of laboratory mice were developed and successfully employed a long time ago (32, 62).[5] However, experiments of these types are costly, labor-intensive, and time-consuming, and because of concerns about animal rights, it may be difficult to get permission to do these experiments with mice or other higher vertebrates. On the other hand, experiments of this type with insects and other invertebrate animal hosts as well as plants would be tenable and valuable as tests of the general theory, albeit less immediately relevant to the evolution and maintenance of virulence in human pathogens.

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[5] Greenwood and colleagues (32), studied microparasites with different transmissibilities ("infectivity") and virulence.In one replica of their study of pasteurellosis (due to infections with a bacterium they call *Pasturella muriseptica* in experimental populations of mice, they report "the appearance of a variant that had gained infectivity and retained, or perhaps increased, its original virulence." However, in general, their study and Fenner's (62) provide little information about the direction of natural selection in these microparasite populations. With respect to evolutionary questions, these were wait-and-see experiments. Only one strain of microparasite was introduced into each population, and it was necessary to wait for mutations that changed their virulence or transmissibility. More information about the direction of selection and a better test of these evolutionary hypotheses could be obtained in these types of experiments if two or more genetically marked strains of microparasites with different virulence and transmissibility were introduced simultaneously and were allowed to compete.

He is a population and evolutionary biologist, who, like a number of others of his ilk, recently discovered infectious disease. Currently he and the postdoctoral fellows and students working with him are doing theoretical (mathematical modeling) and experimental research on the within-host population dynamics of bacterial infections and their treatment, and the epidemiology, population genetics and evolution of antibiotic resistance.

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The Infectious Diseases Impact Statement: A Mechanism for Addressing Emerging Diseases

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The use of an Infectious Diseases Impact Statement (IDIS) is proposed for predictive assessments of local changes in infectious diseases arising from human-engineered activities. IDIS is intended to be analogous to an Environmental Impact Statement. The drafting of an IDIS for specific activities, particularly in developing nations, would provide a formal mechanism for examining potential changes in local health conditions, including infected and susceptible populations, diseases likely to fluctuate in response to development, existing control measures, and vectors likely to be affected by human activities. The resulting survey data could provide a rational basis and direction for development, surveillance, and prevention measures. An IDIS process that balances environmental alterations, local human health, and economic growth could substantially alter the nature of international development efforts and infectious disease outbreaks.

A 1995 report by Aksoy et al. (1) describing the GAP (Turkish acronym for the Southeastern Anatolia Irrigation Project) irrigation project in Turkey suggests that anticipating the emergence or expansion of vector-borne and zoonotic diseases in a limited environment is a useful exercise. According to the report, a number of diseases (e.g., leishmaniasis, malaria, and schistosomiasis) are likely to increase in direct response to the expansion of irrigation and the increases in under water acreage and human population in the GAP region. The succinct overview of the disease and vector conditions in the GAP area could serve as a starting point for creating what will be referred to in this article as an Infectious Diseases Impact Statement (IDIS), a document that would be analogous to the Environmental Impact Statement (EIS) routinely used in the United States to assess the likely effects of construction, irrigation, agriculture, and similar activities on a local environment or region. An IDIS, however, would not assess the environment directly, but rather would predict changes in local disease patterns resulting from changes to the local environment.

Like an EIS, an IDIS would be a predictive and proactive assessment. Drafting an IDIS for a particular region or microenvironment would provide a formal mechanism for asking (and attempting to answer) specific questions about future changes in local health conditions. For example, what are the diseases and vectors in the given area? How are the proposed changes to the environment (e.g., dam-building, forest-clearing) likely to change the incidence and the prevalence of those diseases and vectors? What actions should be taken during the course of a given project and in the future to prevent potential increases in disease and vector populations? If an increase in human disease is likely, is the expense of the proposed project warranted? Will the economic benefits of a particular development or agriculture project be offset by increased costs in health care, vaccination, and vector control?

The 1969 National Environmental Policy Act was designed to provide a legal mechanism in the United States for evaluating potential impact to the environment from development activities and for permitting the public to participate in the evaluation process at the earliest stages (i.e., "scoping"). A Council of Environmental Quality in the executive branch of the federal government was established as a monitor, and the EIS process was implemented to inform decision makers and the public of potential environmental problems and reasonable alternatives to proposed actions. Environmental Protection Agency (EPA) requirements for environmental assessments are outlined in the Code of Federal Regulations (CFR). An EIS is intended to prospectively examine impact "upon the quality of the human environment of the United States and, in appropriate cases, upon the

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environment of the global commons outside the jurisdiction of any nation" (46 CFR sec. 504.7). The requirements of an EIS typically include descriptions of human populations in the designated area, current land use patterns, air quality, noise levels, locations of wetlands and coastal zones, sites of historical or cultural value, and non-point source pollution. The protection of human health is implied in the EIS process, but this concern is usually assumed to pertain to the location of industrial plants and dumps and to exposure to toxic chemicals, heavy metals, ionizing radiation, and pesticides. The EIS process contains no explicit reference to infectious diseases or disease vectors affecting human health in response to deliberate environmental changes. In 1995, the only published EIS references to diseases, infectious or otherwise, were for proposed control measures at two California plant nurseries. Yet past events suggest that attention should be directed toward changes in infectious disease patterns directly attributable to human-engineered events.

For example, the construction of the Aswan Dam in Egypt is widely believed to have precipitated the appearance of Rift Valley fever (RVF) in Egypt during the 1970s (2). Tens of thousands of RVF cases and hundreds of deaths followed. Similarly, completion of the Diama Dam in Senegal, in 1987, led to epidemics of malaria and RVF (3); impoundment of the Volta Lake in Ghana, in 1968, led to an explosive outbreak of schistosomiasis (4). Increased agriculture on the Argentine pampas and along the edges of Bolivian forests has contributed to frequent hemorrhagic fever outbreaks caused by Junin and Machupo viruses, respectively (5). Mining operations in the Brazilian jungles have led to outbreaks of malaria (6). Road-building projects under way in Papua New Guinea are likely to bring large numbers of susceptible human hosts into contact with rare and yet-to-be-discovered viruses. These epidemics and encounters with new diseases are the unforeseen consequences of critically altering the local environment. As a consequence, development and agriculture projects initiated to improve the lives of local populations can have the opposite effect by increasing disease prevalence and causing new epidemics. Embedding an IDIS requirement into the planning and execution of large-scale projects likely to alter local environments could prevent new epidemics and reduce infectious disease–associated morbidity and mortality.

How Would an IDIS Work?

An IDIS would first need to be established as an integral component of any activity likely to affect the health of a local population. In tropical and developing regions of the world, that would include a variety of national and international development activities. The area designated for large-scale alteration would be surveyed for current disease vectors, and the local populations would be examined for diseases likely to be affected by the project in question. The quality of the surveillance and the extrapolation of expected changes brought on by a particular activity would vary, depending on the knowledge of diseases, vectors, local host immunity, and other factors. Although the variables increase the margin of uncertainty in such extrapolations, these estimates would be expected to improve as the state of field and laboratory research improves and experience with preparing an IDIS increases. A retrospective examination of earlier projects in similar environments would also provide information for developing an IDIS. The standards of "existing credible scientific evidence" and "reasonably foreseeable" impact that current environmental impact assessments rely on could also be applied to the early stages of the IDIS process.

The resulting preproject assessment would provide a snapshot of conditions in a defined area, including the following: diseases likely to fluctuate in response to project activities, numbers of infected and susceptible hosts, existing control measures, and vectors likely to be affected by project activities. Such baseline data are frequently absent from development and agriculture activities (7). Knowing what diseases are already present, and how they might be changed, allows one to ask how anticipated changes in disease prevalence and distribution might be prevented or controlled through changes in the proposed project, improved case finding and treatment, changes in local sanitation and housing, increased vaccination or prophylaxis, or pest management programs. Some or all of the above health maintenance measures could then become components of the overall planning, budgeting, and execution of any major development or agricultural activity in the area. Health and health maintenance would become factors in the overall design and cost of the project. In many instances, local disease surveillance would become an ongoing part of the project, with supplemental assessments being made to refine the original IDIS.

Who Would Request an IDIS, and Who Would Respond to the Request?

Initial candidates would likely be donor organizations (the U.S. Agency for International Development and the World Bank, for example) that provide funding and oversight. In the absence of federal or international statutes, these organizations have the stature and financial capability to make infectious disease control an integral part of their development projects. Indeed, they should have an urgent interest in doing so because increases in diseases or new epidemics increase financial demands on them for medicines, vaccines, and pest control. In the end, more money would be spent beating back the outbreaks and epidemics that foresight might have prevented. National health ministries, state and territorial health departments, and local medical communities in developing countries might also request or initiate an IDIS. The practice of drafting an IDIS and implementing its recommendations might also rejuvenate underfunded areas of international health, vector biology, parasitology, and medical entomology as professionals in these fields are called on to conduct infectious disease assessments of development activities. The peer-reviewed literature and electronic services such as ProMED, Outbreak, and the World Health Organization (WHO) and Centers for Disease Control and Prevention World-Wide Web sites could provide the public "scoping" role that posting in the *Federal Register* and allowing a period for public comment provide in the EIS process in the United States.

The first application of an IDIS to a large-scale development or environmental activity could come from western donor organizations working in the developing world. The successful demonstration of an IDIS could encourage other organizations, national health officials, and health activists to push for the routine integration of public health with national development. This could happen in the United States, as well. The United States recently experienced the emergence of Sin Nombre virus in the Southwest and is theoretically open to the introduction of five vector-borne diseases: malaria, Rift Valley fever, yellow fever, dengue, and arbovirus encephalitides (15). Public health officials and citizen activists could initiate independent IDIS for activities perceived to threaten the balance between health, the environment, and domestic productivity.

What are the Strengths and Limitations of the IDIS Process?

A project-embedded IDIS would not be the same as an environmental management program, which seeks to control disease vectors through environmental modification and manipulation and through reduced human contact with vectors (8). An IDIS would, in fact, precede environmental management control measures by first postulating the likely emergence of specific pathogens and vectors. The usefulness of an IDIS lies in its ability to provide a conceptual framework for identifying potential disease problems, and, indeed, preventing them by altering or curtailing the very activities that could lead to disease emergence.

In an activist sense, an IDIS could be wielded as a tool of caution or prevention, much as an EIS is wielded in the United States to alter or halt some activity perceived to be a threat to the environment. That ability to influence potential changes and to affect health could be vital; public health concerns connected with agricultural and developmental projects are usually a low priority among foreign ministries, international donor organizations, and engineers (9); neglecting them can leave the full benefits of development unrealized.

Lest anyone imagines that an IDIS could be used solely as a tool of the political Left, as a kind of "liberation microbiology," it is important to point out that the same IDIS could be used to justify the use of pesticides and other organized control measures, including the relocation of local populations. Recently, for example, pesticide use has come under attack by various environmental groups, and donor organizations have become increasingly reluctant to fund such activities (10). In the United States, EPA's Endangered Species Act has also tended to thwart the use of pesticides because of potentially adverse impact on some birds and mammals (11). However, an IDIS describing the probable emergence of important disease vectors could be used to justify such use. Thus, a health care issue could be twisted into a health scare by either the political Right or the Left. The recent ratification of the North American Free Trade Agreement (NAFTA) was preceded in the United States by an effort to stall the treaty with an EIS requirement. If an IDIS had predicted new disease outbreaks from increased border trade and traffic, that concern might have had greater impact on the public imagination than more abstract concerns about atmospheric particulates in the border

region and could have been effectively used by anti-NAFTA forces. An IDIS should be not a political tool but rather a valuable information source that helps guide economic development and land use.

How Can an IDIS Complement Existing Surveillance Systems?

Almost half of the planet's five billion people are at risk for one or more vector-borne diseases (12, 13). Surveillance remains a key tool for monitoring these diseases and identifying new cases and outbreaks. Four types of surveillance are used in the control of vector-borne diseases: 1) recording human cases, 2) determining vector distribution and infectivity, 3) monitoring vertebrate reservoirs, and 4) tracking weather patterns to predict vector distribution (14). But throughout the developing world and across tropical boundaries, effective and continuous surveillance is extremely difficult, if not impossible. Cases are missed; outbreaks go unreported. Effective case reporting and continuous field monitoring are best conducted in limited, well-defined areas. Within the microenvironments of human activities, an IDIS could provide valuable baseline surveillance data before changes to that area occur and affect disease and vector distributions. This information could provide a rational basis and direction for ongoing monitoring and corrective measures (e.g., vaccination, relocation, pest control). Focusing on a limited area and a limited number of diseases in that area may also expand the use of promising but underutilized technologic methods such as remote sensing and geographic information systems (GIS).

Haines et al. (15) noted the importance of vector-borne disease monitoring and recommended that remote sensing and GIS be used to detect changes in ecosystems and vector populations. To a large extent, however, the advantages of satellite imagery and GIS have not been realized, in part, because of the frequent absence of "ground truth" (data on diseases, vectors, and other factors in the area) and of having to wait to observe natural environmental changes likely to affect disease and disease transmission (16-18). Satellite imagery for much of the planet has been collecting in databases since 1972 (16). By 1998, accumulated satellite data will be in the petabyte (1,000 terabyte) range, 1,000 times larger than the contents of the Library of Congress (B. Montgomery, NASA, pers. comm.). High-resolution, multispectral, multiyear images for many potential development and agricultural sites are available. Using a preproject IDIS to "ground truth" the project's environment with current satellite imagery, it may be possible to more completely describe local disease and vector conditions and make more accurate predictions about their plasticity during periods of construction, flooding, or farming. The result would be a firmer linkage of ground surveillance and satellite imagery to monitor public health changes within a well-defined and limited environment.

In recent years, the sudden emergence of rare or forgotten diseases such as Ebola virus infection, dengue, yellow fever, plague, and hantavirus (Sin Nombre virus) infection has attracted the attention of the public and inspired renewed commitments to surveillance and control. WHO recently formed a rapid response unit (the Division of Emerging, Viral and Bacterial Diseases Surveillance and Control) to deal with outbreaks of new and reemerging infections (19). Similarly, nine Southeast Asian countries held a meeting on emerging diseases and concluded that each country should also develop rapid response teams for epidemics (20). However, these disease control efforts are almost entirely passive, with staff, equipment, and budgets idling in anticipation of something eventually happening somewhere. It is difficult to maintain a high degree of public and financial support for such wait-and-see approaches to disease control. The United States has suffered a serious decline in national surveillance and outbreak investigations, in part, because of decreased support for passive monitoring programs (11).

Is an IDIS Really Needed When the Existing EIS Statutes Already Cover Human Health and Safety Concerns?

In the United States the need is not clear. Infectious diseases caused by environmental manipulation may be assumed to fall under the general EIS category of human health. However, infectious diseases have not often been considered in the past, and it is easy to imagine that if they were a factor in the EIS process, an environmental/infectious disease issue could be smothered under the weight of government regulations and the adversarial legal system. EPA operates under 16 federal statutes and 70 congressional committees and subcommittees and is engaged in some 600 lawsuits at any given time (21). Moreover, emerging infectious disease issues could bring EPA and the EIS process into conflict with the missions of

federal agencies and state and local health departments. Outside the United States, beyond federal statutes and informed public debate, the need for an IDIS is clearer. In the developing world, epidemics and substandard health care are common, and the national goals of healthy environment and healthy economy are usually at odds. An IDIS process that balances environmental alterations, local human health, and economic development could substantially alter the nature of international development efforts and infectious disease outbreaks.

To the ancient Greeks, the past appeared in front of them, real and visible; the future was behind them, unseen and unknowable. With that perspective, they were always glancing nervously backward, looking for a future that usually managed to creep up and tap them on the shoulder. In a sense, we have the same perspective for disease surveillance and control that the ancient Greeks had for time. Past epidemics and our responses to them are readily apparent; it is that unexpected tap on the shoulder by a hantavirus or an Ebola virus that is always so startling. We cannot know when and where such pathogens will emerge. Their appearance is often a chance event initiated by unpredictable changes in weather or the accidental encounter of a single person with a mysterious vector. These taps on the shoulder are an affront to our sense of control and understanding of disease. Moreover, it is unsettling to the public's sense of security and its faith in medical research. Although we cannot expect to eliminate the surprises of emergent pathogens in the near future, we can take control of situations in which our own actions directly lead to the emergence of diseases. Generating an IDIS in areas where human activities are likely to disrupt endemic-disease patterns would be an important step in controlling future outbreaks. Routine application of a preproject IDIS could improve local surveillance and health care planning by 1) providing baseline data on endemic-disease and vector prevalence and competence; 2) embedding projected health maintenance costs into the planning and cost of any project or activity likely to influence the environment and public health; and 3) providing a mechanism for instituting project alterations and health care measures to offset adverse effects on the health of local populations.

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Emerging Disease Issues and Fungal Pathogens Associated with HIV Infection

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Fungal diseases are increasing among patients infected with human immunodeficiency virus (HIV) type 1. Infections due to *Candida* and *Cryptococcus* are the most common. Although mucocutaneous candidiasis can be treated with oral antifungal agents, increasing evidence suggests that prolonged use of these drugs results in both clinical and microbiologic resistance. The optimal therapy for cryptococcal meningitis remains unresolved, although initial treatment with amphotericin B, followed by life-long maintenance therapy with fluconazole, appears promising. Most cases of histoplasmosis, coccidioidomycosis, and blastomycosis occur in regions where their causative organisms are endemic, and increasing data suggest that a significant proportion of disease is due to recent infection. Aspergillosis is increasing dramatically as an opportunistic infection in HIV-infected patients, in part because of the increased incidence of neutropenia and corticosteroid use in these patients. Infection due to *Penicillium marneffei* is a rapidly growing problem among HIV-infected patients living in Southeast Asia. Although the advent of oral azole antifungal drugs has made primary prophylaxis against fungal diseases in HIV-infected patients feasible, many questions remain to be answered before the preventive use of antifungal drugs can be advocated.

Over the last decade, the incidence of fungal infections has increased dramatically. The human immunodeficiency virus (HIV) type 1 epidemic accounts for a large share of this increase. This article is not a general guide to the diagnosis or treatment of fungal diseases but rather a review of emerging disease issues in regard to these infections among HIV-infected patients. The infections discussed include candidiasis; cryptococcosis; the endemic mycoses histoplasmosis, coccidioidomycosis, and blastomycosis; aspergillosis; and penicilliosis. In addition, the role of preventive therapy for fungal infections in HIV-infected patients is assessed. Fungal pathogens and their most common manifestations in HIV-infected patients are listed separately (Table).

Candidiasis

Mucocutaneous candidiasis is one of the most common manifestations of HIV infection. In one prospective study, 84% of HIV-infected patients had oropharyngeal colonization by *Candida* species on at least one occasion, and 55% developed clinical thrush (1). While other yeasts may occasionally cause clinical disease, *Candida albicans* is the organism isolated from most patients (1, 2). *Candida* species normally colonize the gastrointestinal tract of healthy adults, and most infections in HIV-infected patients are endogenously acquired. In some cases, candidal strains can be transmitted from person to person (1).

