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Emerging Infectious Diseases

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

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Molecular Population Genetic Analysis of Emerged Bacterial Pathogens: Selected Insights

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Research in bacterial population genetics has increased in the last 10 years. Population genetic theory and tools and related strategies have been used to investigate bacterial pathogens that have contributed to recent episodes of temporal variation in disease frequency and severity. A common theme demonstrated by these analyses is that distinct bacterial clones are responsible for disease outbreaks and increases in infection frequency. Many of these clones are characterized by unique combinations of virulence genes or alleles of virulence genes. Because substantial interclonal variance exists in relative virulence, molecular population genetic studies have led to the concept that the unit of bacterial pathogenicity is the clone or cell line. Continued new insights into host–parasite interactions at the molecular level will be achieved by combining clonal analysis of bacterial pathogens with large-scale comparative sequencing of virulence genes.

To avert the threat of resurgent and new microbial diseases, it is critical to gain insight into the molecular mechanisms contributing to temporal variation in disease frequency and severity. Although comprehensive, unambiguous understanding of the host and parasite factors mediating these processes is not available for any infectious agent, population genetic research in the last 10 years has provided noteworthy new information about the bacterial side of the equation. This review will summarize the insights accrued from population genetic analysis of bacteria responsible for disease outbreaks or increases in infection frequency and severity. One of the primary themes emerging from this research is that distinct bacterial clones have been responsible for several infection outbreaks (Table 1). Moreover, the distinct clones are frequently characterized by unique combinations of virulence genes or alleles of virulence genes. These observations have important implications for our understanding of infectious diseases and the public health measures required to reduce their detrimental and potentially devastating effect on society.

Population Genetics and Clonal Analysis of Bacterial Pathogens: Basic Concepts

Population genetic study of bacterial pathogens arose largely as an offshoot of research designed to address questions of longstanding interest to students of the molecular evolutionary processes in higher eukaryotic organisms. Bacteria were an attractive group of experimental organisms because of their phenotypic diversity, short generation times, haploid chromosomal genomes, and accessory genetic elements. Hence, bacterial population genetic research was originated by population geneticists interested in bacteria, rather than bacterial geneticists or medical microbiologists interested in population genetics (1, 2). In spite of its important implications for how the field has developed over the last decade, this ontogeny will not be discussed in detail here. However, the reader should recognize that bacterial population genetics is a discipline separate and distinct from the study of the molecular epidemiology of infectious agents. The research tools, methods of data analysis, and general thought processes are very different from the typological thinking used by investigators of disease outbreaks or microbial pathogenesis (3-6).

Early work on the clonal nature of bacterial pathogens was conducted largely with *Escherichia coli*, through a framework supplied by serotyping of one or a few polymorphic surface antigens (7, 8). Only a few of the many possible O and H antigen serotypes were frequently associated with outbreaks of infantile diarrhea in the United Kingdom and other countries, which suggested that isolates expressing these traits had special virulence properties (7-9). Because serotype analysis of relatively few surface structures does not provide robust data for estimating overall

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Table 1. Representative Emerged Bacterial Pathogens Investigated with Molecular Population Genetic Strategies

Organism	Disease	Reference
Borrelia species	Lyme disease	146-149,150,151
Escherichia coli O157:H7	hemorrhagic colitis; hemolytic uremic syndrome	33-35
Haemophilus influenzae	Invasive disease	156
H. influenzae biogroup aegyptius	Brazilian purpuric fever	25,26
Listeria monocytogenes	Food-borne invasive disease	157, 158
Mycobacterium tuberculosis	Tuberculosis	91,105
Neisseria meningitidis	Sepsis, meningitis	52-63,66,69-73
Salmonella species	Food-borne illness	159-161
Staphylococcus aureus	Toxic shock syndrome; methicillin resistant strains	47,143,144
Streptococcus pyogenes	Invasive disease, toxic-shock-like syndrome	82,83,85
S.pneumoniae	Otitis, pneumonia, sepsis, meningitis	125-131,136
Vibrio cholerae	Cholera	153-155

levels of chromosomal diversity and relationships among strains, the primary research tool used to examine the population genetics of emerging bacterial pathogens has been multilocus enzyme electrophoresis (10, 11). This technique indexes allelic variation in sets of randomly selected structural genes located on the chromosome and provides a basis for estimating overall levels of genotypic variation in populations, i.e., the sample of bacteria chosen for analysis. The key concept underlying use of starch gel-based protein electrophoresis in population genetics is that electromorphs (mobility variants) of an enzyme can be directly equated with alleles of the corresponding structural gene. Moreover, electromorph profiles over a sample of different enzymes, therefore, correspond to multilocus enzyme genotypes and are frequently referred to as electrophoretic types or ETs. The proteins analyzed are usually metabolic enzymes expressed by virtually all isolates of a species under the growth conditions used. The allelic variation detected is unaltered by environmental conditions such as culture conditions, laboratory storage, anatomic site of recovery, or specific clinical disease. Allelic variation in these metabolic enzymes is selectively neutral, or nearly so, which means that convergence to the same allele through adaptive evolution is unlikely (4, 12-14). As a consequence, this approach to the study of bacterial and other microbial pathogens provides a convenient strategy for indexing overall levels of chromosomal diversity in the sample and for inferring genetic relationships among strains. Because of the strong correlation of chromosomal divergence indexed by multilocus enzyme electrophoretic data and DNA-DNA hybridization studies (15-18), several cryptic species have been identified once their existence was initially discovered by population genetic analyses that employed starch gel electrophoresis (16, 19-21). In most bacterial species, the number of allelic variants is large, and it is unlikely that recombinational processes would, by chance, frequently generate strains with the identical electromorph profile. Hence, organisms with the same electromorph profile are generally thought to be similar by descent, rather than by convergence through lateral gene flow.

Recently, convenient, rapid, and relatively inexpensive large-scale DNA sequencing techniques have also been adopted by several laboratories. Large-scale automated DNA sequencing has been used to rapidly and unambiguously identify a causative infectious agent and confirm or refute the identity of isolates recovered from temporally-linked patients thought to be involved in a disease outbreak. In addition, sequence-based studies have been employed to define the nature and extent of allelic variation in toxin and other virulence factor genes and to rapidly identify mutations associated with antimicrobial agent resistance (22).

Unless noted, data on the population genetic analysis of emerging bacterial pathogens summarized in this article were generated by multilocus enzyme electrophoresis, sometimes performed in concert with automated DNA sequencing.