Table. Common fungal pathogens in HIV infection and their most frequent clinical syndromes

Organism	Clinical syndrome
Candida albicans	Thrush, vaginal candidiasis, esophageal candidiasis
Cryptococcus neoformans	Meningitis
Histoplasma capsulatum	Disseminated infection with fever and weight loss
Coccidioides immitis	Diffuse and focal pulmonary disease
Blastomyces dermatitidis	Localized pulmonary disease and disseminated infection, including meningitis
Aspergillus fumigatus	Pulmonary disease with fever, cough, and hemoptysis
Penicillium marneffei	Fever alone or with pulmonary infiltrates, lymphadenopathy, or cutaneous lesions

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During the course of HIV infection, patients appear to be colonized with one or a few dominant strains, which tend not to change over time. Powderly and colleagues isolated the same strain of *C. albicans* in 11 of 17 patients with recurrent yeast infection, by DNA probe analysis (2). In another study, using contour-clamped homogeneous electric field electrophoresis, Sangeorzan et al. found that 60% of patients were colonized with one dominant strain of *C. albicans*. In 74% of these patients, recolonization with the same strain occurred after antifungal therapy (1). Using biotyping and restriction fragment length polymorphism analysis of 25S ribosomal DNA, Whelan and colleagues found that strains of *C. albicans* isolated from 24 patients with AIDS were not significantly different from strains from 23 patients without HIV infection (3). Thus, the candidal strains causing disease in patients with HIV infection appear to be the same as those colonizing patients without HIV infection and, in most patients, do not change over time.

In HIV-infected patients, candidiasis is virtually always mucocutaneous, involving the oropharynx, the esophagus, and the vagina. HIV infection by itself is not associated with the syndrome of disseminated candidiasis, which is characterized by candidemia, endophthalmitis, and multiple organ involvement. The precise immunologic processes that control candidal infection in HIV-infected patients are not known. However, mucocutaneous candidiasis is clearly related to the development of clinical cellular immunodeficiency. In fact, oropharyngeal candidiasis is an independent predictor of immunodeficiency in patients with AIDS (4). Moreover, a CD4 lymphocyte count < 200/µl is a major risk factor for the development of clinical thrush in HIV-infected persons (1).

Although oropharyngeal candidiasis is frequent in men, recurrent vaginal candidiasis is a common early manifestation of HIV infection in women. The location and severity of candidiasis in women with HIV infection appear to be closely associated with the degree of cellular immunodeficiency, based on the peripheral blood CD4 lymphocyte count. In a study of 66 women, mucocutaneous candidiasis developed in more than half of the women over a median of 14 months of follow-up; vaginal candidiasis, with a mean CD4 lymphocyte count of 506/µl, developed only in 10, while oropharyngeal candidiasis, with a mean count of 230/µl developed in 16, and esophagitis, with a mean count of 30/µl developed in nine (5).

Mucocutaneous candidiasis can be treated either topically or with systemic antifungal agents (1, 6), but such therapy does not eradicate colonization (1). Recently, several reports have noted the failure of azole drugs, particularly fluconazole, to treat recurrent cases of oropharyngeal candidiasis (1, 7). While factors such as diminishing cellular immunity, drug interactions, or decreased drug absorption may account for some of these treatment failures, increasing evidence suggests that *Candida* organisms are developing drug resistance.

In the past, a lack of consensus on the methods for performing antifungal susceptibility tests made it difficult to establish whether clinical failure of antifungal therapy was due to resistance of the organism. However, the National Committee for Clinical Laboratory Standards (NCCLS) has now developed reference methods allowing for uniform testing of yeast isolates (8). Using an NCCLS method, Sangeorzan et al. found that the MIC of fluconazole for *Candida* isolates increased over time among patients who had received fluconazole compared with those who received clotrimazole. Clinical resistance to fluconazole was associated with an increased MIC required by the isolate as well as the patient's low CD4 lymphocyte count (1). These data strongly suggest that continued use of antifungal agents, particularly fluconazole, leads to both clinical treatment failure and antifungal resistance, especially in highly immunodeficient patients.

Cryptococcosis

A rare disease before the HIV epidemic, cryptococcosis was identified very early in the epidemic as one of the most common life-threatening infections in AIDS patients (9). However, issues regarding its epidemiology and therapy remain unresolved.

A single species, *Cryptococcus neoformans*, is responsible for virtually all clinical cases of cryptococcosis. The species exists in two varieties, *neoformans* and *gattii*, which inhabit different ecologic niches. *C. neoformans* var. *neoformans* has been isolated in many parts of the world from numerous sites, most frequently from soil containing high amounts of dried bird excreta, particularly of pigeons and chickens. It has long been

presumed that inhalation of soil contaminated with such excreta is the most likely source of cryptococcal infection. However, few data support this hypothesis. In contrast, the only known environmental source of *C. neoformans* var. *gattii* is the river red gum tree (*Eucalyptus camaldulensis*), which grows in rural Australia. Although infection due to var. *neoformans* is worldwide, cases due to var. *gattii* have only been identified in tropical and subtropical regions, including areas where *E. camaldulensis* is not found (10).

Virtually all instances of cryptococcosis among HIV-infected persons have been caused by var. *neoformans*. The ubiquity of var. *neoformans* in the environment may be making exposure and subsequent infection likely; however, no clear link has ever been established between environmental sources of *C. neoformans* and the development of cryptococcosis in patients with HIV infection (10). In a recent study, Varma and colleagues, using genomic probe analysis, could not find a direct link between environmental sources of *C. neoformans* and infection in patients with AIDS or a unique strain of *C. neoformans* that was infecting these patients (11). Suppression of cellular immunity appears to be a critical factor in the development of cryptococcosis in HIV-infected patients, with the development of disease relating directly to the risk for AIDS and to the CD4 lymphocyte count (12, 13).

The appropriate therapy for cryptococcal meningitis in HIV-infected patients is unsettled at this time. The combination of amphotericin B plus flucytosine for 4 to 6 weeks has been considered standard for patients without HIV infection. However, concern about increased toxicity and decreased efficacy (12) has led to a reconsideration of this regimen in HIV-infected patients.

Several studies have examined the use of oral fluconazole in lieu of amphotericin B for initial therapy of cryptococcal meningitis in patients with HIV infection. Larsen and colleagues studied 21 patients and found that amphotericin B plus oral flucytosine resulted in fewer clinical failures and faster cryptococcal clearance of the cerebrospinal fluid than fluconazole alone (14). A large collaborative trial compared results with a relatively low dose of intravenous amphotericin B to those with oral fluconazole and found no significant difference in the overall mortality rate (15). However, in this trial, the early mortality rate, defined as death within the first 2 weeks of therapy, was slightly higher, and time to first negative CSF culture was somewhat longer among patients in the fluconazole group.

The recurrence of cryptococcosis, even after initial therapy has rendered the CSF culture sterile, is extremely common in HIV-infected patients (16); continued antifungal therapy appears to reduce the risk for recurrence (12, 16). Fluconazole in daily doses has been shown to be effective in preventing relapse (16) and is superior to amphotericin B in weekly doses (17).

Current information suggests that therapy for cryptococcal meningitis in HIV-infected patients should begin with amphotericin B, with or without flucytosine. Suppressive therapy with fluconazole should be given subsequently to prevent a relapse. When available, the results of a study sponsored by the Mycoses Study Group and AIDS Clinical Treatment Group of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, should clarify these issues.

Histoplasmosis, Coccidioidomycosis, and Blastomycosis

Unlike candidiasis and cryptococcosis, infections caused by *Histoplasma capsulatum*, *Coccidioides immitis*, and *Blastomyces dermatitidis* are acquired in specific geographic regions. Infections due to these fungi were not initially associated with HIV infection because the HIV epidemic in the United States began in the large urban areas of the East and West Coasts, outside the areas in which these fungi are endemic. As HIV infection spread to the Midwest, where histoplasmosis and blastomycosis are endemic, and to the Southwest, where coccidioidomycosis occurs, these fungi became recognized as major opportunistic agents.

Histoplasmosis

H. capsulatum var. *capsulatum* causes infection worldwide and is the organism most associated with disease in HIV-infected patients. A few cases of histoplasmosis in HIV-infected patients have been due to *H. capsulatum* var. *duboisii* (18), which is found in tropical Africa. In the Western Hemisphere, infection is concentrated in the eastern United States but is also found in the Caribbean as well as in Central and South America. *H. capsulatum* var. *capsulatum* is typically isolated from soil contaminated with avian or bat excreta,

and a number of epidemics among persons without HIV infection have been associated with disruption of contaminated soil.

Most cases of histoplasmosis in patients with HIV infection have occurred within the recognized area for endemic *H. capsulatum* in North America, the Ohio and Mississippi River valleys. However, within that area, great variability has been seen in the incidence of disease, with most cases being reported from a single city, Indianapolis, Indiana (19, 20). Since 1978, Wheat has recorded several outbreaks of histoplasmosis in that city, mostly centered in areas where active construction led to soil disruption. In the most recent outbreak, patients with AIDS accounted for more than 50% of the culture-proven cases of histoplasmosis (21); these data suggest that many of these cases represent new infection due to recent exposure rather than reactivation of latent disease.

Cases of histoplasmosis and HIV-infection have also been reported well outside the recognized histoplasmosis-endemic areas (22, 23). In some instances, these cases represent reactivation of infection acquired during residence in or travel to disease-endemic regions. In other instances, they appear to represent acute infection after disruption of microfoci of *H. capsulatum* that exist outside the recognized disease-endemic areas (19).

Patients with progressive disseminated histoplasmosis, the most common form of disease among HIV-infected persons, usually have fever, malaise, and weight loss over a period of weeks. Diagnosis is often established by isolating the fungus from respiratory secretions, blood, or bone marrow, but detecting *Histoplasma capsulatum* var. *capsulatum* polysaccharide antigen (HPA) in the serum or urine is also helpful (19).

Amphotericin B therapy is usually effective for progressive disseminated histoplasmosis in patients with HIV infection. Itraconazole may be useful for less severe cases (24). However, relapses are extremely common when therapy is stopped (19, 25), and maintenance therapy with intermittent amphotericin B (25) or with itraconazole (26) is required to prevent this. Fluconazole is less effective but can be used by those who cannot tolerate amphotericin B or itraconazole (27). Urine and serum HPA levels decline with successful therapy and can be useful in determining a patient's response to therapy as well as assessing a patient for relapse (26-28).

Coccidioidomycosis

Within the disease-endemic area (U.S. Southwest), coccidioidomycosis is one of the most frequent opportunistic infections in persons with AIDS (29). In a prospective study in Tucson, Arizona, active coccidioidomycosis developed in 25% of a cohort of HIV-infected patients over a 41-month period (30). The major risk for developing disease was immunosuppression, as manifested by a CD4 lymphocyte count below 250/µl, a diagnosis of AIDS, or anergy indicated on control skin tests. Length of time in the disease-endemic area, a history of prior coccidioidomycosis, and a positive coccidioidal skin test were not associated with the development of active coccidioidomycosis. These data suggest that most coccidioidomycosis cases among HIV-infected persons in a disease-endemic area are recently acquired and not due to reactivation of latent infection. However, as with histoplasmosis, in a small number of HIV-infected patients, previously acquired infection is reactivated, and clinical coccidioidomycosis develops while the patient is residing outside the disease-endemic area (29).

Despite recent outbreaks of coccidioidomycosis in the San Joaquin Valley and in Northridge, California (31, 32), most cases of coccidioidomycosis among HIV-infected patients have been reported from Arizona, particularly the metropolitan areas of Phoenix and Tucson (30, 33). Whether this represents underreporting of disease in California or a true increase in incidence in Arizona is unknown.

Most HIV-infected patients with coccidioidomycosis seek treatment for pulmonary disease. In many, chest radiographs show a diffuse, reticulonodular pattern. Approximately 70% of patients with this pattern die within 1 month despite antifungal therapy (30, 33). In fact, this radiographic pattern may mimic that seen with *Pneumocystis carinii* pneumonia (34). Sites of dissemination frequently seen in patients without HIV infection, such as skin, soft tissue, bone, joint, and meninges, appear less common among patients with HIV infection (30, 33). Serologic tests can be useful in diagnosing coccidioidomycosis in HIV-infected patients, although they are more likely to have negative results than patients without HIV infection (35). On the other hand, a positive coccidioidal complement-fixation serologic test result in an HIV-infected patient, even in the absence of

clinical illness, predicts impending active coccidioidomycosis (36).

Comparative trials regarding the therapy of coccidioidomycosis in HIV-infected patients have not been carried out. For severe disease, such as for diffuse, reticulonodular pneumonia, amphotericin B is recommended. However, for less fulminant infection, fluconazole has been effective. As with cryptococcosis and histoplasmosis, life-long maintenance therapy with an oral azole is recommended, although relapses have occurred despite this.

Blastomycosis

Blastomycosis is the least common of the three endemic mycoses in North America among patients with HIV infection; fewer than 25 cases have been reported. Only recently has an environmental link been established for infection, when the organism was isolated from riverbank soil in association with an outbreak (37).

Pappas and colleagues have published the largest series of cases associated with HIV infection, consisting of 15 patients from 10 medical centers (38). Ten of the 15 cases were reported from sites within the known disease-endemic area, the midwestern and southeastern United States, and the other five patients had resided within the disease-endemic area at some time before the diagnosis. Most patients were clinically immunodeficient at the time of diagnosis and had either chronic pulmonary infection or disease disseminated beyond the lungs, including meningitis. In all but one case, the diagnosis was established by culture of *B. dermatitidis*, whereas results of serologic tests, when performed, were uniformly negative. Both amphotericin B and ketoconazole were used successfully as therapy in the series.

Aspergillosis

Although disseminated aspergillosis was originally listed as an infection at least moderately predictive of AIDS, it was removed from the list in 1984 because only three cases had been reported among 1,762 patients (39). However, over the last 5 years, the number of cases of invasive aspergillosis among HIV-infected patients has dramatically increased (40-44); more than 75 cases now have been documented. The largest series, containing 33 patients, was reported by Lortholary and colleagues from France (43). All patients had AIDS and a median CD4 lymphocyte count of 27/µl. About half the patients had the traditional risk factors for invasive aspergillosis (neutropenia or corticosteroid use) at the time of diagnosis. Culture of fluid from bronchoalveolar lavage was positive in all cases in which it was done; *A. fumigatus* grew in 29 of 31 patients. In this study, isolating the fungus from bronchoalveolar lavage fluid correlated with histologic evidence of invasive aspergillosis in 14 of 15 patients.

In all series, the death rate has been extremely high despite therapy. Intravenous amphotericin B has been used most often for treatment, but itraconazole has been tried in some instances (40, 43). In a recent study of the use of itraconazole for the treatment of invasive aspergillosis in a variety of patients, therapy was unsuccessful in all 16 patients with AIDS and aspergillosis (45). In seven of these, either toxicity to the drug developed or clinical symptoms worsened while they were receiving itraconazole; nine died, two directly of aspergillosis and seven of other causes.

If the incidence of aspergillosis is increasing among HIV-infected patients, the factors associated with this increase remain unclear. The identified risk factors for aspergillosis have no doubt increased among HIV-infected patients in the last decade with the use of such drugs as zidovudine and ganciclovir, which are associated with neutropenia, and corticosteroids for the treatment of severe *Pneumocystis carinii* pneumonia and other conditions. Others have postulated that prior pneumonia, which may result in diminished macrophage function (43) and contaminated air, inhaled during marijuana smoking (40), may also play a role. Further studies are needed to clarify this.

Penicilliosis

Piehl and colleagues reported the first case of infection due to *Penicillium marneffei* in an AIDS patient in 1988 when they described a patient in Chicago with persistent fever, anorexia, and a papular skin rash. The patient's travel history was not given. Blood, bone marrow, sputum, and skin biopsy specimen cultures all grew *P. marneffei*. The patient responded to therapy with amphotericin B, but relapsed once the therapy was discontinued (46).

Since that report, the number of reported cases among HIV infected patients has risen rapidly, particularly in association with the HIV epidemic

in Thailand. In a series of 80 patients reported by Supparatpinyo and colleagues (47), most patients were men from the Chiang Mai province of northern Thailand. The most common characteristic in these patients was a generalized papular rash; some of the lesions had central ubilication reminiscent of molluscum contagiosum. Diagnosis was usually established by examining Wright-stained samples of bone marrow aspirates or touch smears of skin biopsy specimens.

Treatment with amphotericin B has been successful in most patients. Itraconazole and fluconazole may also be useful. The mortality rate appears most related to a delay in diagnosis. Relapse is common once therapy is stopped (48); therefore, antifungal therapy should be life-long in the HIV-infected patient with penicilliosis.

In northern Thailand, penicilliosis is now the third most common opportunistic infection (after tuberculosis and cryptococcosis) among HIV-infected persons (48). Given that *P. marneffei* appears endemic in Southeast Asia, penicilliosis can be expected to become an even larger problem as the HIV epidemic continues to expand in this part of the world.

Prevention of Fungal Infections in HIV-Infected Patients

With the rise of fungi as opportunistic pathogens among patients infected with HIV and with the success of preventive therapy for other opportunists, such as *P. carinii*, primary prevention of fungal disease in HIV-infected patients should be pursued. The goal of any such prevention would be to increase both the length and the quality of the patient's life.

Since the availability of the oral azoles, using antifungal agents to prevent fungal diseases in HIV-infected patients has become a promising approach. However, several questions must be considered before antifungal chemoprophylaxis is used (49). Is the prevalence of disease high enough to make the drug useful in most patients? Is the efficacy of the drug in preventing disease sufficient? Does the therapy induce the development of drug resistance? Is the cost of the drug reasonable? Finally, does the drug have an acceptable toxicity profile, and does it interact or interfere with the metabolism of other drugs?

Few answers to these questions are available. In a recently published prospective, randomized study comparing fluconazole to topical clotrimazole (13), fluconazole was significantly better than clotrimazole in preventing oropharyngeal and esophageal candidiasis as well as cryptococcal meningitis. The benefit of fluconazole was greatest among patients whose CD4 lymphocyte count was < 50/µl. However, 10% of the patients had at least one episode of candidiasis while taking fluconazole, which suggests drug resistance. Moreover, the overall and fungal-disease-related death rates were no different between the two groups. Given the present data, further studies will be needed before the issue of primary prevention of fungal disease through chemoprophylaxis is settled (50).

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An Outbreak of Ross River Virus Disease in Southwestern Australia

More than 540 serologically confirmed cases of Ross River (RR) virus disease have been reported from the southwest region of Western Australia since November 1995 (Figure 1). Most affected by the mosquito-borne disease are communities on the Swan Coastal Plain south of Perth. Cases have also been reported from towns farther south or inland and from Perth itself. These regions were foci of RR virus activity during previous southwest outbreaks in 1988–89 and 1991–92 (1,2); however, the current outbreak differs somewhat in the timing and location of virus activity. This article is a preliminary overview of the incidence of disease, mosquito and virus activity, and environmental conditions before and during the outbreak.

Monitoring of the incidence of human disease provided no indication of abnormally high levels of RR virus activity until mid-December 1995 when the number of reported cases began to rise sharply. In contrast, monitoring of mosquito breeding sites, adult mosquito populations, and environmental conditions in late October and November 1995 showed a potential for high levels of virus transmission.

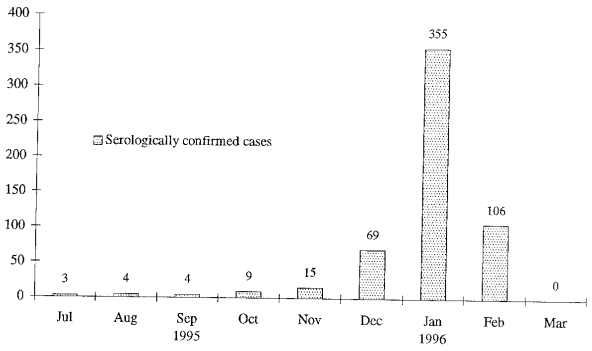

Figure 1. Serologically confirmed cases of Ross River virus disease, by month of onset, in the southwest of Western Australia, July 1995 to February 1996, as reported by doctors to the Health Department of Western Australia (when possible, case follow-up questionnaires were administered by environmental health officers from relevant local authorities). Only a small number of cases diagnosed by state and private laboratories, although the patient was not notified, have been included. Consequently, the number of cases shown is almost certainly an underestimate of the true number of serologically confirmed cases. Almost 65% of cases have dates of onset in January 1996. However, further notifications and analysis of follow-up questionnaires that have not yet been carried out for many January/February cases may alter this pattern. Previous southwest outbreaks also peaked in January or February but were considerably less acute.

The areas affected most, in terms of numbers of cases and attack rates (not shown), are coastal towns and communities around the Leschenault Inlet (including the city of Bunbury), between 165 and 190 km south of Perth and in the shires of Capel and Busselton, as well as on the coast between 190 and 245 km south of Perth (Table 1). These regions are popular tourist destinations during the summer holidays. It appears that many holiday-makers from elsewhere in the southwest, as well as local residents, were exposed to infected mosquitoes in these regions during the Christmas–New Year period.

Many of the Perth cases are from semirural, outlying suburbs, but some are from suburbs closer to the city center, often near the Swan and Canning rivers or fresh water wetlands and lakes. Follow-up questionnaires indicate that a considerable proportion of metropolitan cases were in persons exposed in the southwest, particularly in the Leschenault, Capel, and Busselton regions. However, many cases from Perth also appear to have been locally acquired. Locally acquired cases were also present during the two previously reported outbreaks.

Many cases have also been reported from the Peel region, 70 km to 130 km south of Perth, surrounding the Peel Inlet and Harvey Estuary. However, considerably more cases had been reported by late February in the Peel region during the 1988–89 and 1991–92 outbreaks. Also, during 1988 and 1991 virus activity in the Peel outbreaks commenced earlier than in the Leschenault and Capel-Busselton regions; this is apparently not the case during the current outbreak (Table 1). The reasons for these differences are not yet clear, but extensive control of saltmarsh mosquito breeding has been carried out in the Peel region this season.

Large saltmarshes and brackish wetlands in the Peel, Leschenault, Capel, and Busselton regions provide an ideal breeding habitat for *Ae. camptorhynchus* mosquitoes (5,6). This species is the major vector of RR virus in the southwest of

Table 1. Cases of Ross River virus disease, by month of onset and geographic region, in the southwest of Western Australia, July 1995 to February 1996[a]

Southwest region	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Totals
Metro. area	1	1			6	23	99	17	147
Peel		1	3	1	1	8	34	9	57
Leschenault	2			1	3	9	114	30	159
Capel/Busselton				2	1	26	73	20	122
Inland/south coast		2		1	2	3	30	27	65
North/east of Perth			1	4	2		5	3	15
Totals	3	4	4	9	15	69	355	106	565

[a]Cases are recorded by region in which exposure most likely occurred, where available (from case follow-up questionnaires), or by region of residence. Data are incomplete. Case follow-up questionnaires are available for many January/February cases but have not yet been analyzed, and most pathology laboratory reports (nonnotified serologically confirmed patients (i.e., figures represent underestimate of true number of confirmed cases) are not included.

Western Australia. Surveillance during previous outbreaks has clearly shown that the risk for RR virus transmission in coastal regions of the southwest increases markedly if large populations of adult *Ae. camptorhynchus* persist into late spring and summer (1,2). Adult mosquito populations and RR virus activity are monitored routinely by our laboratory at up to 40 sites between Rockingham and Dunsborough (50 km to 260 km south of Perth) each fortnight through spring and summer. In addition, saltmarsh mosquito breeding sites are regularly monitored by local authorities and the health department.

Widespread breeding of *Ae. camptorhynchus* (larvae) was observed in the Capel—Busselton region in late October 1995 and prompted a health department warning of an increased risk for RR virus transmission in the southwest. However, almost no activities to control mosquito larvae were carried out in the worst-affected regions. The adult mosquito monitoring program subsequently showed that extremely large populations of *Ae. camptorhynchus* survived through November and December. (Figures 2 and 3). The number of mosquitoes collected per trap per night in the Capel–Busselton region during November and December 1995 (up to 10,000 mosquitoes per trap at some sites) is unprecedented in the 5 years of surveillance in the region. Similar results were obtained in the Leschenault region where the number of *Ae. camptorhynchus* mosquitoes collected during December 1995 and January 1996 were similar to those observed during the 1991–92 outbreak. These observations, along with the expected seasonal exodus of city dwellers to these areas during the Christmas holidays, prompted a second warning by the health department in December 1995.

Fourteen isolates of RR virus were obtained from *Ae. camptorhynchus* mosquitoes collected at a major wetland west of Busselton on December 7, 1995 (Figure 3). Large populations of potential vertebrate hosts (western gray kangaroos, *Macropus fuliginosus*) were also observed in close proximity to this site throughout the spring and summer. Case follow-up questionnaires indicate thata large percentage of Busselton patients were exposed in this locality.

The last time RR virus was isolated from this site was during the 1991-92 outbreak in the region.

Mosquito populations in the Capel–Busselton region (Figure 3) and most areas of the Leschenault region dropped rapidly by mid-January 1996. However, further cases with dates of onset in February have been reported (Table 1), which suggests a high infection rate in the remaining adults. The results of processing of adult mosquitoes collected in February are, therefore, eagerly awaited.

Several isolates of RR virus were also obtained from mosquitoes collected in the Peel region (Table 2). The recent isolations from *Ae. vigilax* are of particular concern. This species is regarded as the major vector of RR virus in coastal areas of northern and eastern Australia (3,4), but until now it has had little or no role in transmitting RR virus in the southwest (1,2). *Ae. vigilax* has become the

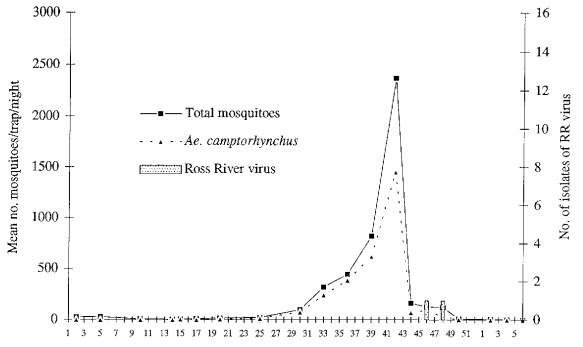

Figure 2. Mean number of adult mosquitoes (total population and dominant species) and isolations of Ross River virus from mosquitoes at Capel–Busselton region, wetland site, January 1994 to April 1995.

Table 2. Isolations of Ross River (RR) virus from mosquitoes collected in the Peel region: 1995–96 season

Date	Species	Isolates of RR virus
14-Sep-95	*Ae. camptorhynchus*	1
24-Oct-95	*Ae. camptorhynchus*	2
7-Dec-95	*Ae.camptorhynchus*	1
27-Dec-95	*Ae. camptorhynchus*	5
15-Jan-96	*Ae. camptorhynchus*	2
15-Jan-96	*Ae. vigilax*	3

dominant species in the Peel region between December and March since the opening of the Dawesville Channel. This mosquito is a vicious biter, even during the day if weather conditions are suitable, and is known to disperse considerable distances from breeding sites. Thus, the potential for interaction between infected mosquitoes and humans in the Peel region may be greater and occur over a wider area than originally thought.

Analyses of environmental conditions before and during the outbreak are not yet complete. However, record-high daily rainfall was recorded in October at numerous centers in the southwest. Above-average rainfall occurred in November in Perth and Mandurah and in December in Mandurah, Banbury, and Capel-Busselton. These were accompanied by above-average October and November temperatures at many southwest centers. A series of extremely high tides was also recorded along the Peel–Leschenault region coast around December 20. This resulted from unusually early cyclonic activity (three cyclones) along the north and west coasts of Western Australia during December. Clearly, a combination of some or all of these factors enabled widespread breeding and survival of vector mosquito species. Late spring and summer rains, a short-term rise in sea level (accompanied by higher tides), and mild spring and summer temperatures were predisposing factors during previous outbreaks in the southwest (2,4).

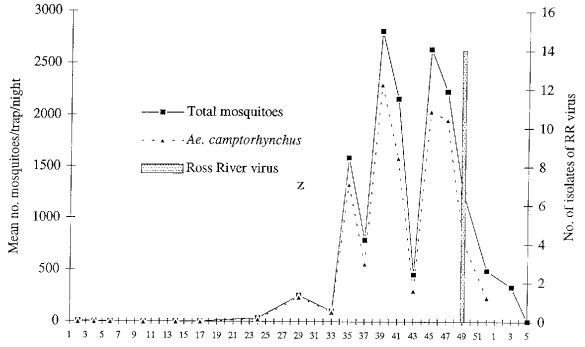

Figure 3. Mean number of adult mosquitoes (total population and dominant species) and isolations of Ross River virus from mosquitoes at Capel–Busselton region, wetland site, January 1995 to January 1996.

Preliminary analysis of the location of virus activity (measured as either human cases or isolations from mosquitoes) shows that activity is far less likely in regions in which virus activity was detected in the previous season. Thus, length of time since the previous outbreak also appears to be a predisposing factor for higher levels of virus activity in the southwest. The reason for this is not yet known but may be due to higher levels of immunity in recently infected populations of enzootic or amplifying vertebrate hosts. This may help explain the comparatively reduced numbers of cases in the Peel region this season following moderate levels of virus activity last year, which coincided with the opening of the Dawesville Channel.

A small number of cases of Barmah Forest virus infection have been diagnosed during the current outbreak. Numerous cases of a RR virus-like disease have also been reported, as in the 1988-89 and 1991-92 outbreaks. Serum from these patients has been tested for IgM antibody to RR and Barmah Forest viruses but is negative for both viruses. Some of these cases may be in persons that had not seroconverted at the time of the first blood sample. However, many have since provided further samples, all of which have had negative test results. Sera from these patients are being tested against a wide range of other Australian arboviruses, and more blood samples will be sought to ensure that the phenomenon is not due to an extremely delayed immunologic response to RR virus.

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Invasive Penicillin-Resistant Pneumococcal Infections: A Prevalence and Historical Cohort Study

More than 25 years ago, isolates of *Streptococcus pneumoniae* were uniformly susceptible to penicillin. However, since a penicillin-resistant pneumococcus was first identified in 1967 (1), the incidence of penicillin-resistant *S. pneumoniae* (PRSP) strains has been gradually increasing. In certain areas of the United States, PRSP strains have become widespread; Alaska has the highest reported prevalence, 26% (2); a recent study conducted in Atlanta, Georgia, found a 25% prevalence of PRSP (3). Outside the United States, an even higher (33%-58%) prevalence of PRSP has been reported (2).

Pneumococcal infections are a leading cause of morbidity and mortality in the United States. *S. pneumoniae* causes more than 500,000 cases of pneumonia, 55,000 cases of bacteremia, and 6,000 cases of meningitis annually, which result in 40,000 deaths (4). The death rate from pneumococcal bacteremia approaches 30%, despite the use of appropriate antimicrobial therapy (5). Reports of refractory illness due to resistant pneumococci demonstrate the clinical relevance of these strains (6,7). Identifying risk factors in the development of PRSP infections is important for both the prevention and treatment of these infections.

The prevalence of invasive infections due to PRSP was previously studied in Denver, Colorado (8,9). We undertook the study described here to determine the prevalence of invasive PRSP infections in the Colorado Front Range and to determine whether invasive PRSP infections have increased in metropolitan Denver since the earlier studies. In addition, we studied a cohort of patients who had invasive pneumococcal disease during 1994 in metropolitan Denver to ascertain risk factors for invasive PRSP infections.

Twenty-six hospital microbiology laboratories in the Colorado Front Range, which comprises the 10 largest counties in Colorado and 80% of the state's population (10), submitted to the Colorado Department of Public Health and Environment reports of all blood and cerebrospinal fluid (CSF) isolates of *S. pneumoniae* that were tested for penicillin susceptibility during 1994. (Penicillin susceptibility testing on invasive pneumococcal isolates was standard practice for the laboratories and did not depend on a clinician's request.) For the part of the study that assessed prevalence, penicillin resistance was defined as a minimum inhibitory concentration (MIC) of ≥ 0.12 µg/ml or an oxacillin zone of inhibition < 20 mm. Isolates that were tested by an MIC method were further classified as intermediately (0.12 µg/ml-1.0 µg/ml) or highly (≥ 2.0 µg/ml) penicillin resistant. For the part of the study in which the cohort was analyzed, only isolates that were confirmed as penicillin resistant (i.e., MIC ≥ 0.12 µg/ml) by either broth dilution or the E test (AB Biodisk, North America, Inc., Culver City, California) were included. Data for the cohort study were collected by chart review by the principal investigator, and telephone interviews of patients were conducted by trained interviewers in the Health Statistics Survey Research Unit of the Colorado Department of Public Health and Environment. For patients under 18 years of age, a parent or legal guardian was interviewed. When patients had died, a relative of the patient (if available) was interviewed.

Invasive pneumococcal infections were found in 363 patients in the Colorado Front Range; 49 (13%) of the infections were resistant to penicillin. In metropolitan Denver, 29 (14%) of the invasive pneumococcal infections were penicillin-resistant, of which 20 (69%) were intermediately penicillin-resistant (i.e., MIC 0.12-1.0 µg/ml), and 9 (31%) were highly penicillin-resistant (i.e., MIC ≥ 2.0 µg/ml). This prevalence rate of invasive PRSP infections is significantly higher than the previously reported rates in Denver of 1% (8) and 7% (9). Previous surveillance of invasive PRSP isolates showed that one region in the United States, which included Colorado, had a significantly higher rate of penicillin resistance among pneumococcal isolates than other U.S. regions (11).

Half of the PRSP strains in the cohort part of the study that were tested for cephalosporin susceptibility were resistant to an extended-spectrum cephalosporin. Our results are similar to the recent Atlanta study which found that 34% to 54% of PRSP infections were resistant to an extended-spectrum cephalosporin (3). These rates are much higher than other reported rates of cephalosporin resistance among PRSP isolates of 27% in Kentucky and 25% in Tennessee, (12). This has important implications for the management of invasive

PRSP infections, especially in meningitis, where MICs of ß-lactam antibiotics in the cerebrospinal fluid may be less than the MICs of ß-lactam antibiotics in the blood (13). The Centers for Disease Control and Prevention recommends that in areas where pneumococcal resistance to cephalosporins is high, empiric therapy with vancomycin plus an extended-spectrum cephalosporin should be considered in all cases of meningitis potentially caused by *S. pneumoniae*, until the results of culture and susceptibility testing are available (14).

A number of studies have addressed the clinical relevance of PRSP infections and have attempted to identify predictive factors for the development of these infections. In our analysis of the demographic and clinical characteristics of the study population (Table), we found that day-care attendance by a member of the patient's household in the 3 months before the patient's illness was associated with invasive PRSP infections. Indeed, 26% of patients with PRSP infections, compared with 7% of patients with penicillin-sensitive infections, had at least one member in their household, excluding the patient, who had been attending a day-care center before becoming ill.

Table. Demographic and clinical characteristics of patients with invasive penicillin-resistant *S. pneumoniae* (PRSP) and penicillin-sensitive *S. pneumoniae* (PSSP) infections

	PRSP; n = 29 (%)	PSSP; n = 180 (%)	Odds ratio (95% confidence interval)
Demographic characteristics			
Age, years			
< 5	11 (38)	39 (22)	1.9 (0.4-9.1)
5-64	8 (28)	93 (52)	Reference
> 64	10 (34)	48 (27)	1.9 (0.5-7.1)
Sex			
Male	12 (41)	104 (58)	Reference
Female	17 (59)	76 (42)	1.8 (0.6-5.4)
Race[a]			
White	16 (55)	100 (56)	Reference
Nonwhite	11 (38)	68 (38)	1.4 (0.5-3.9)
Clinical characteristics			
Site of infection			
Blood	26 (90)	171 (95)	Reference
CSF	3 (10)	9 (5)	0.8 (0.1-5.1)
Underlying medical condition	14 (48)	108 (60)	1.0 (0.3-3.2)
Antibiotic use[b]	14 (52)	56 (39)	2.5 (0.9-7.1)
No history of penicillin allergy[c]	26 (90)	163 (91)	0.7 (0.1-3.4)
Previous hospitalization[d]	5 (19)	33 (23)	0.3 (0.1-1.5)
Day-care attendance			
Patients < 11 years[e]	5 (50)	19 (50)	1.1 (0.4-2.7)
Household member(s)[f]	7 (26)	10 (7)	8.1 (2.2-30.7)
Residence in a long-term care facility (patients > 64 years)[g]	2 (20)	13 (27)	0.7 (0.1-3.6)
Hospital-acquired infection	1 (3)	8 (4)	0.4 (0.04-4.9)
Outcome			
Survived	25 (86)	156 (87)	Reference
Died	4 (14)	24 (13)	1.6 (0.4-6.3)

[a] Missing information on 2 PRSP and 12 PSSP patients.

[b] Includes patients who had taken an antibiotic in the 3 months before illness; missing information on 2 PRSP and 36 PSSP patients.

[c] Missing information on 1 PSSP patient.

[d] Patients who were hospitalized in the 3 months before illness; missing information on 2 PRSP and 34 PSSP patients.

[e] Children < 11 years of age who attended day care in the 3 months before illness; children < 11 years of age, N = 56 (PRSP = 12, PSSP = 44); missing information on 2 PRSP and 6 PSSP patients.