Representative Insights

Brazilian Purpuric Fever

Brazilian purpuric fever (BPF), a serious invasive disease of children, was first characterized in 1984 after an outbreak in Promissao, Sao Paulo State, Brazil. Children with BPF have acute onset of fever and usually die within 48 h with disseminated purpura, vascular collapse, and hypotensive shock (23). BPF is caused by *Haemophilus influenzae* biogroup aegyptius, an organism associated with sporadic or epidemic conjunctivitis (24). Multilocus enzyme electrophoresis and other molecular techniques have demonstrated that isolates recovered from BPF patients represent a distinct clone (25).

As a first step toward identifying the evolutionary origin of this pathogenic *H. influenzae* biogroup aegyptius clone, chromosomal variation and genetic relationships were indexed among 17 biogroup aegyptius isolates, and 2,209 encapsulated *H. influenzae* strains were recovered worldwide (26). Biogroup aegyptius isolates form three distinct evolutionary lineages of the species *H. influenzae*. Isolates of the case clone are only very distantly related to other isolates classified as biogroup aegyptius; that is, the case clone was no more related to other biogroup aegyptius isolates than are (for example) two *H. influenzae* isolates selected at random from the species. The BPF case clone was genetically allied with *H. influenzae* isolates expressing serotype c polysaccharide capsule, a result that explains an earlier observation (27) that BPF isolates, like serotype c strains, produce type 2 IgA1 protease, whereas other isolates of biogroup aegyptius express type 1 IgA1 protease. Thus, the population genetic evidence showed that biogroup aegyptius is polyphyletic and that the BPF organism is a genetically distinct clone unrelated to other isolates with the phenotypic criteria of biogroup aegyptius.

The genetic diversity in the sample of all biogroup aegyptius strains was approximately equal to that recorded for entire species of certain pathogenic bacteria (16, 17). Therefore, the effective population size of aegyptius must be large; however, this interpretation is difficult to reconcile with the observation that strains in the biogroup are rare pathogens associated only with human disease. A possible explanation for the relatively extensive genetic diversity among biogroup aegyptius strains is that they represent cell lineages spawned from a much larger base population of diverse nonpathogenic precursor clones. According to this hypothesis, acquisition or loss of one or more genes (or, perhaps, a shift in ecological niche) may produce a pathogenic form with the characteristic viscerotropism for human conjunctivae.

Although population genetic analysis did not provide a simple reason for the BPF outbreak, the demonstration that the causative clone of biogroup aegyptius was highly differentiated from other phenotypically similar organisms provided an explanation for the unique infection manifestations and the unique group of characters associated with the clone (28-30). Moreover, population genetic analyses demonstrated distinct medical correlates to isolates classified as biogroup aegyptius. The results of numerous subsequent studies have confirmed that, as a population, *H. influenzae* biogroup aegyptius strains vary in their behavior, as one would expect of a genetically diverse set of organisms.

Escherichia coli O157:H7

Strains of *E. coli* expressing serotype O157:H7 were recognized in the early 1980s as important causes of hemorrhagic colitis and hemolytic uremic syndrome in North America (31). Disease usually occurs after consumption of contaminated beef or other food. Several large outbreaks have occurred, and more than 60 case clusters have been reported in the United States (32). Because several *E. coli* reference laboratories rarely identified organisms expressing this serotype before the early 1980s, reporting of these isolates has increased dramatically. Because of the medical and economic importance of *E. coli* strains considerable effort has been directed toward elucidating genetic relationships among and between them as well as between them and other members of the species; as a result extensive information is now available about clonal relationships among these important bacteria (33-35).

The observation that O157:H7 strains synthesize one or more Shiga-like toxins and lack the ability to rapidly ferment sorbitol initially suggested that strains of this serotype had shared a recent common ancestor. To directly test this idea, multilocus enzyme electrophoresis was used to assess genetic relatedness of 100 strains of *E. coli* serotypes recovered from patients with hemorrhagic colitis or hemolytic uremic syndrome (33). Although 25 distinct multilocus enzyme genotypes

were identified, cluster analysis found that O157:H7 isolates are closely related organisms. The results were interpreted to mean that O157:H7 organisms recovered from epidemiologically unassociated North American outbreaks belong to a single geographically widespread pathogenic clone with specific virulence properties (33). Subsequent analysis of O157:H7 strains by pulsed-field gel electrophoresis has supported this idea (36).

To delineate clonal relationships among O157:H7 organisms and other *E. coli* strains that cause hemorrhagic colitis and infantile diarrhea, 1,300 isolates representing 16 serotypes from patients with these diseases were studied by multilocus enzyme electrophoresis and probing for genes encoding Shiga-like toxins (34). The O157:H7 clone was closely related to a clone of O55:H7 strains that has a long history of worldwide association with outbreaks of infantile diarrhea (34). The data strongly suggested that the O157:H7 and O55:H7 clones have recently radiated from a common ancestral cell. The O157:H7 clone arose from an O55:H7-like ancestor, perhaps through horizontal transfer and recombination events adding Shiga-like toxin genes and adhesion genes to an *E. coli* genome preadapted for causing diarrheal disease (34, 35). If, as the multilocus enzyme electrophoretic data indicate, O157:H7 and O55:H7 organisms have shared a recent common ancestor, it is likely that the close genetic affiliation would be reflected at the nucleotide level. To test this notion, the gene (*eae*) (34) encoding intimin, a protein involved in bacterial attachment to enterocytes and subsequent effacement of the microvilli, was sequenced from representative isolates of these two serotypes. The resulting sequence data were consistent with the hypothesis that O157:H7 and O55:H7 organisms share a close genetic affinity and thereby provide a plausible explanation for the observation that these bacteria cause similar attaching and effacing lesions in cells grown in culture (38) and in animal models (39). Because conventional serotyping of *E. coli* does not provide a reliable basis for analyzing population structure and can be grossly misleading as to genetic relationships among isolates (40-42), many important medical correlates of the population structure will not be recognized and understood fully until *E. coli* isolates are sorted out along clonal lineages.

Staphylococcus aureus Toxic Shock Syndrome

Toxic shock syndrome (TSS) was described in 1978 (43) as a severe acute illness (characterized by high fever, erythematous rash, hypotension or shock, multiorgan involvement, and desquamation of the skin) of young children associated with infection with *Staphylococcus aureus*. Two years later, it was recognized that TSS is a geographically widespread disease affecting mainly young, healthy, menstruating women, especially those using certain high absorbency tampons (44). Most vaginal isolates of *S. aureus* recovered from patients with TSS produce a chromosomally encoded toxin, designated as toxic shock syndrome toxin-1 (TSST-1) (45). Evidence implicating TSST-1 as a major virulence factor in the pathogenesis of TSS has accumulated (46). Almost all strains recovered from patients with menstrual TSS, which account for approximately 90% of TSS cases, synthesize TSST-1, whereas only 50%-60% of isolates from cases of nonmenstrual TSS and 5%-25% of strains causing other diseases produce this protein.

Several questions of importance to both medical bacteriology and evolutionary genetics were addressed in a study of 315 TSST-1-producing strains of *S. aureus* (47). It was discovered that the organisms responsible for most cases of TSS with a female urogenital focus are members of a single distinctive clone (designated as ET 41), a result that explains the observation that isolates recovered from patients with TSS share many traits (48, 49). The investigation also showed that TSST-1 is expressed by isolates of a great variety of clones representing virtually the full breadth of genotypic diversity in the species as a whole. In addition, isolates of ET 41 represented 24% of a sample of TSST-1-producing strains recovered before 1978, which meant that the *tst* gene encoding the toxin neither evolved nor was acquired recently by this species. The failure to recover isolates of ET 41 from non-human hosts effectively eliminated the likelihood that animals are important in the transmission of this clone.

Twenty-eight percent of isolates of *S. aureus* cultured from the introitus, vagina, or cervix of unassociated healthy carriers or women with non-TSS urogenital symptoms were ET 41 or closely allied clones; no other single multilocus enzyme genotype accounted for more than 12% of normal vaginal isolates. These observations led to the hypothesis that isolates of ET 41 are more readily able to colonize the human vagina and, hence, are

widely dispersed in an ecological niche of great consequence in TSS. Under this "adapted clone" hypothesis, isolates of ET 41 are responsible for most vaginal cases because this clone has a special affinity for the cervicovaginal milieu, perhaps (but not necessarily) as a consequence of variation in regulation of toxin- or other virulence-gene expression. In summary, data derived from clonal analysis of TSST-1-producing *S. aureus* are consistent with the notion that the "bloom" in TSS cases happened because of a change in the character of catemenial products (perhaps associated with decreasing levels of anti-TSST-1 antibody in human populations), not because of a new *S. aureus* strain.

Two additional points are noteworthy regarding population genetic analysis of *S. aureus* strains producing TSST-1. First, if the gene encoding TSST-1 were evolutionarily old, allelic variants differing in nucleotide and, perhaps, amino acid sequence would exist in natural populations. This prediction was borne out by the identification of a variant of TSST-1 associated with goat, sheep, and occasionally bovine mastitis that is encoded by a gene which differs from the "human" form by 14 nucleotides, resulting in 9 amino acid changes. The variant toxin retains mitogenic activity for mouse splenocytes but differs significantly in other functions ascribed to TSST-1, including ability to induce a TSS-like disease in rabbits (50). Second, if the rapid increase in TSS cases were caused by a change in host character rather than by the rapid spread of a single, new, hypervirulent clone, subclonal heterogeneity would be present among isolates classified as ET 41. Examination of RFLP patterns for the gene (*coa*) encoding coagulase has shown at least three distinct subclones of ET 41 (51).

Neisseria meningitidis

Extensive work in the last 10 years has examined the molecular population genetics of *Neisseria meningitidis*, predominantly by clonal analysis, and more recently by DNA sequencing of putative virulence genes. This work suggests that temporal variation in disease frequency and severity is usually associated with clonal replacement much like influenza epidemics are driven by antigenic shift.

Serogroup B ET-5 Complex Organisms

Caugant et al. (52) demonstrated that an epidemic of serogroup B meningococcal disease that began in the 1970s in Norway and subsequently spread through much of Europe was caused by a group of 22 very closely related clones, designated as the ET-5 complex, that have no close genetic relationship to other clone groups. Clones of this complex were traced intercontinentally to Chile and South Africa, where they also caused contemporary outbreaks of invasive disease. Clonal analysis also showed that a severe epidemic of meningococcal disease in Cuba (characterized by a high attack rate and incidence of septicemia) was due to ET-5 complex organisms. The recovery of these same bacteria from outbreaks in Miami, Florida, in 1980 and 1981, strongly suggested that Cuban refugees imported the clones to Miami. Members of the ET-5 complex have seldom been recognized as important pathogens in the United States. However, ET-5 complex organisms were responsible for a recent increase in meningococcal disease rates in Washington and Oregon (53). In addition, a serogroup B epidemic in greater Sao Paulo, Brazil, was also caused by ET-5 complex members (54).

Recently, clonal analysis has been used to study serogroup B meningococcal isolates that caused invasive disease in The Netherlands between 1958 and 1986 (55). Significant temporal variation in the clonal composition of meningococcal populations was identified. Recent disease episodes were caused predominantly by isolates of three clonal lineages (designated I, III, and VI) that were not represented in samples collected before 1975. In addition, an epidemic in 1966-1967, and a hyperendemic disease wave in 1972 were caused mainly by two closely related clones (ET-11 and ET-17) expressing serotype 2b protein. Strong statistical deviation in the sex ratio was recorded for disease caused by clones of two lineages. Clones of lineage V were cultured far more frequently from female than for male patients; whereas, clones of lineage IX were recovered from disease in male patients approximately four times more often than average. The cause(s) of these differences are unknown but warrant further investigation.

For most bacterial pathogens, few data are available regarding the frequency with which distinctive clones are recovered in asymptomatic persons. Caugant et al. (56) studied the clonal composition of meningococcal isolates cultured

from the nasopharnyx of healthy carriers in Norway and discovered that the frequency of recovery of clones (ET-5 complex and ET-37 complex) causing 80% of disease episodes were represented by only 7% and 9%, respectively, of carrier isolates. This same study demonstrated that the clones most commonly represented among carrier isolates (19%) have never been recovered from patients with invasive meningococcal infection. The data reinforce the concept that bacterial clones vary dramatically in virulence potential.

Serogroup C Disease

An increase of invasive disease due to serogroup C *N. meningitidis* strains has been reported in several countries in recent years (57-60). Study of 121 isolates recovered from patients in Greater Sao Paulo, Brazil, between 1976 and 1990 identified a striking increase in isolates assigned to ET 11 complex (58). The percentage of invasive disease episodes caused by complex 11 organisms increased from 8% in 1988 to 66% in 1990. Outbreaks of serogroup C meningococci have also been recently reported from distinct regions of the United States (59) and Canada (57, 60). Analysis of organisms collected from 13 U.S. outbreaks identified five distinct multilocus enzyme types, all very closely allied in overall chromosomal relatedness (59). Moreover, strains causing 4 of these 13 outbreaks were identical in multilocus enzyme type (designated ET-15) to organisms responsible for outbreaks in eastern Canada (60). Canadian investigators have reported (60) that ET-15 organisms had a significantly higher case-fatality ratio than other invasive meningococcal disease isolates, which may be due to a lower herd immunity to the newly emerged clone.