[f] Patients with at least 1 child < 11 years of age (excluding the patient) in the household who attended day care in the 3 months before illness; missing information on 2 PRSP and 39 PSSP patients.

[g] Adults > 64 years of age who resided in a LTC facility in the 3 months before illness; adults > 64 years of age, N = 58 (PRSP = 10, PSSP = 48).

This study is unique in that, to our knowledge, day-care attendance among household members of patients has not been studied. Even though most studies have not specifically considered family members as a potential mode of PRSP transmission, rates of nasopharyngeal carriage of PRSP are significantly higher in family contacts of children colonized with PRSP who were attending day-care centers (7, 15).

Our finding that patients with PRSP infections were more likely to have had a child in their household who had attended day-care during the months before their illness suggests that day-care settings may serve as foci for spreading resistant pneumococcal strains. Antibiotics are extensively used to treat upper respiratory infections that children attending day-care centers often have; the practice of administering a prolonged course of prophylactic antibiotics to children with recurrent otitis media who

attend day-care centers (16) may promote the selection of resistant bacteria in these settings (6, 15). These children may subsequently transmit resistant *S. pneumoniae* to susceptible persons in their households. Thus, patterns of antibiotic treatment of children who attend day-care centers may explain why day-care attendance might facilitate PRSP transmission. The likelihood that day-care settings may serve as reservoirs for antibiotic-resistant pneumococci indicates that the efficacy of prophylactic antibiotics for otitis media should be reassessed, especially when PRSP is present in a community.

Furthermore, carriage of or infection with PRSP has been associated with recent use of antibiotics (17). Our study showed that patients with PRSP infections were more likely to have taken an antibiotic in the 3 months before their illness than patients with penicillin-sensitive pneumococcal infections. This finding supports the theory that antibiotic resistance has developed because of the widespread availability and use of antibiotics. Since the beginning of the antibiotic era 50 years ago, it has been well recognized that antibiotics have been and continue to be inappropriately used (18).

The emergence of drug-resistant *S. pneumoniae* emphasizes the importance of following the recommendation of the Immunization Practices Advisory Committee that all persons 2 years of age and older who are at high risk for pneumococcal disease receive the 23-valent pneumococcal capsular polysaccharide vaccine. Because of its lack of immunogenicity and efficacy, the pneumococcal vaccine has not been licensed for children under 2 years of age (14). The high prevalence of PRSP among young children (3, 17), and the potential for these children to transmit PRSP to susceptible persons, underscore the need for an effective pneumococcal vaccine for this age group.

Antimicrobial resistance contributes to increased morbidity, mortality, and health care costs (19). The solution lies in changing antibiotic prescribing patterns, changing patient attitudes about the necessity of antibiotics, increasing surveillance of drug-resistant organisms, improving techniques for antibiotic susceptibility testing, and investing in research and development of newer antimicrobial agents.

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Nosocomial Transmission of Multidrug-Resistant *Mycobacterium tuberculosis* in Spain

Before 1990, outbreaks of multidrug-resistant tuberculosis (MDRTB) were uncommon (1); since then, more than 10 outbreaks have been reported, all in hospitals and prisons in the eastern United States (2-7). Persons traditionally considered at risk for MDRTB (foreign-born TB patients and those inadequately treated for TB) have not been associated with these outbreaks. Instead, the presence of patients with active TB near immunocompromised patients in HIV-dedicated wards has led to MDRTB-infected HIV patients whose TB cases often go unrecognized. The patients receive inadequate treatment in facilities without effective procedures for isolating acid-fast bacilli; these circumstances favor nosocomial transmission. Health officials in other geographic areas where HIV and TB are major public health threats have been alerted to this emerging problem, and surveillance systems have been designed (8).

Spain has the highest reported incidence rate of AIDS in Europe (143.4 cases per million in 1994) (9). Although in Spain TB is not notifiable at the national level, reported rates in the autonomous community of Madrid for 1994 were among the highest in Europe (33.5/100,000) (10). During a 45-month period starting in September 1991, a number of patients and one health care worker became infected with MDRTB in a 120-bed, infectious disease reference hospital in urban Madrid. In May 1995, the Field Epidemiology Training Program of the Spanish Ministry of Health was invited to assist the Madrid Department of Health and hospital officials in investigating the outbreak. This report describes the findings of the epidemiologic and molecular laboratory investigation and analyzes risk factors associated with the outbreak. The study was designed in three parts: 1) a description of the reported MDRTB cases, including a laboratory investigation of isolates; 2) a case-control study comparing HIV-infected patients who also had MDRTB to HIV-infected patients who did not have MDRTB; and 3) a study of tuberculin conversion among hospital employees.

We reviewed the medical records and laboratory specimen testing results of a series of patients with MDRTB in the HIV-dedicated ward of the hospital for January 1991 through June 1995. Cases were defined as patients with culture-confirmed TB and drug resistance to at least rifampin and isoniazid and with no previous history of inadequate treatment. Demographic and clinical variables were collected to characterize the cluster. Drug susceptibility testing and DNA subtyping analysis were performed with resistant strains available at the time of the study. Drug susceptibility testing was performed by the method of proportions in Middlebrook 7H11 medium distributed in petri plate compartments (reference).

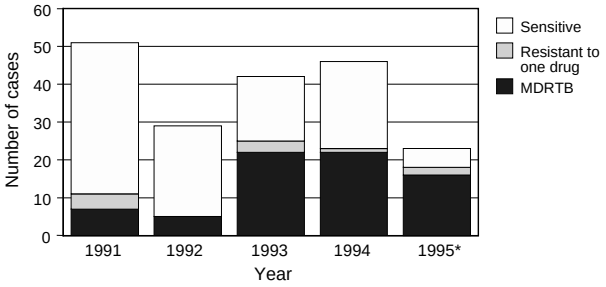
All 48 reported cases of isoniazid and rifampin resistance were among HIV-infected patients hospitalized in the HIV-dedicated ward from September 15, 1991, to May 1995. One patient was an HIV-infected nurse who worked on the ward from 1990 to 1994. The mean age of patients was 34.1; 81.3% were male, and 66.6% were intravenous-drug users (Table 1). Of the 47 (97.9%) who died, the mean interval from diagnosis to death was 77.6 days.

The epidemiologic curve suggests a propagated transmission pattern of MDRTB among HIV ward patients, starting in 1991 and continuing until June 1995 (Figure 1). By the first 6 months of 1995, 65% of *Mycobacterium tuberculosis* strains seen among HIV ward patients were multidrug-resistant.

Table 1. MDRTB patient characteristics

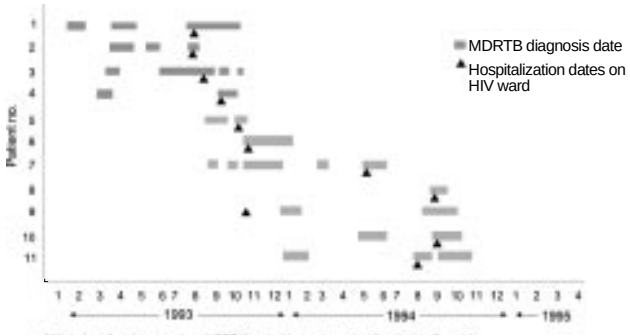
Variable	Number (n = 48)	%
Sex		
Male	39	81.3
Female	9	18.7
Mean age	34.1	(6.8)[a]
HIV status		
Infected	48	100.0
HIV risk group		
Intravenous drug users	32	66.6
Homosexual	10	20.8
Others	6	12.6
Outcome		
Death	47	95.9
Discharge	1	4.1
Mean survival after MDRTB diagnosis (in days)	77.6	(107.8)[a]

[a] Standard deviation.



*1995 data are for first 6 months only.

Figure 1. Hospital outbreak, Madrid, Spain, 1995.



*All strains of resistance pattern HSER (isoniazid, streptomycin, ethambutol, rifampin) had similar DNA subtyping.

Figure 2. Evolution of MDRTB strain* over time, hospital outbreak, Madrid, Spain, 1995.

At the beginning of the outbreak, no consistent antibiogram pattern was observed among isolates. However, beginning in 1993, strains were consistently resistant to isoniazid, streptomycin, ethambutol, and rifampin (HSER); of the 26 patients with an MDRTB diagnosis since the last trimester of 1993, 24 (92.3%) had HSER isolates. DNA subtyping analysis was performed on 12 of these HSER isolates that were available. Eleven of the 12 strains had the same band patterns (Figure 2). Two additional isolates with a different antibiogram pattern had different banding patterns.

Subtyping of *M. tuberculosis* strains was performed (11) by analyzing DNA located between two copies of repetitive sequence IS6110. A 10-µl sample of the extracted DNA was amplified in a reaction mixture containing 0.5 pM of each of the four primers (Ris1, Ris2, Pntb1, and Pntb2), 200 UM DNTPs, 50 mM Tris-HCL, 50 mM KCL (pH 8.8), 2.5 mM MgC12, 0.1% Triton x-100, and 0.5% Taq polymerase. The samples were denatured by incubation at 95°C for 10 min and amplified by 30 cycles of denaturation at 94°C for 1 min, primer alignment at 56°C for 2 min, and primer extension at 72°C for 1 min. The amplification products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and observed with an ultraviolet transilluminator.

HIV-infected patients hospitalized on the HIV-dedicated ward between September 15, 1991, and December 31, 1994, who had TB diagnosed in 1994 with a known drug-susceptibility pattern were included in a case-control study. Case patients' isolates were resistant to isoniazid, rifampin, streptomycin, and ethambutol; control patients' isolates were sensitive to the same antimicrobial drugs.

A time-person line diagram was prepared for each of the case patients and control patients, including all hospitalization dates, ward and room number, potentially infectious days, and possible days exposed to infective patients. Prior admission was defined as all admissions to the HIV-dedicated ward since the MDRTB patient was diagnosed (9/15/91) until the end of the period (12/31/94). TB patients were classified as "potentially infective" from the 2 week-period before sputum or culture results were positive for *M. tuberculosis* until the time sputum results were negative or the patient died. TB-negative patients were classified as "possibly exposed" if they were hospitalized on the HIV-dedicated ward during the time a potentially infective patient was also present, beginning on September 15, 1991, until 2 weeks before TB diagnosis.

Thirty-five patients (18 with cases and 17 controls) met the established study case/control definition. Case patients and control patients were not significantly different with respect to age, sex, HIV risk factors, interval between HIV and TB diagnoses or count of CD4 lymphocytes at the time of TB diagnosis (Table 2).

Before the hospitalization during which TB was diagnosed, 76.4% of the case-patients had been hospitalized on the HIV-dedicated ward of the hospital versus 23.5% of control patients (OR = 7.8 [1.4,50.5]) (Table 3). Of the five case patients with no prior hospitalization, three were family members of previously hospitalized HIV-infected patients who had visited the HIV ward frequently during the outbreak period.

Case patients and controls were compared with respect to possible exposure to a potentially infective wardmate beginning on 9/15/91 until 2 weeks prior to the diagnosis of TB. Case patients were more likely to have been exposed to potentially infective wardmates (72.2% with a median of 26.4 days) than control patients (41.2% with a median of 7.6 days). When we stratified possible ward exposure by days (0 days vs. 1 to 30 days vs. 30 days), we observed a dose-response effect, and the chi-square for linear trend was statistically significant (Table 3). Case patients were more likely to die during their initial hospitalization for MDRTB(94.4%) than were control patients (29.4%) during their hospitalization for TB (OR = 40.8 [3.6,1842]) (Table 3).

A TB screening clinic visit was offered to all hospital employees after the outbreak was identified. Of the 591 active employees, 565 (95.6%) participated. Of these, 288 (51%) had not participated in previous hospital employee screening programs conducted in 1990 and 1994. The overall prevalence of TB infection among participating employees was 450 (80%) of 565; only 115 (20%) of the current employees tested were tuberculin-negative.

Employees currently working at the hospital, who had a documented negative (< 6 mm) tuberculin test between January 1993 and June 1995 were eligible for this skin test conversion study. Many of the employees had received BCG vaccine. For those who had not received BCG vaccine, conversion was defined as an induration of 10 mm or greater with a change of at least 6 mm of induration since the last negative tuberculin test (12). For BCG vaccinees, conversion was defined as a 15-mm induration change since the last negative (< 6 mm) skin test.

Employees were defined as occupationally exposed if they worked in parts of the hospital where exposure to patients or *M. tuberculosis* was likely (the HIV-dedicated ward, HIV outpatient clinic, radiology unit, the mycobacteriology laboratory, and the internal medicine ward). Employees were asked to quantify the cumulative number of months spent in these high-risk areas, regardless of their usual place of work, during the 30-month study period.

According to the Mantoux technique, 2 tuberculin units of purified protein derivative (PPD) tuberculin RT-23 were administered in the anterior forearm of the screened employees, and the results were read within 48 to 72 h. Of the participants, 92 (16.3%) were eligible for the conversion study. The incidence of conversion during the 30-month period was 24 of 92 (26%). Employees who had occupational exposure to high-risk areas had higher conversion rates than employees who did not have occupational exposure to high-risk areas (RR = 5.0 [2.7,9.6]) (Table 4). The conversion had a dose-response effect, that is, the more months the person is occupationally exposed to high risk-areas, the higher the risk for conversion.

This is the first nosocomially transmitted MDRTB outbreak reported in

Table 2. Population characteristics: case control study

Variable	Case patients (%) n = 18	Control patients (%) n = 17	OR (95% CI)[a]
Mean age (yr)	34.4	35.2	$p = 0.30$[b]
Male sex	13 (72.2)	14 (82.3)	1.8 (0.3-12.1)
IVDU	11 (61.1)	11 (64.7)	1.2 (0.2-6.7)
Interval HIV to TB diagnosis in days	1403	1293	$p = 0.79$[b]
CD4 lymphocyte count, median	112.9	199.5	$p = 0.30$[b]

[a] OR (95% CI) = odds ratio (95% confidence intervals); Fisher's Exact Test.
[b] *p* value for analysis of variance (ANOVA). IVDU = intravenous drug user.

Table 3. Variables associated with MDRTB

Variable	Case patients (%) n = 18	Control patients (%) n = 17	OR (95% CI)[a]
Prior admission to HIV ward			
Yes	13 (76.4)	5 (29.4)	6.2 (1.2-37.2)
No	5 (23.6)	12 (70.6)	
Possible ward exposure days			
None	5 (27.8)	10 (58.8)	1.0
1-30	6 (33.3)	5 (29.4)	2.4
30	7 (38.9)	2 (11.8)	7.0, $p = 0.03$[b]
Outcome			
Death	17 (94.4)	5 (29.4)	40.8 (3.6-1842)
Discharge	1 (5.6)	12 (70.6)	

[a] OR (95% CI) = odds ratio (95% confidence intervals); Fisher's Exact Test.
[b] Chi-square for linear trend.

Table 4. Employee tuberculin screening conversion study results

Variable	Convertors (%) n = 24	Nonconvertors (%) n = 68	RR (95% CI)
Occupational exposure to high-risk areas			
Yes	14 (70.0)	6 (30.0)	5.0 (2.7, 9.6)
No	10 (13.9)	62 (86.1)	
Months exposed in high-risk areas			OR[b]
0	6 (10.7)	50 (89.3)	1.0
1-6	4 (28.6)	10 (71.4)	3.3
7-36	14 (63.6)	8 (36.4)	14.6, $p < 0.01$

[a] RR (95% CI) relative risk (95% confidence intervals).
[b] Chi-square for linear trend.

Spain, which, with recent outbreaks in the United Kingdom (13) and Italy, is among the first in Europe. Its characteristics are similar to the other reported outbreaks on that it occurred in an HIV-dedicated ward among non-foreign-born patients who had not been treated for TB; it had a high mortality rate within 3 months of onset during which mycobacteria laboratory surveillance recognized similar antibiogram resistant patterns; and identification of MDRTB isolates was followed by DNA subtyping, which confirmed that the same strain was responsible for the outbreak. Risk factors identified include admission to the HIV-dedicated ward and more "possible exposure" days to potentially infective wardmates; additionally, skin test conversion rates among employees were directly related to working in high risk areas of the hospital. The consistency of these findings with those reported in similar outbreaks and the fact that the same isolate was cultured over an extensive period among many different patients on a hospital ward without proper room isolation techniques support the conclusion that nosocomial transmission was the leading cause of the outbreak.

CDC's recommended guidelines for hospital TB prevention and control (14) were not fully implemented in the hospital. Acid-fast bacilli room isolation techniques were not in place; moreover, no ventilation system was available to provide negative pressure to prevent bacilli from passing from the MDRTB patients' rooms to the hallway or to provide the six air interchanges per hour recommended for removing bacilli from room air. Surgical masks used in the HIV-dedicated ward during the outbreak as protective masks are not recommended for this purpose because of filtering and facial sealing problems. Observational visits to the HIV-dedicated ward revealed that MDRTB patients without masks were walking, talking, and smoking in the halls and in the lounge next to the unprotected patients, families, and staff.

Complete follow-up of employees is an essential component of a hospital control program (14). Because 80% of the employees in this outbreak were tuberculin-positive, chest radiographs became an important component for disease screening; yearly surveillance of the tuberculin-negative employees will help determine if the interventions in place to prevent future outbreaks have worked in this setting.

Once the case-control study findings were analyzed, a TB control committee was established. All MDRTB patients were placed in a separate area of the hospital. Hospital staff were informed about the outbreak and alerted about future possible cases and the patient management and treatment schemes. The mycobacteria laboratory expanded the antibiogram service to include all second-line antibiotics used by the hospital clinicians. Clinicians have elaborated a treatment flow chart for MDRTB patients. Masks that fulfilled the sealage and filtering criteria (15) were purchased, and mask use was aggressively implemented. An employee health clinic was also instituted, with a prophylaxis and chest X-ray follow-up program for the 410 infected employees, along with a computerized follow-up surveillance system which included all employees graded by occupational risk category. Plans were made to screen tuberculin-negative employees every 3 months to identify those who had recently seroconverted. Family, community members and wardmates of all patients whose MDRTB had been diagnosed within the previous 6 months were notified of their risk and were scheduled for follow-up evaluations. The HIV-dedicated ward will be transformed into an acid-fast bacilli isolation zone, with an exclusive ventilation system that provides 11 individual rooms, each with 12 air interchanges per hour and negative pressure relative to the hallway.

Health officials in Europe need to be updated about this emerging problem, especially in areas

of high HIV and TB prevalence. Basic universal TB control and prevention measures (16,17) should be implemented by all general community hospitals in Spain, especially those with HIV-dedicated wards in areas where TB is prevalent. TB surveillance of health care employees is necessary to identify emerging problems as well as to protect employees, patients, and visitors.