Serogroup A Disease

Unlike other serogroups of *Neisseria meningitidis* , which are usually associated with endemic disease, isolates expressing serogroup A capsular polysaccharide are unusual in that they may cause large epidemics. For example, serogroup A organisms have been responsible for epidemics of invasive disease in Africa, China, Iran, Greece, Finland, Brazil, and Nepal (61). Major epidemics every 5-10 years in the Sahel region of sub-Saharan Africa have led to the description of a "meningitis belt" and to detailed studies by clonal analysis of the molecular epidemiology of serogroup A organisms responsible for these and other outbreaks (62, 63).

A group led by M. Achtman assembled 423 serogroup A meningococcal isolates, recovered primarily from invasive episodes, and representing organisms responsible for 23 epidemics or outbreaks between 1915 and 1983. Thirty-four distinctive clones were assigned to four complexes representing groups of related clonal genotypes (61). Most epidemics were caused by a single clone, and the same clone often was responsible for concurrent epidemics in contiguous countries. For example, serogroup A clone I-1 caused a pandemic that began in North Africa and certain Mediterranean countries in 1967 and spread throughout West Africa in the subsequent 2 years; clone III-1 has been responsible for disease outbreaks in Finland, Brazil, Nepal, and China.

Recently Achtman's group has extensively characterized more than 300 serogroup A isolates from patients or carriers in one epidemic in The Gambia in 1982-1983 and in 1984-1985 after an immunization program at the end of 1983. Analysis of a representative subgroup of 64 isolates showed that all were assigned to clone IV-1 (64, 65). Isolates of this clone were examined for subclonal variation with SDS-PAGE profiling, LPS profiling, and genomic restriction endonuclease profiling, and rare variants were detected. Two cell-surface antigens (class 5 outer membrane protein and pili) were unusually variable, and the hypothesis was formulated that variation in the class 5 OMP occurs as a consequence of recombinational events affecting the translational reading frame. The role, if any, of this subclonal microheterogeneity in serogroup A meningococcal epidemics is being assessed. Clonal analysis has provided a framework that is being exploited to rationally select strains for further characterization by molecular and serologic techniques that may provide insight into the forces driving a bacterial epidemic (66-68).

Clonal analysis also has demonstrated that serogroup A isolates are a restricted phylogenetic subpopulation of the species *N. meningitidis* (69). This result may mean that the genotype bestowing the epidemic phenotype has arisen a single time and that it has not been successfully transferred horizontally to unrelated phylogenetic lineages of the species.

Moore et al. (70) employed clonal analysis to document the intercontinental spread of an epidemic group A meningococcal clone complex by Muslim hajis pilgrims in 1987. Apparently this clone was carried from South Asia (Nepal and/or

India) to Mecca, Saudi Arabia, where it was disseminated in epidemic form to other hajis (pilgrims) and to indigenous Saudis. The report of invasive serogroup A meningococcal disease in other Gulf nations and among hajis returning to the United States, Europe (France and the United Kingdom), and Africa (Ethiopia, Sudan, and Chad) and the recovery of isolates of the same clone complex (designated ET III-1) from persons in these diverse geographic localities strongly suggested that an unusu ally virulent organism had been rapidly dispersed intercontinentally. Spread of clone III-1 from Mecca to France by hajis was independently confirmed by Riou et al. (71). This meningococcal clone also caused recent episodes of invasive disease in Sweden (72) and Kenya (73).

Streptococcus pyogenes Invasive Disease

Severe invasive infections caused by *S. pyogenes* have been reported with increased frequency in recent years in the United States (74, 75), Europe (76-78), and elsewhere (79, 80). These include both soft tissue infections, such as cellulitis, and deeper infections, including osteomyelitis, necrotizing fasciitis, and sepsis, many of which have occurred in previously healthy persons. The observation that many patients have multiorgan failure and other signs and symptoms mimicking staphylococcal toxic shock syndrome led to the characterization of a streptococcal "toxic-shock-like syndrome" (TSLS) (81). Most *S. pyogenes* isolates recovered from such patients produce one or more pyrogenic exotoxins with significant amino acid sequence homology and functional similarity with several enterotoxins synthesized by *S. aureus* (82).

To determine the genetic diversity and clonal relationships among *S. pyogenes* isolates recovered from patients with TSLS or other invasive diseases in the United States, 108 organisms were studied by multilocus enzyme electrophoresis and analyzed for exotoxin A, B, and C synthesis (82). The analysis showed that 33 distinctive clones were present among isolates comprising the sample, but nearly half the disease episodes, including more than two-thirds of the cases of TSLS, were caused by strains of two related clones, designated ET-1 and ET-2 (82). The production of pyrogenic exotoxin A (scarlet fever toxin, which is bacteriophage-encoded), either alone or in combination with other pyrogenic exotoxins, was associated with recovery in patients with TSLS. This association was present with isolates of the same clone, as well as those of distantly related phylogenetic lineages. The data were interpreted as strong circumstantial evidence that scarlet fever toxin A itself, or, possibly, the product of a gene tightly linked to it, is a factor in the pathogenesis of TSLS.

Because an increase in disease caused by strains expressing the M1 serotype protein had also been observed in England, Sweden, Norway, Germany, other European countries, and elsewhere, we sought to determine if strains recovered from these diverse localities were genetically allied. Chromosomal diversity and relationships among 126 M1 strains from 13 countries on five continents were analyzed by multilocus enzyme electrophoresis and restriction fragment profiling by pulsed-field gel electrophoresis (83). All isolates were also examined for the *speA* gene by PCR, and to increase the possibility of identifying intrastrain variation, strain subsets were examined by automated DNA sequencing for allelic polymorphism in genes encoding M protein (*emm*), streptococcal pyrogenic exotoxin A (*speA*), streptokinase (*ska*), pyrogenic exotoxin B (*speB*), and C5a peptidase (*scp*). Seven distinct *emm1* alleles were identified that would express M proteins differing at one or more amino acids in the N-terminus variable region. Although substantial levels of genetic diversity existed among M1 organisms, most invasive episodes were caused by two subclones marked by distinctive multilocus enzyme electrophoretic profile and PFGE restriction fragment length polymorphism (RFLP) types. One of these subclones (ET 1/RFLP pattern 1a) has the *speA* gene, and was recovered worldwide. Identity of *speA* , *emm1* , *speB* , and *ska* alleles in virtually all isolates of ET 1/RFLP type 1a means that these organisms have shared a common ancestor, and that global dispersion of this M1 subclone has occurred very recently. The occurrence of the same *emm* and *ska* allele in strains that are well-differentiated in overall chromosomal character demonstrated that horizontal transfer and recombination play a fundamental role in diversifying natural populations of *S. pyogenes* .

The population genetic framework constructed for *S. pyogenes* has been exploited to rationally choose strains for comparative molecular characterization of the gene (*speA*) encoding scarlet fever toxin (84, 85). An analysis by Nelson et al. (84) identified four alleles of *speA* in natural

populations, one of which (*speA1*) occurs in many distinct clonal lineages and is, therefore, probably evolutionarily old. The presence of identical exotoxin A structural genes in diverse phylogenetic lineages means that the gene has been horizontally distributed among clones, presumably by bacteriophage-mediated transfer. Two other alleles (*speA2* and *speA3*), characterized solely by single nucleotide changes resulting in single amino acid substitutions, were each identified in single clones (ET 1 and ET 2) that together have caused most of TSLS episodes. The restriction of *speA2* and *speA3* to single clonal lineages can be interpreted as evidence that these two alleles are evolutionarily younger than *speA1* . A fourth allele (*speA4*) also is present in a single phylogenetic lineage and is 9% divergent from the other three toxin alleles. The absence of synonymous (silent) nucleotide changes in *speA2* and *speA3* is unusual and suggests that the allelic variation is not selectively neutral, which implies that the toxins are not functionally equivalent. Moreover, the mutations occur in a segment consisting of five amino acids that are highly conserved in the aligned sequences of staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), SEC1, SEC3, SED, SEE, and streptococcal pyrogenic exotoxin C (86). The segment of SPE A containing these variations is immediately adjacent to a region containing cysteine residues involved in the formation of a disulfide loop believed to be required for mitogenicity of SPE A and other bacterial superantigens. Population genetic analysis then suggests that there are functional correlates of the allelic variation and that the alleles have been subject to natural selection. Recent studies have shown that the ability of the SPEA2 and SPEA3 variants to stimulate human peripheral blood mononuclear cells exceeds that of SPEA1 (87).

Mycobacterium tuberculosis

Perhaps no bacterial infection in recent years has generated as much interest nationally as resurgent tuberculosis (TB) (88). Largely because of the success of public health strategies, the incidence of TB declined steadily in the United States since the early 1950s, and the disease was thought to be eradicable by the end of the first decade of the 21st century (89). However, the yearly decline in TB incidence ended in 1984, and after several years of a plateau phase, resurged from 1988 through the present. An estimated 63,000 excess cases occurred through 1993 (90). The HIV/AIDS epidemic, immigration from countries with high TB prevalence, and outbreaks in correctional institutions, nursing homes, shelters for the homeless, and other congregative environments have contributed to the resurgence (88). On a global scale, one-third of the world's population is infected with this pathogen, and 8 million new TB cases occur each year. Moreover, nearly 3 million people die annually of TB, making it the leading cause of death due to an infectious agent worldwide (88). Hence, there is a need to understand the nature and extent of molecular variation in this pathogen.

Although the population genetics of *M. tuberculosis* has not been examined by multilocus enzyme electrophoresis, a recent study (91) analyzed DNA sequence diversity in eight loci (192,875 nucleotides) from unassociated isolates recovered in North America and Europe. The data showed almost a complete absence of coding sequence nucleotide variation. To rule out the possibility that restricted geographic sampling biased the data set, 350-bp fragments of genes encoding the beta subunit of RNA polymerase (*rpoB*), a 65-kilodalton heat shock protein (*hsp65*), the A subunit of DNA gyrase (*gyrA*), an enzyme involved in aromatic amino acid biosynthesis (*aroA*), RecA protein (*recA*), and a 1435-bp region of the gene (*katG*) encoding a catalase-peroxidase enzyme important in isoniazid resistance (92-94) and host-parasite interactions (95), were sequenced from one randomly selected isolate from each of seven countries with well-differentiated human populations (Switzerland, Turkey, Algeria, Somalia, Papua New Guinea, Vietnam, and Tibet). A virtual lack of nucleotide variation was also found in these seven isolates, and sequencing of several genes from many additional TB isolates has reinforced the concept of extremely restricted structural gene polymorphism. The paucity of sequence variation was surprising for several reasons. First, paleopathologic evidence suggests that humans got TB as early as 3700 BC in Egypt and 2500-1500 BC in Europe and also pre-Columbian North and South America (96). Moreover, *M. tuberculosis* DNA recovered from lung lesions in a 1000-year-old Peruvian mummy confirmed that the disease existed in the pre-Columbian New World (97). Second, as noted above, there is a very large global pool of infected persons (88), and third, considerable chromosomal restriction fragment length polymorphism has been identified by probing with

mobile elements such as IS *6110* (98, 99). Based on a population genetic interpretation of the data, it was posited that *M. tuberculosis* may be only 15,000 to 20,000 years old, an age that dates speciation and global dissemination to roughly the same time as paleomigration into the New World. The time frame is also consistent with speculation (100) that the agent of human TB arose from the very closely related cattle pathogen *M. bovis* by host specialization occurring since the domestication of this animal some 8,000 - 10,000 years ago. Recent large-scale DNA sequencing results are also consistent with an interpretation that *M. tuberculosis* and *M. bovis* have shared a recent common ancestor.

These molecular population genetic findings have considerable implications for *M. tuberculosis* pathobiology research. First, the virtual absence of naturally occurring nucleotide substitutions greatly increases the likelihood that missense mutations identified in genes associated with resistance to antimicrobial agents actually confer resistance rather than simply acting as convenient surrogate markers of resistance (93, 94, 101, 102). Second, restricted allelic diversity means that it is probable that only nominal amino acid variation will occur in proteins of potential immunoprophylaxis, diagnostic, or virulence interest.

Clones W and Son of W

Commensurate with the rise of TB cases in the United States was an increase in the number of organisms resistant to one or more anti-TB medications (103). This trend has been viewed with great concern by public health authorities and clinicians, in part because no new first-line anti-TB agents have been introduced in several decades. Certain communities have contributed disproportionately to the documented increase is resistant organisms, the most notable being New York City (104), which has accounted for up to 60% of all drug-resistant *M. tuberculosis* reported nationally in some surveys. Although strains with several antimicrobial agent susceptibility patterns have been identified, approximately 300 organisms are invariably resistant to isoniazid, streptomycin, rifampin, ethambutol, and variably resistant to ethionamide, kanamycin, capreomycin, and ciprofloxacin (105, 106). Early reports based on IS *6110* restriction fragment length polymorphism typing (99), other molecular techniques (98, 107), and classic epidemiologic investigations suggested that many of these organisms were clonally related. More recent analysis using IS *6110* typing, several other molecular typing strategies, and automated DNA sequencing to identify the exact nucleotide changes responsible for resistance to isoniazid, rifampin, and streptomycin has unambiguously demonstrated the existence of two abundant closely related subclones (arbitrarily named W and W1, son of W) that have clearly shared a recent common origin (105). Multidrug resistance in these strains is due to sequential accumulation of amino acid substitutions conferring resistance to each drug alone, rather than a single-step molecular event, such as acquisition of a multidrug-resistance-conferring plasmid. Progeny of these two subclones have now spread well beyond the New York City borders. The organisms have been isolated from patients in other New York communities (108), Atlanta, Miami, Denver, Las Vegas, and Paris, France (109). Thus far, all patients documented to have infection caused by W or W1 organisms can readily be epidemiologically connected with New York City, that is, secondary, tertiary, or quaternary spread has not yet sufficiently obscured this important epidemiologic thread. Dissemination of these difficult to treat W and W1 organisms throughout New York City and other cities demonstrates the devastating consequences of clonal origin and spread of a bacterial pathogen. Because some persons now infected latently with W and W1 will later experience reactivation disease, dissemination of W and W1 has adverse implications for TB control in the 21st century.