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Application of Pulsed-Field Gel Electrophoresis to an International Outbreak of *Salmonella agona*

Between 1 December 1994 and 31 January 1995, *Salmonella agona* infections increased abruptly in England and Wales; isolates of *S. agona* from 41 patients with diarrheal illness were referred to the Laboratory of Enteric Pathogens of the Public Health Laboratory Service, compared with nine cases in the previous 12 months. Most isolates were from children under 10 years of age. Many of the cases were in Jewish children in London and several other parts of England; two children required hospital admission. *S. agona* can be subdivided by phage typing and, by using a method developed in the Laboratory of Enteric Pathogens (L. R. Ward, unpublished manuscript), we found that the isolates belonged to *S. agona* phage type (PT) 15. The outbreak was traced to a kosher savory snack imported into the United Kingdom from Israel, and all isolates from the contaminated product and from patients who had a history of consuming this product were found to belong to *S. agona* PT 15 (1). (Full details of this outbreak will be published elsewhere.)

After notification of the outbreak in the United Kingdom, health authorities in countries where the contaminated snack had also been distributed were warned of the possibility of an upsurge in *S. agona* infections; these countries included Israel, the United States, and Canada. Countries in the European Union (EU) were also notified through SALM-NET, the European salmonella surveillance network (2). As a result of such notification, isolates of *S. agona* subsequently identified as PT 15 were received from patients in Israel, the United States, and France and from food samples in Israel, the United States, and Canada.

For outbreak investigations, the policy of the Laboratory of Enteric Pathogens is to use serotyping, phage typing, and antibiogram analysis (R-typing) for the primary identification and subdivision of isolates, supplemented when appropriate by a range of DNA-based methods, including plasmid analysis, ribotyping, insertion sequence (IS) *200* fingerprinting, and pulsed-field gel electrophoresis (PFGE). The use of these techniques has recently been reviewed (3). Of these methods, PFGE has been used for the molecular fingerprinting of *S. typhi* (4, 5) and for subdivision within epidemic phage types of *S. enteritidis* (6, 7).

Because a small number of patients in England and Wales had been infected with *S. agona* PT 15 in the 11 months before the kosher snack outbreak, we decided to characterize the outbreak isolates by genotypic methods and, if possible, to use such methods for subdivision within the phage type. For *S. agona*, strains of this serotype do not possess IS*200* elements (8), and studies by the Laboratory of Enteric Pathogens have demonstrated that the serotype is unlikely to be subdivided by ribotyping (M.D. Hampton, E.J. Threlfall, unpublished observations). PFGE was, therefore, considered the method most likely to provide a genotypic fingerprint suitable for epidemiologic investigations. Isolates from the food product and from all patients infected both during the outbreak and in the preceding 11 months were, therefore, examined by PFGE. Similarly, because of the international distribution of the contaminated food product, *S. agona* PT 15 organisms isolated in Israel and Canada from the food product and also from specimens from patients in Israel, the United States, and France were examined by PFGE.

Analysis by PFGE of the fragments resulting from *Xba* I digestion of genomic DNA from 78 isolates of *S. agona* PT 15 made in the United Kingdom, Israel, the United States, Canada, and France between December 1993 and April 1995 showed 11 distinct pulsed-field profiles (PFP) and one variant profile, with 14 to 17 resolvable chromosomal fragments, ranging from approximately 25 kb to 680 kb (Figure). These profile types have been designated *S. agona* PFP (*Xba*I) 1 through to *S. agona* PFP (*Xba*I) 9, and *S. agona* PFPs (*Xba*I) 11 and 12; the variant type has been designated *S. agona* PFP (*Xba*I) 6a. The pattern designated *S. agona* PFP (*Xba*I) 10 had been assigned to an isolate of *S. agona* of an unrelated phage type, which has been used as a control in PFGE analysis of this serotype.

The predominant PFGE profile, *S. agona* PFP (*Xba*I) 6, gave at least 15 resolvable fragments, ranging from 25 kbp to 485 kbp; this profile type was exhibited by 51 of the 78 isolates examined. The PFP 6 variant, designated PFP 6a and identified in one of the Canadian food isolates, could be differentiated from PFP 6 by the presence of an additional fragment of approximately 395 kbp; in

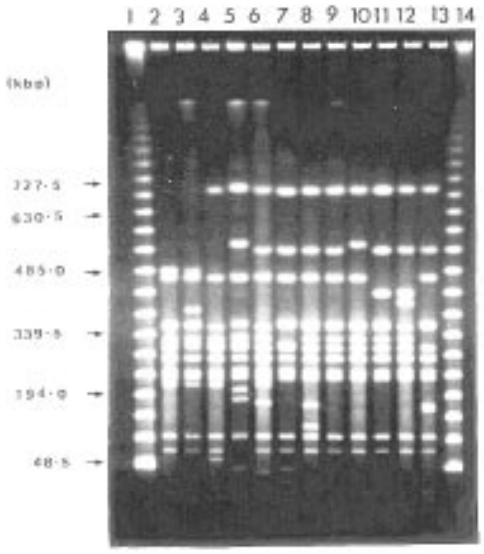

Figure. PFGE profiles of *Xba*I-digested genomic DNA from strains of *S. agona* PT 15. *Legend*: Tracks 1 - 14 contained: 1 and 14, lambda 48.5-kb ladder (Sigma); 2, *S. agona* PFP (*Xba*I) 6; 3, PFP 6a; 4, PFP 4; 5, PFP 10 (= control PFP type for *S. agona*); 6, PFP 9; 7, PFP 7; 8, PFP 3; 9, PFP 2; 10, PFP 5; 11, PFP 1; 11, PFP 8; 13, PFP 9. Gels were run at 6.0 V cm^{-1} for 36 h with a 25- to 75-s pulse ramp time.

all other respects, *S. agona* PFP 6a was indistinguishable from *S. agona* PFP 6.

The distribution of *Xba*I-generated PFGE types between source group and country of isolation in isolates of *S. agona* PT 15 is shown in the Table. Of the 10 PFGE profiles in 46 isolates from persons in the United Kingdom, 26 isolates (56.5%) belonged to PFP 6. All isolates with this profile pattern were made between 25 November 1994 and 17 February 1995 and were from patients associated with the kosher snack–related outbreak. Two isolates of *S. agona* PT 15 from the contaminated food product were also examined and both belonged to PFP 6. In contrast, PFP 6 was not identified in any isolates of *S. agona* PT 15 made in the United Kingdom in the 11 months before the outbreak. In one case, *S. agona* PT 15 with the PFGE profile PFP 9 was isolated from a patient just after the outbreak began, in November 1994. However, the PFGE profile of this isolate was sufficiently unrelated to that of the outbreak isolates (PFP 6) to warrant exclusion of the patient from the kosher snack–related cases. Nine PFGE profiles were identified in the remaining 19 U.K. isolates, with PFPs 5, 2, and 1 predominating (Table). Four of five patients infected with isolates with the PFP 2 pattern lived in northwestern England, and three of five patients with the PFP 5 profile lived in southwestern England or southern Wales. None of these nine PFGE profiles were identified in isolates from case-patients associated with the kosher snack outbreak.

Nine isolates of *S. agona* PT 15 from patients and two isolates from the contaminated food product that had been made in Israel were also examined by PFGE and, without exception, all strains belonged to PFP 6. Likewise, all of 10 isolates from outbreak-associated cases in the United States belonged to PFP 6 as did two of three strains isolated in Canada from the contaminated food product; the PFP of the remaining Canadian strain, PFP 6a, was closely related to PFP 6. Three PFPs were identified in the six isolates of *S. agona* PT 15 from France, of which two (PFP 2 and PFP 5) had been observed in isolates of PT 15 made in the United Kingdom before the outbreak. However, none of the isolates of *S. agona* PT 15 made in France were of PFP 6 (Table).

In conclusion, 11 distinct PFGE profile types and one variant type have been identified in 78 isolates of *S. agona* PT 15 made in the United Kingdom, Israel, the United States, Canada, and France between December 1993 and April 1995. One PFGE profile type, PFP 6, was specifically associated with isolates from a contaminated savory snack and from persons who consumed this product in the United Kingdom, Israel, and the United States. These results demonstrate that one strain of *S. agona* PT 15, with a characteristic PFGE profile, was responsible for outbreaks in the United Kingdom, Israel, and the United States in 1994-95. In contrast, the PFGE profile types of isolates of *S. agona* PT 15 identified in the United Kingdom either in the 11 months before the outbreak (or in one case at the start of the outbreak) and of isolates of PT 15 obtained from patients in France were unrelated to PFP 6. We conclude that PFGE fingerprinting provides a genotypic method for subdivision within phage type 15 of *S. agona* and that this method has provided an invaluable supplement to phage typing for investigation of international outbreaks. It is, however, important to realize that PFGE typing may not be applicable to all salmonella serotypes and phage types; thus, the method's results should be carefully evaluated by using both epidemiologically-related and unrelated isolates before it is used for outbreak investigations.

Table. Distribution of *XbaI* pulsed-field profiles in isolates of *Salmonella agona* phage type 15 made in the United Kingdom, Israel, USA, Canada, and France, 1993–1995[a]

Country	Source	Number studied	Pulsed-field profiles (PFP)												
			1	2	3	4	5	6	6[a]	7	8	9	10	11	12
United Kingdom	Human	46	3	5	1	2	5	26[b]		1	1	1		1	
	Snack	2						2							
Israel	Human	9						9							
	Snack	2						2							
United States	Human	10						10							
Canada	Snack	3						2	1						
France	Human	6		1			4								1
Total		78	3	6	1	2	9	51	1	1	1	1	0	1	1

[a] Isolates from Israel, the United States, and Canada were made in 1995; of the isolates from France, 3 were obtained in 1994 and 3 in 1995.

[b] Isolated 25 November 1994 to 17 February 1995.

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Potential Risk for Dengue Hemorrhagic Fever: The Isolation of Serotype Dengue-3 in Mexico

The Americas have a long history of dengue epidemics, which present public health problems because of the potential emergence of dengue hemorrhagic fever (DHF) (1). Efforts to control *Aedes aegypti*—the only demonstrated vector of dengue virus in the Americas—were effectively deployed in the 1950s and 1960s when the Pan American Health Organization launched a continental eradication campaign against yellow fever (2). *Aedes aegypti* was eliminated in Mexico in 1963 (3). However, subsequent social and economic changes in the Americas have permitted the rapid reinfestation of the vector throughout the region. In Mexico, population movement from rural areas to urban centers—brought about by intensive industrialization—were not matched with adequate housing and sufficient water, sewage, and waste management systems. The introduction and proliferation of nonrecyclable products provided numerous and effective breeding sites for urban mosquitoes. For example, from 1960 to 1990, the annual production of bottles in Mexico increased from 500,000 to 3.5 million, and the annual production of tires increased from 2 to 17 million (4). Tourism and travel, promoted as essential to the national economy, have also become important mechanisms for transporting dengue viruses. Additionally, surveillance, prevention, and control programs lack the infrastructure and human resources needed to tackle this neglected health problem (1,4). Millions of people living in the tropical and subtropical areas of the region face the reemergence of dengue and DHF (2).

In Mexico from 1984 to 1993, DHF cases were sporadically reported; only 26 cases were identified, followed by 30 cases in 1994 (4). During 1995, however, the General Directorate of Epidemiology of the Ministry of Health in Mexico confirmed 358 DHF cases in 18 states with a case-fatality rate of 7.8% (unpublished data). The widespread distribution of DHF cases and of the vector and the circulation of different serotypes demonstrate the risk of serious illness throughout the country.

Dengue fever in endemic-disease areas is often not diagnosed properly because of its nonspecific clinical manifestations. Furthermore, only patients with symptoms are treated, and patients rarely demand medical care; thus, the proportion of infected persons in the population is usually underestimated (5). On the other hand, DHF is an acute, life-threatening disease that requires specialized treatment in a medical setting. Identifying dengue serotypes in the continent is one of the most serious problems faced by every surveillance system in the region. The serotype, strain, and sequence of infections by different serotypes are among the most meaningful risk factors for DHF; thus, creating a strong dengue virus surveillance system in every country in the Americas should be a high priority (6, 7).

Serologic evidence of dengue in the Americas can be traced back to 1941 in Panama (8). DEN-2 was isolated in Trinidad in 1953 (9). DEN-3 was isolated in the Caribbean and Venezuela in 1963 (2,10), DEN-1 was introduced to the Americas in 1977, and DEN-4 affected the region 4 years later. In 1981, Cuba had a major DHF epidemic caused by a new strain of DEN-2 (11). DEN-3 was detected in Nicaragua and Panama in 1994 and in Costa Rica in 1995 (12), after a long absence from the region; a strain similar to one in Sri Lanka and India in the 1980s caused the DHF epidemics in those countries (12). The identification of DEN-3 in the region increases the probability of DHF cases associated with a newly circulating serotype. In Mexico, this particular situation may have important epidemiologic consequences for several reasons: 1) DEN-3 has not been identified in the country, and the population is totally susceptible to infection by this serotype; 2) infection by DEN-3 would most likely be of the secondary type; 3) population movements through Mexico and towards other countries, might disseminate this new serotype to areas where susceptible persons will be exposed to a new serotype; and 4) intensive transmission of dengue would naturally increase the risk for DHF epidemics.

Surveillance of dengue virus in Mexico began in 1982 when seven isolates of DEN-1 and DEN-2 were identified from outbreaks reported in the south and southeastern regions of the country. From 1982 to 1995, the National Institute of Epidemiological Diagnosis and Reference (INDRE) identified 681 dengue virus isolates. Serotypes were identified by indirect immunofluorescence with specific monoclonal antibodies donated by the Division

of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado.

DEN-1 was the serotype most frequently isolated from 1982 to 1995 (47% of all isolates), followed by DEN-4 (30%) and DEN-2 (21%) (Table). In 1995, DEN-3 was identified in 19 patients with classic dengue fever with no hemorrhagic manifestations (Table). Beginning in 1995, active surveillance for dengue cases was begun in areas where transmission had been documented. Sentinel surveillance centers were implemented to obtain serum samples from febrile patients with a clinical picture suggestive of dengue and to isolate and identify the serotype involved. From August to December 1995, 245 isolates of dengue virus were obtained, which represented 36% of isolates obtained during the 14-year period. The prevalence of serotypes isolated in 1995 differed from those isolated from 1982 to 1994; DEN-1 was isolated in only 16% of the samples processed, whereas 40% were DEN-2, 8% were DEN-3, and 36% were DEN-4 (Figure 1). It is unclear whether the change in distribution of serotypes is due to more intensive surveillance in certain areas or in a manifestation of herd immunity to serotype 1. This is the first report of DEN-3 in Mexico and reflects the strengthening of the surveillance at INDRE for dengue viruses in areas at risk.

The geographic and temporal distribution of DEN-3 isolated in 1995 in Mexico (Figure 2) shows a pattern similar to the one followed by the first dengue epidemics in the early 1980s (2) and may be related to population movements towards the northern border. The role of DEN-3 in increasing DHF cases is still to be determined; to date none of the DHF cases in which dengue virus was isolated have been associated with this serotype. Five cases were associated with DEN-1 and 20 cases with DEN-2. Nevertheless, the infection of DEN-3 in persons sensitized by previous infections with other serotypes and the widespread susceptibility of the Mexican population to this serotype must be considered a potential risk factor for future outbreaks.

The cost of each DHF case has not been fully determined, but the resources needed to face a DHF epidemic are certainly not available in countries where the health sector has financial constraints due to unstable economic conditions. The development of dengue vaccines is encouraging, but the widespread dispersion of mosquito breeding sites exceeds the capabilities of vector control programs. Moreover, the potential role of

Table. Number of isolates of dengue virus serotypes in Mexico*

Year	DEN-1	DEN-2	DEN-3	DEN-4	Total
1982	2	5	0	0	7
1983	5	6	0	2	13
1984	89	2	0	38	129
1985	30	8	0	9	47
1986	65	0	0	24	89
1987	13	0	0	0	13
1988	28	0	0	0	28
1989	21	0	0	0	21
1990	6	0	0	0	6
1991	4	0	0	20	24
1992	1	5	0	19	25
1993	0	10	0	0	10
1994	15	9	0	0	24
1995	40	98	19	88	245
Total	319	143	19	200	681

*Serum samples from suspect cases were added to C6-36 cells grown in D-MEM with 5% fetal calf serum for 7 days at 28° C and incubated for 1 hour. Cells were washed and further incubated in D-MEM with 0.4% bovine albumin for identification of cytopathic effect.

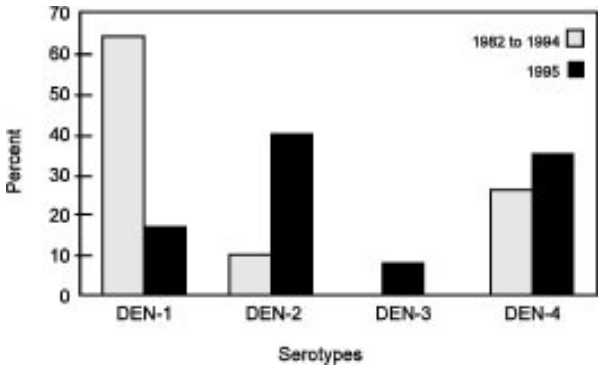

Figure 1. Frequency of dengue serotypes isolated in 1982 to 1994 and in 1995.

Figure 2. Geographic and temporal distribution of DEN-3 serotype in Mexico.

Aedes albopictus in the transmission of dengue virus in Mexico must be evaluated because DHF cases have appeared in areas where *A. albopictus* has been identified (14). The role of this vector in dengue transmission could increase should its geographic distribution expand and its susceptibility to infection increase (15).

The challenge faced by national health services is to improve the early detection of dengue transmission, prevent DHF, and decrease the case-fatality rate in DHF patients. This strategy must be supported by a strong surveillance network for viral diseases, which is now being implemented on a regional basis according to the risk of dengue transmission in the country. The detailed knowledge of the serotypes involved in future epidemics will provide useful information that will define the role of each serotype in the genesis of DHF cases and target control measures. The threat of a major epidemic requires a control strategy that will prevent the emergence of this public health problem.

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Improved Serodiagnostic Testing for Lyme Disease: Results of a Multicenter Serologic Evaluation

The diverse clinical manifestations of Lyme disease (1-3) have led to frequent confusion in clinical diagnosis, a confusion compounded by problems in the accuracy and precision of diagnostic serologic tests (4-11) and the difficulty of isolating the causative organism (12-14), *Borrelia burgdorferi*. In 1990, more than 20 commercially prepared serologic test kits for Lyme disease were being sold in the United States, but no nationally standardized reference test was available. A collaborative evaluation of a selected sample of the commercial test kits by the Centers for Disease Control and Prevention (CDC) and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) demonstrated poor concordance of results among these test kits and among a selected group of state health department laboratories (11). Because of the lack of a rigorously defined reference serum panel, conclusions could not be drawn about the sensitivity and specificity of the test kits evaluated. An unexpected finding in this study was the low concordance in test results between CDC and two consulting academic reference center laboratories. A number of other studies also have demonstrated low concordance of Lyme disease serologic test results obtained by a variety of laboratories (4-10).

As a result of those findings, the study described here was designed to fulfill the following objectives: 1) to assemble a serum panel from patients who had clinically well-defined Lyme disease (preferably confirmed by isolation of *B. burgdorferi*); healthy controls, and persons residing in non–endemic-disease areas whose potentially cross-reactive specimens had yielded equivocal ELISA results in earlier CDC tests; 2) to test this panel in a blinded fashion by several recognized Lyme disease reference and research laboratories; and 3) to compare the accuracy and precision of tests as a prelude to developing national recommendations for standardized serologic testing for antibodies to *B. burgdorferi*.

Tests were performed by five academic centers active in Lyme disease research (the Marshfield Clinic, Marshfield, Wisconsin; University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, New Brunswick, New Jersey; State University of New York at Stony Brook, Stony Brook, New York; Tufts/New England Medical Center, Boston, Massachusetts; and the University of Connecticut Health Center, Farmington, Connecticut) and CDC's Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, based in Ft. Collins, Colorado.

Serum samples from Lyme disease case-patients were obtained from the participating academic investigators (n = 72) and from the CDC Lyme disease reference serum collection (n = 37). All case-patient serum samples (total = 109) were from patients who met the CDC clinical case definition for surveillance of Lyme disease (15). The clinical manifestations in these patients ranged from acute erythema migrans (EM) to late neurologic disease accompanied by Lyme arthritis. *B. burgdorferi* had been cultured by the method of Berger et al. from 14 of 34 (41%) acute-phase specimens provided by CDC (14). Duplicate specimens (n = 85) were randomly selected from the 109 case-patient samples for precision analysis, making a total of 194 case-patient samples in the panel.

Control serum samples were provided by CDC from unpaid healthy blood donors (n = 113) who resided in areas where Lyme disease is not endemic (Cincinnati, Ohio, and Atlanta, Georgia; travel histories were not available from these donors, however. Duplicate specimens (n = 87) also were randomly selected, resulting in 200 noncase samples in the serum panel. Additional control samples were obtained from persons who resided in areas where Lyme disease was not endemic but whose physicians submitted their serum for Lyme disease testing to CDC through their state health department (n = 113). These specimens from patients with suspected cases had borderline (equivocal) seroreactivity in the whole cell sonicate (WCS) enzyme-linked immunoassay (ELISA) used by CDC before 1992 and are referred to hereafter as "WCS-suspects" (16). The addition of duplicate specimens (n = 87) brought this group to 200 equivocally seroreactive samples.

Serum was separated and frozen by the original collectors and shipped frozen to CDC's facilities in Ft. Collins, Colorado. The specimens were divided into aliquots and coded; code labels were applied

by CDC staff not involved in serologic testing of the specimens (n = 594). The panels were then refrozen and shipped on dry ice for blind testing by participating investigators. All specimens were received frozen. To calculate test sensitivity and specificity, only the result of the sample with the lower random code number of each pair was used.

Each laboratory employed the testing method that it used routinely at the time this study was undertaken (1992). CDC used an ELISA with a WCS antigen prepared from highly passaged strain B31 (gift of A. Barbour, University of Texas Health Sciences Center, San Antonio, Texas) and an ELISA with a strain B31 flagellar antigen (FLA) then being evaluated (16, 17). The other five participating investigators used ELISA tests that employed a WCS antigen of *B. burgdorferi*. Four used assays developed in their own laboratories, and one used a commercially available test kit (18-22). Three investigators also tested all specimens by Western blotting using published methods (19, 20). Two of these three performed immunoblotting for IgM and IgG antibodies separately. One laboratory tested for IgM and IgG together.

Each participating laboratory submitted the raw data of its results, along with a dichotomous interpretation of those results as either positive or negative. By prior agreement, ELISA results that fell into a range ordinarily reported as "equivocal" by that laboratory were treated as negative for this analysis. Statistical analyses undertaken at CDC included calculations of sensitivity (true positives correctly identified), specificity (true negatives correctly identified), precision (frequency of obtaining the same result on duplicate analysis of a specimen), and a measure of concordance (agreement among investigators) of results among the tests using the kappa statistic.

The accuracy and precision of the serologic tests as performed in 1992 by all six laboratories is summarized in Table 1. The test methods of investigators 1, 2, and 3 produced essentially equivalent results, with moderately high sensitivity (73% to 79%) for the aggregate of all case-patient samples tested and high specificity (98% to 99.5%). Precision was high in these three laboratories for both blood donor samples (97% to 99%) and the WCS-suspects samples submitted from areas where Lyme disease is nonendemic (94% to 98%). Precision was somewhat lower for the case-patient samples (82% to 91%).

Table 1. Accuracy and precision of serologic tests for Lyme disease performed in 1992

	Accuracy (%)		Precision (%)		
Investigator	Sensitivity	Specificity	Case patients	Non-case-patients	WCS suspects[a]
CDC (WCS)	93	71	93	77	69
CDC (FLA)	92	82	92	79	62
1	73	99.5	89	99	98
2	76	99	82	99	97
3	79	98	91	97	94
4	49	91	79	94	93
5	40	72	63	74	77

[a] Specimens from patients with suspected cases that had borderline (equivocal) seroreactivity in an enzyme-linked immunosorbent assay with whole-cell sonicate antigen (WCS).

The performance of the other three laboratories, including CDC's, was poor. Both CDC ELISA tests had high sensitivity (92% to 93%), but low specificity (71% to 82%). Precision for case-patient specimens was fairly high (92% to 93%), but low for both non-case-patient (77% to 79%) and WCS-suspects groups (62% to 69%). The method of investigator 4 gave very low sensitivity (49%), moderately high specificity (91%), poor precision with Lyme disease case-patient specimens (79%), but good precision with blood donor and WCS-suspects samples (93% to 94%). Investigator 5, who used a commercial test, obtained results with low accuracy and precision.

Concordance was high (kappa statistic 0.700) between the results of investigators 1, 2, and 3. The CDC FLA test showed moderate concordance (kappa 0.400) with results from investigators 1, 2, and 3. The results of investigator 4 showed moderate concordance with those of investigators 1 and 2 (kappa 0.400) and low concordance (0.400) with the other results. The results of investigator 5 had low concordance with all other results. The CDC WCS test showed moderate concordance with the FLA test, but low concordance with results of the ELISA tests of the other laboratories.

The three investigators with the best results all used Western blot to supplement their ELISA. Two of these three investigators submitted their dichotomous test interpretation with and without using Western blot results. The sensitivity improved by 20% for one investigator and by 30% for the other when Western blot results were included. The improvement resulted from identifying as

positive by Western blot those case-patient specimens from which an equivocal result was obtained by ELISA and which by study design would have been counted as negative by ELISA results alone. Specificities were not affected by Western blot analysis in this group of three investigators, since the serum panel in this study did not contain cross-reactive sera; and the negative controls and WCS-suspects had negative results by both ELISA and Western blot.

Test sensitivity from the three laboratories with the best test specificity (98%) was analyzed according to the clinical manifestations in the case-patients (Table 2). As expected, the sensitivities of the tests were lowest in specimens from patients with early disease, 59% to 66% for erythema migrans and 63% to 75% for early neurologic disease. Sensitivities were much higher for samples of patients with late disease. Sensitivities of 89% to 95% were obtained for Lyme arthritis patients and 91% to 100% for persons with late neurologic disease, primarily encephalopathy or polyneuropathy.

The emergence of a disease can outstrip the development of reliable methods for its laboratory diagnosis. The serodiagnosis of Lyme disease has been fraught with problems of precision and accuracy. This study provided an opportunity for selected academic research centers and CDC to compare the performance of their individual tests by using a serum panel from clinically well-characterized patients and controls from non–endemic-disease areas. The clinical diagnosis of early Lyme disease was supported by the isolation of *B. burgdorferi* from skin biopsy specimens (14), when possible. The panel, which was coded blind had a sufficiently large number of samples (n = 335) to provide adequate statistical power for the comparison.

Laboratories that supplemented their primary test, an ELISA, with immunoblotting achieved greater test accuracy than those that did not. The use of Western blot as a second test enabled the best performing laboratories to increase test sensitivity without a concomitant loss of specificity. This increase in sensitivity occurred as a result of identifying as true positives by Western blot a number of those specimens from patients with clinical cases of Lyme disease that were interpreted as equivocal by ELISA and would have been otherwise considered in this study as dichotomously negative results. Although the investigators employing Western blot tested all panel specimens with this method, they did so at that time to evaluate the potential value of Western blot in Lyme disease serologic diagnosis.

The observation that Western blotting could be employed to resolve equivocal ELISA results gave additional impetus for evaluating its potential adjunctive role in Lyme disease serodiagnosis and eventually led to the finally recommended two-test approach (23). The potential utility of Western blotting, however, pointed out the lack of standardized methods for producing blots and standardized interpretive criteria.

Performance of the CDC WCS and FLA ELISA in this study that did not include known cross-reactive sera suggested that the positive cut-off value for these tests was inappropriately low, thereby increasing sensitivity at the expense of specificity. These results then explained the large number of borderline WCS ELISA results obtained by CDC when it tested the sera of patients residing in areas where Lyme disease was not endemic. This group of WCS suspects was nearly uniformly found to be negative on ELISA by the three laboratories with the best performance (Table 1) (23).

Table 2. Test sensitivity of laboratories demonstrating a test specificity of 98%

Clinical Manifestations	Sensitivity, % (positive samples/total)		
	Laboratory 1	Laboratory 2	Laboratory 3
Erythema migrans, all	59 (55/94)	60 (56/94)	66 (62/94)
Acute phase[a]	65 (11/17)	65 (11/17)	76 (13/17)
Convalescent phase[b]	57 (44/77)	58 (45/77)	64 (49/77)
Carditis	100 (2/2)	100 (2/2)	100 (2/2)
Lyme arthritis[c]	89 (58/65)	95 (62/65)	92 (60/65)
Neurologic, all	85 (28/33)	88 (29/33)	91 (30/33)
Early	63 (5/8)	63 (5/8)	75 (6/8)
Late	91 (10/11)	100 (11/11)	91 (10/11)
Late and arthritis	93 (13/14)	93 (13/14)	100 (14/14)
Total	74 (143/194)	77 (149/194)	79 (154/194)

[a] ≤ 30 days from onset of erythema migrans to blood collection.

[b] > 30 days from onset of erythema migrans to blood collection.

[c] Without neurologic signs or symptoms.

Specificity in this study was determined by testing specimens from blood bank donors. With these samples, specificity in the three laboratories that used immunoblotting was very high (98% to 99.5%). The test panel did not, however, contain specimens from patients with conditions known to produce cross-reacting antibodies (e.g., syphilis) or polyclonal B-cell activation (e.g., Epstein-Barr virus infection or systemic lupus erythematosus). Thus, reported specificities in this study are likely higher than they would have been if cross-reactive specimens were included in the evaluation. Subsequent studies that included cross-reactive sera demonstrated that Western blotting correctly identifies many false-positive ELISA reactions (23, 24).

This study confirmed in the reference and research laboratory setting the previously documented problems with accuracy and precision of serodiagnostic tests by using WCS antigens of *B. burgdorferi* (4-11). The study confirmed that a serious disparity existed between the test results obtained by CDC and those obtained by academic reference centers with the best testing performances. These results guided corrective action and led to the adoption by CDC and ASTPHLD of a two-test approach to serodiagnosis (23), which forms the basis for the future national standardization of Lyme disease serologic testing methods.

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Emergence of *Bartonella quintana* Infection among Homeless Persons

Bartonella quintana has episodically emerged as a cause of infection among distinct and diverse populations during the 20th century. The organism was first identified as an important human pathogen during World War I when it caused epidemics of louse-borne trench fever that affected an estimated 1 million troops in Europe (1, 2). Trench fever was characterized by fever, rash, bone pain, and splenomegaly and ranged in severity from a mild flulike illness to a more severe, relapsing disease. *B. quintana* infections were rarely recognized from the end of World War II until the 1980s when the organism reemerged as an opportunistic pathogen among HIV-infected persons. In this population, *B. quintana* has been identified as a cause of bacillary angiomatosis, endocarditis, and bacteremia (3-5) and has been isolated from AIDS patients in France (6) and the United States (3-5).

In the 1990s, *B. quintana* has emerged among homeless persons in North America and Europe. In 1993, the organism was isolated from the blood specimens of 10 patients at a single hospital in Seattle, Washington, within a 6-month period (7). These patients had illnesses characterized by fever and persistent bacteremia. Endocarditis developed in two patients, one of whom required a heart valve replacement. All 10 patients had chronic alcoholism, eight were homeless, and the six who were tested for HIV infection were HIV-negative. These six were the first cases of invasive *B. quintana* infection among HIV-negative persons reported in the United States. Results of a case-control study indicated that the patients with *Bartonella* bacteremia were more likely than controls (other hospitalized patients from whom blood specimens were obtained at approximately the same time) to be homeless ($p = 0.001$), to have a history of alcohol abuse ($p = 0.001$), and to be nonwhite ($p = 0.007$). The isolates from the 10 patients were identical by polymerase chain reaction restriction-fragment-length polymorphism testing, which further suggests that the cases were epidemiologically linked. Patients' characteristics were obtained by retrospective medical record review, and at the time they sought treatment, three patients reported a recent cat scratch, five had scabies, and one had lice. More complete information, however, on patients' past exposures to animals and ectoparasites was not available.

In 1995, Drancourt and co-workers reported three cases of *B. quintana* endocarditis among HIV-negative, homeless, alcoholic men in France (8). One of the patients had reported contact with a dog, and one had reported contact with dogs and cats; however, a current or past history of infection with lice or scabies was not documented for any of the patients. In 1995, Stein and Raoult also reported serologic evidence of *B. quintana* infection in an HIV-negative, homeless man from Marseilles, who had a relapsing febrile illness and a history of louse infestation (9).

As a follow-up to the 1993 *B. quintana* outbreak in Seattle in 1994, we conducted a seroprevalence study of anti-*Bartonella* antibodies among patients at a community clinic in the "skid row" section of Seattle, which serves a primarily homeless and indigent population (10). The median age of the 192 patients included in the study was 45 years, 156 (81%) of the 192 were male, and 126 (66%) were classified as homeless. *B. quintana* IgG titers ≥ 64 were detected by an indirect fluorescence antibody assay (11) in 39 (20%) of the 192 clinic patients. In contrast, only 4 (2%) of 199 banked blood specimens from an age-matched and sex-matched comparison group of Seattle volunteer blood donors had titers ≥ 64 ($p < 0.001$). Among clinic patients, seropositivity (titer ≥ 64) was associated by univariate analysis with older age, homelessness (relative risk [RR] 2.0; 95% confidence interval [CI] 1.0-4.1), alcohol abuse (RR, 2.5; 95% CI 1.4-4.2), smoking (RR, 2.0; 95% CI 1.2 -3.4), and injection drug use (RR, 2.5; 95% CI 1.3-4.8). By multivariate analysis, only alcohol abuse remained independently associated with seropositivity (odds ratio 3.3; 95% CI 1.6-6.9), and of 39 seropositive patients, 21 (54%) had a history of chronic alcoholism. Reliable data on past exposure to animals or ectoparasites were also not available for patients in this study.

The study was limited by the well-described cross-reactivity of the assay between *Bartonella* species (12, 13), and most (62%) clinic patients with *B. quintana* titers ≥ 64 also had titers ≥ 64 to *B. henselae*. It is, therefore, possible that some of the seropositive patients may have been exposed

to *Bartonella* species other than *B. quintana*. These findings do, however, show that a surprisingly high proportion of clinic patients without a history of documented *Bartonella* infection had detectable anti-*Bartonella* antibodies and may have been exposed to *B. quintana*.

Multiple factors, including those related to disease transmission, host susceptibility, and ability to detect the organism, have likely contributed to the emergence of *B. quintana* infection among the homeless. Transmission of *B. quintana* from human to human by the body louse, *Pediculus humanus*, has been experimentally documented (1) and is believed to have been the predominant mode of transmission of epidemic trench fever in World Wars I and II. Lice reside primarily in the seams of clothing and are easily killed by immersion in water 50°C or warmer (14), which explains the propensity for louse-borne infections among displaced persons or wartime troops. Although these reports of *B. quintana* infection among homeless persons lack sufficient information to conclusively determine the disease vector, louse-borne infection remains a plausible hypothesis. Lice, however, have not been associated with bacillary angiomatosis among AIDS patients, although exposure to cats (and, therefore, possibly to fleas) has been associated with bacillary angiomatosis and bacillary peliosis caused by *Bartonella* species (15) and with cat-scratch disease caused by *B. henselae* (16). Thus, it is possible that *B. quintana* infection is spread among homeless persons by as yet unidentified vectors or reservoirs.

Homeless persons are also at risk for non-vectorborne infectious diseases. An increased risk for tuberculosis in this population is well documented (17, 18), and outbreaks of meningococcal disease (19, 20), pneumococcal disease (21), and diphtheria (22, 23) have been reported. It is likely that factors such as crowding, altered immunity due to alcoholism or other co-existing health problems, and inadequate or infrequent access to medical care affect the transmission and spread of infectious diseases among the homeless. Previous studies have shown that the clinical response to a standard inoculum of *B. quintana* varies substantially in experimental study patients (1); this variation indicates that host factors are likely important determinants of the risk for clinical infection following exposure to the organism.

Although cases of *B. quintana* bacteremia among homeless persons have thus far been reported only from France and Seattle, Washington, the problem is probably not confined to these locations. *B. quintana* is a fastidious and slow-growing bacterium that generally requires special culturing techniques for isolation (3-5, 24), and many clinical laboratories do not routinely use blood culturing methods that are sensitive for isolating this organism. Moreover, *B. quintana* infection can result in a broad range of often nonspecific clinical manifestations (1, 3-5); therefore, case-patients evaluated for suspected bacteremia may represent only a small proportion of infected persons, as suggested by the results of the Seattle seroprevalence survey. To better define the geographic distribution and prevalence of *B. quintana* infection among homeless populations, a heightened awareness for this infection on the part of clinicians and the use of appropriate culture techniques by microbiology laboratories serving this population are needed. In addition, more specific serologic tests would aid in the diagnosis and assessment of the epidemiologic characteristics of *B. quintana* infections.

The optimal treatment regimen for HIV-negative patients with suspected or confirmed *B. quintana* infection has not been established. Minimal published data exist regarding antimicrobial therapy for this infection, and in vitro susceptibility testing has proven unreliable (25). Nonetheless, on the basis of limited data, we believe it is reasonable to treat immunocompetent patients who have uncomplicated *B. quintana* bacteremia with at least 14 days of oral therapy with erythromycin, azithromycin, doxycycline, or tetracycline. In the 1993 Seattle outbreak, most patients had a satisfactory response to treatment with a beta-lactam agent followed by either erythromycin or azithromycin for 14 days (7). Although the number of patients identified with *B. quintana* endocarditis is small, most of these patients have required cardiac valve replacement despite intravenous antimicrobial therapy (5, 8, 26). Therefore, we recommend that patients with *B. quintana* endocarditis receive a more prolonged course of at least 4 to 6 months of antimicrobial therapy and cardiac valve replacement if needed. Further study is needed to determine the role of bactericidal agents, such as third generation cephalosporins or quinolones, as monotherapy or in combination with a bacteriostatic agent for treating invasive *B. quintana* infections.