Penicillin-Resistant *Streptococcus pneumoniae*

The Gram-positive bacterial pathogen *S. pneumoniae* is a major cause of illness and death worldwide (110, 112). In the United States, the organism is responsible for more than 500,000 cases of pneumonia, 55,000 episodes of bacteremia, 6,000 cases of meningitis, and 40,000 deaths each year (113). Until relatively recently, antibiotic resistance in *S. pneumoniae* was rare, but it is now a global public health problem (114-116).

Resistance to penicillin in many organisms is due to the expression of altered high molecular-weight penicillin-binding proteins (PBPs) that have reduced antibiotic affinity (117). Among resistant strains, alterations in four (1A, 2X, 2A, and 2B) of the five high molecular-weight PBPs expressed by isolates of the species have been

identified in resistant patient isolates. Research has shown that two processes have contributed to the rise of these organisms. First, many distinct susceptible strains are independently evolving to the resistance phenotype. Acquisition of penicillin binding protein gene segments from foreign donors, such as oral streptococci, is apparently a primary driving force (118). At the molecular level, the result is generation of mosaic genes, and thereby molecularly remodelled PBP proteins with decreased affinity for penicillin (119-123). Evidence shows that the Hex recombinational pathway (124) participates. Once a distinct drug-resistant cell has been generated, progeny can be transmitted locally and over intercontinental distances by person-to-person spread (125-130). Multilocus enzyme electrophoresis has been applied to analyze genetic relationships among penicillin-resistant strains of *S. pneumoniae* from global sources and to infer patterns of epidemiologic spread of these resistant organisms (125-131).

The importance of rapid local clonal spread of antibiotic-resistant *S. pneumoniae* is illustrated by events in Iceland. Monitoring of antibiotic resistance patterns of pneumococci in Iceland showed no detectable penicillin-resistant organisms in 1983 to 1988. The first penicillin-resistant strain was recovered in December 1988 (132). The frequency of penicillin-resistant organisms rose sharply over the next 3 years from 2.3% to 17% of all isolates in the first quarter of 1992 (132). Almost 70% of the resistant isolates expressed serogroup 6 capsule polysaccharide and were also resistant to tetracycline, chloramphenicol, erythromycin, and trimethoprim-sulfamethoxazole. To test the hypothesis that these Icelandic isolates were clonally related, Soares et al. (128) examined 57 organisms for serotype, PBP pattern, pulsed-field chromosomal restriction endonuclease digestion pattern, and multilocus enzyme electrophoretic genotype. All isolates were serotype 6B and had closely similar or identical patterns for each of the molecular markers examined. Surprisingly, the Icelandic organisms were indistinguishable from a subgroup of multiresistant serotype 6B pneumococci that occurs with high incidence in Spain. The authors concluded that the Spanish clone was imported to Iceland and noted that in recent years a favored vacation locality for Icelandic families with young children had been Spain. The factors responsible for the precipitous spread of the clone in Iceland are largely unknown. The frequency of use of beta-lactam antibiotics in Iceland and Sweden (a country where resistant pneumococci are rare) in 1989 was similar (133), and low compared to other industrialized countries (134). However, Iceland has a very high use of antimicrobial agents such as trimethoprim-sulfamethoxazole, metronidazol, and tetracycline (133, 134), which means it is conceivable that selection for the multiresistant clone occurred. A second possible factor contributing to the clonal spread of the organisms is that 57% of Iceland's population of about 250,000 live in Reykjavik and its suburbs, where most of these strains have been recovered. Moreover, almost 80% of Icelandic children 2 to 6 years of age in Reykjavik attend day-care centers. Together these factors may have provided a unique set of circumstances for introduction and rapid spread of the multiresistant clone.

Investigating the molecular population genetics of pneumococci has led to the realization that horizontal transfer and recombinational processes are also serving to generate variation in capsule type and immunoglobulin A1 (IgA1) protease gene alleles (135, 136). Coffey et al. (135) analyzed European resistant strains expressing serotype 9 or 19 through a combined approach employing clonal analysis and RFLP profiling of genes encoding PBP1A, PBP2B, and PBP2X. Analysis of a resistant isolate synthesizing serotype 19 capsule showed that it was identical in overall chromosomal character to a clone of organisms resistant to multiple antibiotics which expressed serotype 23F, a result that was interpreted as evidence that horizontal transfer of capsular biosynthesis genes had occurred. More recently, Lomholt (136) has shown that recombinational processes contribute to allelic variation in the gene (*iga*) encoding IgA1 protease.

Methicillin-Resistant *Staphylococcus aureus*

Very soon after methicillin entered clinical use in the 1950s, strains of *S. aureus* resistant to this antimicrobial agent were reported in the United Kingdom (137). Within a few years, hospital outbreaks caused by methicillin-resistant *S. aureus* (MRSA) occurred in Europe. MRSA were recognized as an important hospital infection control problem in the United States in the mid-1970s, and these organisms have now achieved global distribution (138).

Intrinsic methicillin resistance is due to the expression of an altered penicillin-binding protein (PBP) termed PBP 2a (139) that is encoded by the chromosomal *mec* gene (140, 141). Evidence has been presented that *mec* originated as a consequence of a recombinational event fusing about 300 bp of a staphylococcal beta-lactamase gene and a segment of a gene encoding a PBP from an unknown donor bacterium, perhaps *E. coli* (142).

Multilocus enzyme electrophoresis and other molecular population genetic techniques were used to determine the extent of *mec* distribution among phylogenetic lineages of the species and genetic relationships among MRSA strains circulating in various geographic regions at different times (143, 144). The *mec* gene is harbored by many divergent phylogenetic lineages representing a large portion of the breadth of chromosomal diversity in the species *S. aureus*. On the basis of additional evidence, it was proposed that multiple episodes of horizontal transfer and recombination have contributed to the spread of the *mec* resistance determinant in natural populations. The identification of a single multilocus enzyme genotype among MRSA organisms recovered in the United Kingdom, Denmark, Switzerland, Egypt, and Uganda, soon after the widespread introduction of methicillin into clinical use in the 1960s, meant that MRSA isolates recovered from those localities at that time were progeny of a single ancestral cell that had probably acquired the *mec* determinant recently. The multilocus enzyme electrophoretic data demonstrating association of *mec* with highly divergent members of the *S. aureus* species effectively ruled out the idea (145) that all extant MRSA are lineal descendants of a single clone, and that *mec* was acquired just once by methicillin-sensitive clones of this pathogen.