Many aspects of the acquisition and pathogenesis of *B. quintana* infections, and specifically *B. quintana* infections among the homeless, are not well defined. Changes in the organism itself that have led to increased virulence may in part account for its reemergence; however, microbiologic data that can support or refute this hypothesis are lacking (27). The absence of recently identified cases in Seattle and in other areas with laboratories that use culture techniques appropriate for isolating *Bartonella* species suggests an episodic pattern of disease, with few or no cases occurring during interepidemic periods. It seems clear, however, that this most recent emergence of an old disease is related, at least in part, to societal factors that have contributed to urban decay and the existence of large homeless populations in our cities. As with other emerging infectious diseases, further efforts to identify, evaluate, and control *B. quintana* infections among homeless persons are challenges that will require the coordinated effort of clinicians, microbiologists, and public health officials.

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The Reemergence of Visceral Leishmaniasis in Brazil

Because of a complex array of factors, an increasing number of new and reemerging infectious diseases are being recognized in both industrialized and developing countries in the Americas (1,2). The expanding population, living in overcrowded conditions with inadequate housing and sanitary facilities, has been exposed to new diseases and human pathogens. For example, the appearance of the South American arenaviruses (Junin, Machupo, and Guanarito) illustrates how exploitation of new areas for human settlement and agriculture increases the likelihood that new infectious diseases will emerge. Cholera, plague, AIDS, dengue hemorrhagic fever, and urban/periurban visceral leishmaniasis are examples of new and reemerging diseases in the region.

In tropical America, zoonotic visceral leishmaniasis caused by *Leishmania chagasi,* an intracellular protozoon, is a long-lasting infectious disease characterized by weight loss, cough, fever, diarrhea, hepatosplenomegaly, and lethargy. Since not all symptoms appear simultaneously, and many other conditions manifest the same symptoms, the diagnosis is not always opportune. This disease is different from the anthroponotic form of visceral leishmaniasis found in Sudan and India. In the New World this disease is more common among poor, malnourished children (3) under 15 years of age who live in semiarid regions. The case-fatality rate is low if pentavalent antimony therapy is introduced promptly (4). The disease has also been reported in immunodepressed persons, especially those with HIV infection.

The domestic dog is the principal animal reservoir of *L. chagasi* and, as the constant companion of humans throughout the endemic-disease area, contributes to the dispersal of diseases during human migrations. The protozoon is transmitted from one mammalian host to another, including humans, primarily by the bite of a sand fly who has fed on an infected dog. In the Americas, the principal sand fly vector is *Lutzomyia longipalpis* (5). This 2- to 3-mm long fly has peridomestic and intradomiciliary habits (6) and avidly bites humans at night, primarily during twilight, while the host is resting.

Visceral leishmaniasis is usually diagnosed by identifying the parasite in spleen aspirates; microscope examination of bone marrow aspirates offers a satisfactory alternative. Specific antibodies may be detected by immune-enzymatic reactions and immunofluorescence, but these methods lack specificity and are too complex to be carried out in the field. TRALd, a recently developed diagnostic test, consists of a 60-second dipstick, based on a recombinant protein rK39 of a sequence of 298 amino acids and an improved serologic procedure (7). The procedure is undergoing field testing and appears to be a promising tool for control programs.

Although visceral leishmaniasis was previously known as a rural disease, large outbreaks and epidemics of visceral leishmaniasis have been reported recently in large cities in Brazil because of the favorable epidemiologic conditions associated with the reduction of the natural ecologic space of this zoonosis. Waves of droughts, lack of available farm land, and famine have led to a large migration of the population, to the peripheral suburbs of large cities, creating densely populated settlements of shanties (*favelas*) with minimal infrastructure and sanitation. Most families that migrate are young, less-established farmers of peak child-bearing age; children under 15 years of age account for a large percentage of the entire population. In these communities, the newly introduced disease (parasite) encounters a vast number of nonimmune hosts who, because of poor living conditions, are malnourished; malnutrition is one of the prime risk factors for *L. chagasi* infection and visceral leishmaniasis (3). The habit of keeping domestic animals such as dogs, chickens, and horses in the back yard provides an abundance of blood meals for sand fly vectors and raises vector population densities dramatically.

Two recent examples of reemerging visceral leishmaniasis in Brazil occurred in the cities of Teresina (population = 678,000), the capital of Piauí State, and São Luís (population = 918,000), the capital of Maranhão State. In Teresina, which has the best-documented records, an epidemic of visceral leishmaniasis occurred from 1981 to 1985; it was initially limited to rural settings, but later spread to peripheral areas of the city (9) (Figure 1). Spraying with residual insecticide helped bring the disease under control during the subsequent 3 years. Since 1989, visceral leishmaniasis has gradually reemerged; it reached epidemic levels by the end of 1992 and peaked in 1994. In the State of Maranhão, the disease reemerged in 1993,

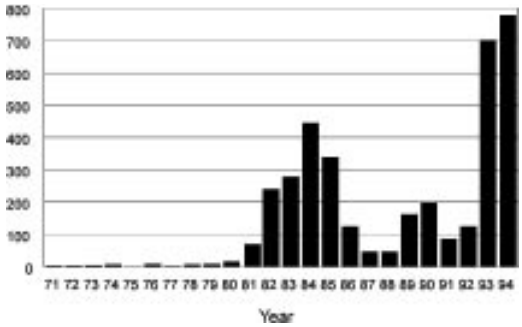

Figure 1. Number of cases of visceral leishmaniasis from the state of Piauí, Brazil, 1971 to 1994.

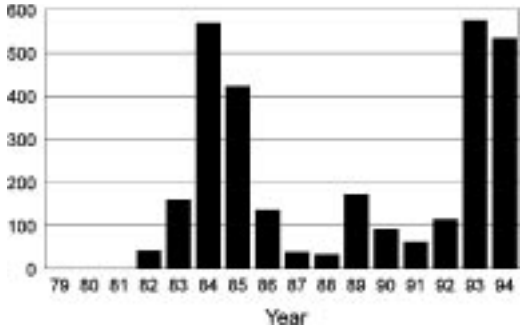

Figure 2. Number of cases of visceral leishmaniasis from the state of Maranhão, Brazil, 1979 to 1994.

(Figure 2), 10 years after a previous epidemic (1982 to 1986). In both Teresina and São Luís, the epidemics were preceded by prolonged, severe drought. The disease was found predominantly among young people who came from rural areas and lived in periurban, substandard housing and kept domestic animals in their back yards. The two states accounted for approximately 40% to 50% of the number of cases reported in the country during 1993 to 1994 (3,000/year).

Emergency control plans based on rapid diagnosis, complete treatment of human patients, spraying of residual insecticide, ultra-low volume spraying, elimination of infected (seropositive) dogs, and health education were initiated in these two cities in July 1994 (10). The incidence rate dropped markedly from January 1995, as expected, since the incubation period of visceral leishmaniasis is 4 to 6 months (11, 12). The remaining reported cases include those with longer incubation periods, as well as others, because of the lack of complete coverage of the control interventions and reduced sensitivity of the current diagnostic tools.

Technical expertise and effective mechanisms to prevent visceral leishmaniasis epidemics and urban transmission exist, but they require social and political commitment as well as the allocation of funds to provide adequate sanitary and satisfactory housing conditions to the population at risk. Visceral leishmaniasis in the Americas must be addressed before it becomes a serious public health problem. Even though it is a zoonosis, control interventions are available that, when properly used, can eliminate urban transmission and keep disease incidence in rural areas at a very low level.

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Molecular Epidemiology of *Pneumocystis carinii* Pneumonia

Pneumocystis carinii pneumonia (PCP) was first recognized as a distinct clinical entity in orphanages in Europe during World War II (1). Today it is the most frequent serious opportunistic infection in AIDS patients. Despite advances in research, numerous questions remain regarding the basic biology and epidemiology of *P. carinii*.

Transmission and Patient Care

Although reactivation of latent infections has long been considered the primary explanation for PCP in immunosuppressed patients, over the years a steady flow of reports has described clusters of PCP cases (2). In addition, recent studies have suggested that the duration of latency is very limited, i.e., usually less than 1 year (3,4). Still other studies have demonstrated genetic variation in PCR-amplified *P. carinii* DNA from the lungs of patients during subsequent PCP episodes (5). Together, these observations provide strong circumstantial evidence of person-to-person transmission of *P. carinii*. Consequently, establishing the role that person-to-person transmission plays in the epidemiology of PCP is urgent.

Another important area of PCP epidemiology is determining the predisposing factors for disease. The most frequently discussed predictor of disease is CD4+ cell count, specifically as it relates to care and management of AIDS patients (6); however, it has long been known that malnutrition can be an important contributor (7). The degree to which other factors such as viral infections or pneumonitis of other causes, may come into play, is yet to be shown.

Much can also be learned regarding the epidemiology of PCP in HIV-infected infants. Recent studies report that primary infections in these infants often develop when the child is 3 to 6 months old (8,9). The source of these patients' *P. carinii* infections (i.e., the hospital setting, their mothers, other children, or an environmental source) is not known.

Clinicians working with AIDS patients need a sensitive, reliable, and noninvasive tool for early detection and diagnosis of PCP infections (10,11). Besides the standard procedures of bronchoalveolar lavage (BAL) and induced sputum (IS) sampling, recent studies indicate that it is possible to amplify *P. carinii* DNA sequences by polymerase chain reaction (PCR) directly from blood or serum samples and from nasopharyngeal aspirates of PCP patients (11,12). Further studies are needed to confirm that a PCR-based diagnostic tool superior to microscopy can be adapted for use in clinical settings. A serologic tool that will distinguish recent PCP infections from those past is also needed.

Prophylaxis failures have been reported for both trimethoprim-sulfamethoxazole (TMP-SMX) and pentamidine (13-16). Studies evaluating these cases, however, are frequently complicated by the difficulties in assessing and confirming patient compliance with the prophylaxis regimen. The only factor that has a significant correlation with failure in most cases, however, is the patient's CD4+ T lymphocyte count (14). Although this correlation would be expected because of the general increased risk for PCP associated with CD4+ cell depletion (6) and the increase in prophylaxis complications in HIV-infected patients (17), these drugs may not eliminate all organisms, and some degree of patient immunity may be required to clear or control the infection. What role, if any, specific antimicrobial resistance mechanisms play in the reported treatment failures has not been shown; however, the emergence of resistance is always a threat. Likewise, long-term TMP-SMX prophylaxis increases the possibility for the selection of antimicrobial resistance in bacterial pathogens, some of which are important potential causes of pneumonia in HIV-infected patients (18). Identifying potential antimicrobial resistance mechanisms in *P. carinii* is difficult because of the lack of an established culture system for human *P. carinii* that would allow traditional antimicrobial sensitivity testing.

At least three separate lines of data suggest that *P. carinii* is a commonly encountered organism: the high seroprevalence rates reported in normal populations (19), the rapid rate at which infants acquire primary infections (8) and AIDS patients become reinfected after successful treatment (20), and the amplification of *P. carinii*-specific DNA from ambient air sampled from the environment (e.g., an apple orchard) (21) and from rooms of animals and patients with PCP (22). Airborne transmission has been demonstrated for PCP in rats (23-26) and is by far the most likely mechanism proposed for natural exposure to *P. carinii* in humans (2,22). Given the similarities

between *P. carinii* and various fungal agents and the enigma surrounding the issue of environmental sources for *P. carinii*, it has been suggested that *P. carinii* may in fact be a dimorphic fungus, ubiquitous in the environment and disseminated by airborne spores (27). Identifying the specific environmental source or sources of *P. carinii* is critical to understanding the epidemiology of PCP and establishing guidelines for preventing its transmission.

It is generally accepted that *P. carinii* strains from rats do not infect humans and that human strains do not infect rats; however, we do not know the host boundaries for a given *P. carinii* strain or if all isolates from a given host display the same degree of host restrictions (28,29). In fact, a careful evaluation of the available data concerning *P. carinii* of numerous hosts suggests that *P. carinii* may represent a collection of diverse fungal species (30). Like drug resistance research, studies aimed at strain/species characterization are generally hindered by the difficulties in culturing human *P. carinii* and the lack of refined molecular biological methods that allow strain identification and characterization.

Molecular Biologic Techniques and Specific Epidemiologic Issues

One of the essential reasons for cultivating any particular pathogen is for strain identification and characterization that would elucidate such specific phenotypic characteristics as virulence factors, antimicrobial sensitivity levels, and factors associated with transmissibility. The isolation and cultivation of individual strains, and ultimately of clones, would provide a homogeneous population of organisms from which the desired information can be obtained and a pure source of genetic material for constructing DNA libraries and identifying relevant genes.

In the absence of cultivation, investigators have been able to begin addressing some of the basic epidemiologically important issues by applying PCR-based technology. In these studies, the DNA sequence of specific genetic loci from *P. carinii* is usually amplified from BAL, IS, or serum samples from PCP patients, using highly specific oligonucleotide primers. Inherent problems exist in this approach (which are discussed below); however, the approach has allowed the identification of genotypic differences in *P. carinii* populations sampled from the lungs of different patients and even from the lungs of the same patient during different PCP episodes. Great potential exists in applying this technology to develop molecular profiles of *P. carinii* isolates that could ultimately allow the particular genotypes to be linked to specific epidemiologically relevant phenotypes.

Molecular Typing

Five to ten different genetic loci have been identified as potentially informative for molecular characterization and typing (30-33). Concerning the typing that has actually been performed on human samples, the primary loci evaluated include: 1) a 346-bp region of the mitochondrial large subunit rRNA gene (*mt lsrRNA*) (10) and 2) a 550-bp fragment containing the nuclear ribosomal internal transcribed spacer regions 1 and 2 (*ITS1* and *ITS2*) (34). When these loci are considered collectively, nucleotide variation can be detected at approximately 37 different positions. Work is in progress in several laboratories, both to type patient isolates according to the available loci and to identify additional genetic loci to more thoroughly define a given genotype.

The primary obstacles to the development of a molecular typing scheme based on PCR-amplified DNA sequence data obtained from PCP patients include the following: 1) multiple strains may infect a single patient at a given time; 2) a diploid organism of a single strain may be heterozygous with respect to a particular polymorphic locus; 3) presumed single genes could have multiple copies in a single genome, which could give the appearance of genetic polymorphism; 4) amplified DNA sequence data might be confounded because of other fungal agents such as *Cryptococcus* or *Candida*; and 5) inferences that can be drawn from restricted sequence data (i.e., gene typing versus strain typing) are limited. Although these problems are not insurmountable, they must be considered when evaluating data obtained by this approach. We propose the following recommendations.

Recommendations

Molecular Epidemiology

1. Recent advances in molecular-based typing should be combined with epidemiologic studies to investigate the transmission of *P. carinii* and new strategies for control.

2. Additional genomic regions must be identified for use in typing, along with the genetic loci that are available. These new loci must be shown to represent single-copy genes. Also, new molecular approaches should be developed that will increase the current capacity to resolve genotypic variation among *P. carinii* strains.

3. Genetic variation should be investigated among *P. carinii* strains that could be linked to variations in factors such as strain virulence, drug resistance, or transmissibility.

4. The critical issue regarding person-to-person transmission is not so much whether it occurs, as whether it contributes to infection significantly more than airborne sources in the environment. Thus, it must be determined whether there is any benefit to establishing complex protocols that ensure that patients are carefully protected from each other if they can become infected from other sources in the environment. Consequently, the importance of person-to-person transmission in the epidemiology of PCP should be defined.

5. The role of latent *P. carinii* infection as a source of PCP in immunocompromised persons should be clarified.

Diagnosis, Treatment, and Prevention

1. New tools for noninvasive early diagnosis of PCP, including culture systems, molecular approaches, and serologic tests that can distinguish recent and past PCP infections are needed.

2. In the United States, clinician compliance with recently published U.S. Public Health Service/Infectious Diseases Society of America guidelines on the treatment and prophylaxis of PCP should be evaluated.

3. Studies should be initiated to develop additional drugs for PCP treatment and prophylaxis.

4. New approaches for improving patient compliance with prescribed PCP prophylaxis must be devised and evaluated.

5. Methods for detecting the possible emergence of drug resistance to *P. carinii* should be standardized.

6. Standard decontamination procedures for respiratory therapy equipment and pulmonary diagnostic instruments should be evaluated to confirm that they effectively eliminate all viable *P. carinii*.

Environmental Reservoirs and General Biology

1. Environmental sources and the coinciding infective stage(s) of *P. carinii* should be detected and evaluated.

2. The host range of *P. carinii* from various sources (i.e., to what degree are humans susceptible to *P. carinii* from nonhuman sources?) should be determined.

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Needed: Comprehensive Response to the Spread of Infectious Diseases

In his article "Globalization, International Law, and Emerging Infectious Diseases," Fidler recognizes that biological agents travel by themselves or with their hosts without any recognition of, or regard for, political borders. He notes that with the continued expansion of economic commerce across continents and more rapid transport and travel, persons infected with infectious diseases of very short incubation periods can act as vectors across several nations before they even become symptomatic. The protective effect of clipper ship travel is long gone.

Fidler examines the need for international treaties, agreements, and policies to manage the spread of new or reemerging infections diseases. His concern is that the current international climate requires more enforceable treaties with adequate resources to identify, track, interfere with, and contain the spread of infectious diseases perceived as an international or global threat.

International cooperation within the existing legislative mechanisms has, on occasion, been very successful. International eradication of smallpox was successful because a specific, cost-effective, efficient vaccine was developed; the disease attacked persons regardless of their economic, political, racial, religious, or social affiliations; the amount of funding was adequate; and all nations recognized the benefits of the eradication program. A similar effort currently in progress to eradicate poliomyelitis will also be successful because of international cooperation.

In contrast, international control of other infectious diseases, such as malaria and tuberculosis, has been attempted for decades with considerably less success. Notwithstanding the lack of efficacious vaccines, the reality is that only very limited resources are being committed to prevent and treat all infectious diseases. Outbreaks of Ebola virus infection and plague are routinely reported in the local, national, and international press. However, the continued increased incidence and prevalence of tuberculosis, AIDS, and other sexually transmitted diseases are accepted by many as problems of the poor, the immoral, and the expendable portion of society. Local, national, and international awareness and continued interest are significant problems.