Borrelia Species Associated with Lyme Disease

Molecular population genetic strategies have also been used to delineate accurate phylogenetic relationships among emerging organisms. For example, Boerlin et al. (146) studied 50 isolates classified as *Borrelia burgdorferi* by multilocus enzyme electrophoresis and identified three distinct genetic clusters that were well differentiated from one another in overall chromosomal character. The investigators proposed that each cluster represented a genospecies, and this idea was subsequently supported by DNA-DNA reassociation studies (147), 16S rRNA gene sequencing (148), genomic fingerprinting by arbitrarily primed polymerase chain reaction (149), and other techniques (Valsangiacomo C, Balmelli T, Piffaretti J-C, pers. comm.). Moreover, different genospecies of *B. burgdorferi* have been associated with distinct clinical manifestations of Lyme borreliosis (150-152).

Although population genetics has only been applied to the study of pathogenic bacteria for approximately a decade, considerable insight has been gained into the molecular mechanisms of temporal variation in disease frequency and severity, host adaptation of clonal lineages, and the relationship of disease severity and naturally occurring bacterial clones. The work cited in this review represents only a small part of the contribution of molecular population genetic investigations to an understanding of temporal variation in disease frequency and severity, microbial pathogenicity, and evolution of virulence genes. For example, contributions have also been made from studies of *Vibrio cholerae* (153-155), encapsulated *H. influenzae* (156), *L. monocytogenes* (157-158), *Salmonella* spp. (159-161), and other pathogens (162).

Changes in human behavior, simple processes of microbial evolution, and increasing resistance to antimicrobial agents will continue to supply mankind with new infectious disease challenges and, therefore, motivation for molecular population genetics studies. The genomes of two bacterial pathogens have now been sequenced (163-164), and it is likely that the genomes of most major human and veterinary viral and bacterial pathogens will be sequenced in their entirety in the next decade. Hence, the trend toward molecular dissection of microbial populations by large-scale DNA sequencing will accelerate. DNA sequence-based and conventional molecular population genetic studies are cost-effective and should be encouraged in the fight to limit the detrimental impact of infectious agents on human, animal, and plant health.

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Emergence of the Ehrlichioses as Human Health Problems

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Ehrlichiae are small, gram-negative, obligately intracellular bacteria that reside within a phagosome. The first human ehrlichial infection was recognized in the United States in 1987. It was later shown to be caused by a new species, *Ehrlichia chaffeensis*. In 1994, an ehrlichial pathogen within neutrophils that is closely related to the known veterinary pathogens *E. equi* and *E. phagocytophila* was found to infect humans. Molecular methods were required to detect, characterize, and identify these fastidious and uncultivated bacteria. Subsequently, *E. chaffeensis* infection was documented in more than 400 patients in 30 states, Europe, and Africa. Likewise, approximately 170 cases of human granulocytic ehrlichiosis have been diagnosed, most since 1994, predominantly in the upper midwestern and northeastern states, but also in northern California. The disease caused by ehrlichiae is generally undifferentiated but is often associated with leukopenia, thrombocytopenia, and elevated serum hepatic transaminase levels in tick-exposed patients. Infection ranges from subclinical to fatal; tetracycline appears to be an effective therapy. The emergence of these two newly recognized tickborne infections as threats to human health is probably due to increased clinical cognizance, but as in other emerging tickborne infections, it is likely that the rapid increase in identified cases signals a true emergence of disease associated with a changing vector-host ecology.

During the last decade, two previously unknown human diseases caused by *Ehrlichia* species have emerged as public health problems in the United States. Each of these infectious diseases is designated by the major target cell: human monocytic ehrlichiosis is caused by *Ehrlichia chaffeensis,* and human granulocytic ehrlichiosis by an *E. equi*-like organism. The taxonomic positions of these bacteria have been determined with much greater scientific clarity by the recent application of molecular methods, which were critical for the identification of *E. chaffeensis* and essential for that of human granulocytic ehrlichia (HGE) (1,2). Extreme difficulty in developing methods for cultivation of these ehrlichiae and, until recently, the lack of molecular approaches to the study of uncultivated organisms partly explain why these far from rare diseases were not detected sooner (3-5). However, it was the observation of Romanowsky-stained blood smears by classic microscopy that actually opened the door to the discovery of both diseases (6,7). This article reviews ehrlichiae and describes their taxonomic and ecologic niches within the scheme of bacterial evolution and the substantial knowledge, developed largely by the veterinary sciences, that served as a foundation for the discovery and elucidation of the human ehrlichioses.

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Evolution of Ehrlichiae in Unusual Ecologic Niches

Among the obligately intracellular gram-negative bacteria, a genetically related set is classified among the *Protobacteria* of the α subgroup on the basis of sequence analysis of the 16S rRNA gene (1,2,8,9). The overview of the evolution of these organisms provided by this approach lacks many details essential to the understanding of human disease; however, the evolution of these organisms correlates well with the clonal divergence of many species that do not have opportunities for genetic recombination because of their intracellular isolation from other organisms. These bacteria have evolved in close association with ticks, mites, chiggers, fleas, other arthropods, and fish flukes into six genetically defined clusters (1,2,8-11) (Table 1 and Figure 1). Future definition of the details determined by many other important genes may designate these six clusters as separate taxonomic genera. Currently, however, the presence of organisms named as *Ehrlichia* species within three of

Table 1. Six related clusters of obligately intracellular gram-negative bacteria determined by 16S rRNA comparisons

Prototype species of the cluster	Other genera within genetic confines	Vector relationship
I. *Ehrlichia canis*	*Cowdria*	Ticks
II. *E. phagocytophila*	*Anaplasma*	Ticks
III. *E. sennetsu*	*Neorickettsia*	Fish flukes
IV. *Wolbachia pipientis*	*Rhinocyllus, Nasonia* incompatibility intracellular symbionts	Insects
V. *Rickettsia ricke ttsii*	None	Ticks, fleas, Mites, lice
VI. *Orientia tsutsugamushi* * (*R. tsutsugamushii*)	None	Chiggers

* Proposed new taxonomic designation (20).

these clusters is confusing, particularly since other genus names also fall into each of the clusters (Tables 1 and 2).