International cooperation must extend beyond merely restricting the natural spread of specific diseases. One also has to recognize the need for effective international treaties to prevent the use of biological agents in either tactical or strategic circumstances. Fear of combatants using biological agents on military and civilian targets intensified during and since the Gulf War. The possibility of biological terrorism is no longer limited to the imagination of fiction writers. Fidler does not stress the issue of nonnatural outbreaks of diseases; a global need for an improved non-ad hoc response to emerging infectious disease agents should be completely considered by civilian and military planners. The threat of infectious diseases as weapons provides an additional incentive for cooperation among governments.

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Transfusion-Associated Malaria

To the Editor: A recent article by Zucker (1) described two cases of malaria that were probably transfusion associated. A case of transfusion-associated malaria in which the source *was* identified was reported in San Francisco in 1991. The case was in an elderly man in whom malaria infection developed after coronary bypass surgery.

The patient was born in China and immigrated to the United States in 1940. His only travel outside the United States was a trip to Hong Kong in 1951 for 6 months. The patient's wife was born in China and had malaria in 1941 during World War II. She received no treatment at that time or at any other time. She came to the United States in 1960 and has not left the country since.

The patient had six donors, five of whom had no history of malaria, and had negative serologic test results for all four malaria species. Both the patient and his wife had blood smears positive for *P. malariae*. The patient's wife had positive serologic test results for *P. vivax* and *P. ovale* (1:64), for *P. falciparum* (1:258), and for *P. malariae* (1:1024).

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Reply to F. Taylor: Dr. Taylor's letter calls attention to the small but important number of induced malaria cases that occur in the United States. From 1957 to 1994, 101 such cases were reported to the Centers for Disease Control and Prevention (CDC); these (including the 1990 case described by Dr. Taylor [1]) are reviewed annually and reported by CDC (2). The occasional occurrence of induced malaria further emphasizes the importance of including malaria in the differential diagnosis of fevers of unknown origin, even in patients who have not traveled to countries where malaria is endemic. Preventing induced malaria requires screening potential blood, tissue, and organ donors and deferring those with a history of malaria or travel to malarious areas. Furthermore, timely surveillance must be maintained to detect induced cases promptly, identify infected blood donors, and prevent additional cases.

The case described by Dr. Taylor was not included in "Changing patterns of autochthonous malaria transmission in the United States: a review of recent outbreaks" (3) because it was a case of induced rather than autochthonous malaria. Each reported malaria case is classified according to standardized terminology (4). *Imported* malaria (which accounts for most cases in this country) is acquired outside the United States and its territories. Malaria acquired within the United States is rare and occurs by one of three mechanisms: *Autochthonous* malaria is acquired through the bite of an infective mosquito. *Congenital* malaria is acquired when a child is infected in utero. *Induced* malaria is transmitted by mechanical means such as transfusion of blood or blood products, organ transplant, deliberate infection for malariotherapy, or contaminated needles or injection equipment. Congenital and induced cases were not included in this review.

When an investigation fails to identify the source of transmission and a case cannot be epidemiologically linked to another case of malaria, the case is classified as *cryptic*. Most cryptic cases are believed to be autochthonous, and there is often evidence to suggest mosquito-borne transmission, even when the source of infection remains unidentified. For this reason, most cryptic cases were included in this review of autochthonous malaria. The two exceptions noted in the article were excluded because both patients had recent histories of blood transfusion, suggesting that their infections were induced.

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An Outbreak of Hemolytic Uremic Syndrome due to *Escherichia coli* O157:H-: Or Was It?

To the Editor: Since the first reported outbreaks of hemolytic uremic syndrome (HUS) and related conditions more than 10 years ago (1), outbreaks of HUS due to *Escherichia coli* O157 have been reported from many parts of the world, particularly North America and Europe. While most of these reports have incriminated the motile strains of serotype O157:H7, nonmotile serotypes (e.g., O157:H-) have also been associated with HUS; these two serotypes are most commonly associated with both outbreaks and sporadic cases of HUS and related conditions. Over the last decade, a number of techniques for the rapid identification of these organisms have been developed. Of these, the use of sorbitol-MacConkey agar (2) has perhaps been the most valuable. This technique is based on the fact that these organisms rarely ferment sorbitol on primary isolation, while most other *E. coli* usually ferment this substrate. We believe that outbreaks due to other enterohemorrhagic *E. coli* may have been attributed to serogroup O157 because of the limited technology used in investigating these outbreaks.

No outbreaks of HUS due to serogroup O157 have occurred in Australia despite sporadic cases of HUS caused by such strains. Other serogroups (particularly serotype O111:H-) have been associated with most cases of HUS and related conditions in Australia (3). No outbreak of HUS had been reported in Australia until January 1995, when an outbreak associated with the consumption of contaminated mettwurst (fermented sausage) was reported from South Australia (4). Twenty-three children with HUS were hospitalized. Most required hemodialysis; one died. Verocytotoxigenic strains of *E. coli* O111 producing Shiga-like toxin (SLT) I and II were isolated from 19 patients and from samples of mettwurst. In addition, strains of *E. coli* O157:H- that produced SLT-I and SLT-II were isolated from three of the patients and the mettwurst. These strains did not ferment sorbitol on the sorbitol-MacConkey agar, which facilitated their isolation. The predominant O111 strains were sorbitol-positive, unlike the O111 strains, recently described as being sorbitol-negative (5). Symptoms of the patients from whom the O157:H- strains were isolated, in addition to *E. coli* O111:H-, were not significantly different from those of the patients whose specimens yielded only *E. coli* O111:H-. In addition to O111 (and O157), other serotypes of enterohemorrhagic *E. coli*, including strains of serogroup O23, O26, and O91, were isolated from the patients. However, antibodies to O111 were detected in nearly all patients, which indicates the serogroup's leading role in the outbreak. The isolation of serogroup O157 is comparatively easy; therefore, it is less likely that these strains would have been missed, than it is that O111 and other serotypes would have been. Even though a negative finding can never be considered conclusive, we consider the inability to isolate serogroup O157 more conclusive than the same result for other serotypes. It has frequently been suggested that the O157 serogroup is cleared from the patient relatively rapidly, which makes its isolation difficult or impossible. We found a similar situation with other enterohemorrhagic *E. coli* serotypes. The fact that most patients elicited an O111 antibody response (and no anti-O157) almost certainly proves this serotype's causal role in this outbreak.

The laboratory in South Australia was particularly well disposed to deal with such an outbreak because some of its ongoing research programs included studies on aspects of enterohemorrhagic *E. coli* and related organisms. The most sophisticated molecular biologic techniques were immediately available to investigate the outbreak accurately and confirm epidemiologic leads regarding a common source. Polymerase chain reaction (PCR) played a major role not only in identifying SLT-I, SLT-II, and SLT-I and SLT-II producing bacteria in the stool of patients, but also in identifying the suspected source (mettwurst). In addition, PCR, utilizing sequences specific for the O111 serogroup, enabled this serogroup to be rapidly identified in patients' feces samples and suspected source material. Without this technology, the outbreak would not have been contained so rapidly. On the other hand, if the laboratory had to rely on conventional microbiologic culture procedures, including sorbitol-MacConkey agar, strains of serogroup O157 would have been identified from three patients, as well as from the epidemiologically incriminated mettwurst. The laboratory would not have found the O111 strains because they all fermented sorbitol readily and would have been discarded as normal flora as would the other enterohemorrhagic *E. coli* serotypes. The outbreak would have been reported as another O157 outbreak, from which only about 15% of the patients yielded the incriminating strains. This outbreak could be recognized as one caused by a number of different enterohemorrhagic *E. coli* serotypes, of which serotypes O111:H- and O157:H- were the most prominent.

Other serotypes, however, such as O23, O26, and O91, were also present. With the widespread nature of verocytotoxigenic strain of different serotypes as has been reported from many environmental studies, it is not surprising that a product, such as mettwurst, which is made from meats from various sources, would contain a number of these potential pathogens.

A large number of E. *coli* serotypes can be verocytotoxigenic and, in a few cases, outbreaks due to such strains have been reported. Most notable have been reports from Italy of outbreaks due to enterohemorrhagic *E. coli* O111 (6); however, the impression is that these are the exception and that the most prominent serotype is O157:H7. Some of the reported outbreaks due to O157 strains may in fact have been due to other serotypes and the O157 strains were only present in comparatively small numbers; however, because of the ease with which these strains can be identified using sorbitol-MacConkey agar, they were believed responsible for the outbreaks. For example, in Argentina, *E. coli* O157:H7 was found in only one (2%) of 51 children with HUS (7) and in the Netherlands, only 5 (19%) of 26 HUS patients yielded *E. coli* O157:H7 (8). In a 10-year, retrospective, population-based study of HUS, this serotype was isolated in 13 (46%) of 28 patients (9), and in their review, Su and Brandt (10) put an overall figure of 46% to 58% as the incidence range of E. *coli* O157:H7 infection in cases of HUS. Finding SLT sequences in a fecal specimen by PCR, or free fecal toxins in many patients of an outbreak while isolating strains of O157 from only a few, does not exclude the presence of other serotypes, but culture methods now available would rarely pick these up. Thus there is ample room to speculate that approximately half the cases of HUS may be caused by serogroups other than O157 and, by inference, at least half the outbreaks may be wrongly attributed to this serogroup. We recognize that enterohemorrhagic *E. coli* O157 have become extraordinarily widespread throughout the world since their first description (1); this does not mean that other serotypes are not also causing infections, either alone, in conjunction with O157, or even with other known or unknown enteric infections. It is important to be aware of the existence of these other serotypes and be vigilant for them. The isolation and characterization of strains of serogroup O157 from patients with HUS is certainly noteworthy, but so is the finding of O111 or any other serogroup. Serogroup O111 has amply demonstrated the ability to cause extensive outbreaks (6). Even though many laboratories are becoming aware of the importance of testing for serogroup O157:H7, we think that testing for this serotype only is a disservice; simple culture techniques can identify this serogroup, but always at the risk of missing other serogroups. The development of simple methods to detect all enterohemorrhagic *E. coli* is now required.

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The Dilemma of Xenotransplantation

To the Editor: I read with considerable interest Robert E. Michler's commentary on xenotransplantation (1).

From my point of view, that of a basic virologist, the dilemma is not to know in what "foreseeable future, clinical xenotransplantation may achieve its targeted goal of extended graft survival," but what deadly emerging infectious disease, most probably viral in nature, would arise in a recipient of a baboon or chimpanzee heart. While we face the terrific threat of AIDS, which clearly emerged from Africa and non-human primates 40 to 50 years ago, we are preparing a new infectious "Chernobyl."

Monkeys and apes harbor approximately 50 simian viruses; some of them pose a serious threat to humans, especially the heavily immunosuppressed. Recently, an outbreak of encephalitis related to a new type of reovirus (2) occurred among baboons from a colony used in human organ transplants. Moreover, once unknown or unrecognized simian viruses, like HIV, may be efficient invaders of the entire earth's population.

Xenotransplantation does not simply pose an ethical problem; it concerns the survival of the human species, an endangered species if transplant practitioners continue their course. Ronald Montalero, a virologist, was right when he said "unknown viruses were always a major concern in xenotransplants" (2). A moratorium on these procedures seems the best solution until all simian pathogens are identified and the risks they pose to humans are clearly established.

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The Thucydides Syndrome: Ebola Déjà Vu? (or Ebola Reemergent?)

To the Editor: The plague of Athens (430-427/425 B.C.) persists as one of the great medical mysteries of antiquity (1-5). Sometimes termed "the Thucydides syndrome" for the evocative narrative provided by that contemporary observer (6, 7), the plague of Athens has been the subject of conjecture for centuries. In an unprecedented, devastating 3-year appearance, the disease marked the end of the Age of Pericles in Athens and, as much as the war with Sparta, it may have hastened the end of the Golden Age of Greece (3). Understood by Thucydides to have its origin "in Ethiopia beyond Egypt, it next descended into Egypt and Libya" and then "suddenly fell upon" Athens' walled port Piraeus and then the city itself; there it ravaged the densely packed wartime populace of citizens, allies, and refugees. Thucydides, himself a surviving victim, notes that the year had been "especially free of disease" and describes the following major findings: After its "abrupt onset, persons in good health were seized first with strong fevers, redness and burning of the eyes, and the inside of the mouth, both the throat and tongue, immediately was bloody-looking and expelled an unusually foul breath. Following these came sneezing, hoarseness . . . a powerful cough . . . and every kind of bilious vomiting . . . and in most cases an empty heaving ensued that produced a strong spasm that ended quickly or lasted quite a while." The flesh, although neither especially hot nor pale, was "reddish, livid, and budding out in small blisters and ulcers." Subject to unquenchable thirst, victims suffered such high temperatures as to reject even the lightest coverings. Most perished "on the ninth or seventh day . . . with some strength still left or many later died of weakness once the sickness passed down into the bowels, where the ulceration became violent and extreme diarrhea simultaneously laid hold (2.49)." Those who survived became immune, but those who vainly attended or even visited the sick fell victim (2.51).

By comparison, a modern case definition of Ebola virus infection notes sudden onset, fever, headache, and pharyngitis, followed by cough, vomiting, diarrhea, maculopapular rash, and hemorrhagic diathesis, with a case-fatality rate of 50% to 90%, death typically occurring in the second week of the disease. Disease among healthcare providers and care givers has been a prominent feature (8, 9). In a review of the 1995 Ebola outbreak in Zaire, the Centers for Disease

Control and Prevention reports that the most frequent initial symptoms were fever (94%), diarrhea (80%), and severe weakness (74%), with dysphagia and clinical signs of bleeding also frequently present. Symptomatic hiccups was also reported in 15% of patients (10).

During the plague of Athens, Thucydides may have made the same unusual clinical observation. The phrase *lugx kene*, which we have translated as "empty heaving," lacks an exact parallel in the ancient Greek corpus (5). Alone, *lugx*, means either "hiccups" or "retching" and is infrequently used, even by the medical writers. Although contexts usually dictate "retching," we note unambiguous "hiccups" in Plato's Symposium (185C). In his thorough commentary on the Thucydides passage, the classicist D. L. Page remarks: "Hiccoughs is misleading, unless it is enlarged to include retching." Regarding "empty, unproductive retching [he] has noted no exact parallel . . . in the [writings of the] doctors, but . . . tenesmus comes very close to it" (5). A CD-ROM search of Mandell, Bennett, and Dolin discloses no reference to either "hiccups" or "singultus" in the description of any disease entity (6).

The profile of the ancient disease is remarkably similar to that of the recent outbreaks in Sudan and Zaire and offers another solution to Thucydides' ancient puzzle. A Nilotic source for a pathogen in the Piraeus, the busy maritime hub of the Delian League (Athens' de facto Aegean empire), is clearly plausible. PCR examination of contemporaneous skeletal and archaeozoological remains might test this hypothesis against the 29 or more prior theories.

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BSE Meeting at CDC

The recent report of a new variant of Creutzfeldt-Jakob disease (V-CJD) in Great Britain and the possible link between the disease and bovine spongiform encephalopathy (BSE) has raised a number of health and safety concerns (1,2). On April 8, 1996, CDC organized a meeting of U.S. agency representatives to review information about the report of U.K. cases and about efforts to identify the existence of BSE and V-CJD in the United States. The meeting covered the scientific evidence for the report of V-CJD; recommendations from a meeting of international experts organized by the World Health Organization on April 2-3; and the current and proposed activities of U.S. agencies with regard to BSE and V-CJD.

Among the observations made during the meeting were the following:

- There is no evidence from U.S. surveillance activities or from scientific studies to indicate that BSE exists in the United States.
- Active surveillance for BSE is conducted by the U.S. Department of Agriculture (USDA). All cattle presented for slaughter in the United States are observed for signs of central nervous system (CNS) disorders. Livestock showing CNS signs are condemned and not allowed to enter the slaughter plant or to become part of the human food supply. Since 1990, laboratory testing of nearly 2,800 brain specimens from cattle with CNS signs has shown no evidence of BSE.
- No U.K. cattle or ruminant-based feed has been imported into the United States since July 1989, when USDA banned the importation of cattle and cattle products from the United Kingdom. U.K. cattle imported before the ban will be destroyed as a precaution to ensure that these animals do not enter the food chain (human or animal).
- The Food and Drug Administration plans to issue a ban on ruminant-to-ruminant feeding in the United States.
- Additional research is needed on the characterization of the causative agent of BSE and on the epidemiology, rapid laboratory diagnosis, and pathogenesis of BSE and CJD.
- The Centers for Disease Control and Prevention (CDC) monitors the occurrence of CJD in the United States through surveillance and special epidemiologic studies (3). On the basis of mortality surveillance from 1979 to 1993, the annual incidence of CJD remained stable at approximately one case per million persons. In the United Kingdom, five of eight patients who died of V-CJD since May 1995 were younger than 30 years of age; by comparison, in the United States, CJD deaths among persons younger than 30 years are extremely rare (fewer than 5 per billion per year). CDC's efforts will be expanded to include active surveillance studies at four Emerging Infections Program sites (Connecticut, Minnesota, Oregon, and the San Francisco area) and in Atlanta to provide more up-to-date information on the occurrence of CJD and to verify the absence or presence of V-CJD.

Future cooperative efforts among U.S. agencies, industry, and other interested parties in response to the report of V-CJD are planned. The report of the April 8 meeting at CDC can be accessed on the CDC NCID Web site (connect to http://www.cdc. gov/ncidod/ncid.htm; the report is under New, Reemerging, and Drug-Resistant Infections).

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CDC Foundation Supports Emerging Infectious Disease Projects

The National Foundation for the Centers for Disease Control and Prevention, Inc. (NFCDC), a not-for-profit corporation established by Congress to support CDC's mission, announced in August 1995 that one of its initial funding efforts would be in the area of antibiotic-resistant diseases.

Also in the area of infectious diseases, NFCDC has recently received a gift from a Pennsylvania foundation to establish fellowships for two repre-

sentatives from nongovernmental organizations to spend 1 month at CDC learning about the agency's AIDS/HIV support services and activities and advising CDC on the type of support nongovernmental agencies need in this area.

The foundation creates new and enhanced financial and program partnerships between CDC and American and international businesses and industries, philanthropic organizations, foreign governments, international organizations, and concerned individuals. An initial grant of $1 million from the Robert W. Woodruff Foundation was awarded to help establish NFCDC in Atlanta.

The NFCDC board of directors plans to first fund projects that reflect the following themes: 1) strengthening global health capacity; 2) promoting healthy lifestyle choices with initial emphasis on adolescent health; 3) improving the quality and communication of public health information, with initial emphasis on development of information technology and networks; and 4) promoting prevention in health care delivery systems, in particular, managed-care systems.

According to foundation executive director Charlie Stokes, tax-deductible gifts to NFCDC represent an investment in CDC's efforts to address current or emerging health problems that threaten U.S. citizens and citizens of the world. Gifts may be designated for a specific purpose or be given without restriction to be used for CDC's greatest needs. Federal employees can make gifts to the foundation through the annual Combined Federal Campaign.

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