Historical Foundations for Recognizing the Human Ehrlichioses in the United States

The current, albeit incomplete, knowledge of the human ehrlichioses has a long history. The emergence of ehrlichiae began in 1910, when Theiler described *Anaplasma marginale* , the etiologic agent of an economically important, severe worldwide disease of cattle. The contributions of veterinary medical science to the understanding of ehrlichiae have continued ever since; they include the description of *Cowdria ruminantium* by Cowdry in 1925, of *E. canis* by Donatien and Lestoquard in 1935, and *E. phagocytophila* by Gordon in 1940. The genus *Ehrlichia* was established in 1945 in honor of the German microbiologist Paul Ehrlich, and the general scientific distinction of rickettsiae and ehrlichiae from viruses and protozoa followed soon thereafter, mainly as a result of the advent of antibiotics and electron microscopy. Molecular methods are now used to identify various species. Indeed, *E. sennetsu* , the etiologic agent of a human infectious mononucleosis-like illness described in Japan in 1954, was considered a *Rickettsia* for many years. Numerous veterinary discoveries preceded the recognition of human ehrlichial infections in the United States: *Neorickettsia helminthoeca* in 1950, *N. elokominica* in 1964, *E. equi* in 1969, *E. ewingii* in 1971, *E. platys* in 1978, and *E. risticii* in 1984 (12). Substantial emphasis on the study of ehrlichiae was stimulated by a disastrous epizootic of canine ehrlichiosis that resulted in 200 to 300 deaths among military working dogs in Vietnam from 1968 until 1970. After the discovery, in 1984, that the etiologic agent of Potomac horse fever was a novel species designated as *E. risticii* ,

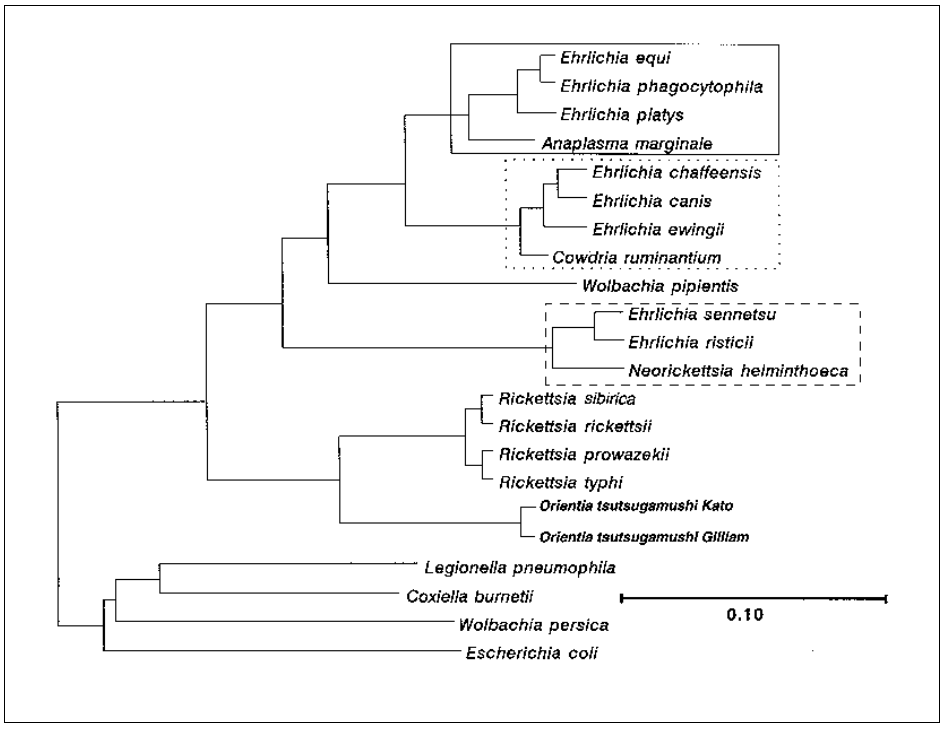

Figure 1. Dendrogram representing the phylogenic relationships of ehrlichiae and other *Protobacteria* as determined by 16S rDNA sequence similarity. The three clusters of bacteria enclosed in rectangles include organisms designated as *Ehrlichia* , although they differ substantially.

Table 2. Three related clusters of bacteria-containing species designated as *Ehrlichia*

	Vertebrate host	Transmission	Major target cell
Genogroup I			
Ehrlichia canis	Canids	*Rhipicephalus sanguineus* tick bite	Monocyte/macrophage
E. chaffeensis	Humans, deer	*Amblyomma americanum* tick bite	Monocyte/macrophage
E. ewingii	Canids	*A. americanum* tick bite	Polymorphonuclear leukocytes
E. muris	Vole	Unknown	Monocyte/macrophage
Cowdria ruminantium	Cattle, sheep, goats, antelope, African buffalo	*Amblyomma* spp. tick bite	Endothelium
Genogroup II			
E. equi	Horses	*Ixodes* spp. tick bite	Neutrophils
E. phagocytophila	Sheep, cattle, deer	*Ixodes ricinus* tick bite	Neutrophils
Human granulocytic ehrlichia	Humans, deer, rodents (?)	*I. scapularis* (*dammini*) tick bite	Neutrophils
E. platys	Canids	Unknown	Platelets
Anaplasma marginale	Cattle	*Boophilus, Rhipicephalus*, and other tick bites	Erythrocytes
Genogroup III			
E. sennetsu	Humans	Possibly ingestion of raw fish	Monocyte/macrophage
E. risticii	Horses	Unknown	Monocytes, enterocytes, mast cells
Neorickettsia helminthoeca	Canids	Salmon fluke ingestion	Macrophage
N. elokominica	Canids	Salmon fluke ingestion	Macrophage
Stellantchasmus falcatus (SF) agent	None known	Grey mullet fluke	Unknown

major advances in the scientific investigation of ehrlichiae have been achieved, particularly in the laboratory of Yasuko Rikihisa at the Ohio State University College of Veterinary Medicine. Indeed, blinded to the potential relevance of *Ehrlichia* ssp. to human medicine, we missed numerous opportunities during the 1980s to investigate canine ehrlichiosis.

Emergence of Human Monocytic Ehrlichiosis

A single, somewhat unusual case in 1986 led to the recognition of human ehrlichial infections in the United States (6). A 51-year-old man who had been bitten by a tick in Arkansas and had been sick for 5 days was admitted to a hospital in Detroit. He was critically ill with fever, headache, myalgia, confusion, azotemia, hypoxemia, and thrombocytopenia and had cytoplasmic inclusions in his peripheral blood leukocytes. Peripheral blood smears and electron micrographs of the inclusion-bearing leukocytes examined at the Centers for Disease Control (CDC) suggested that the organisms were an *Ehrlichia* sp. Serologic evaluation showed a high titer of antibodies reactive with *E. canis* that fell sharply during convalescence. The patient had a prolonged hospitalization complicated by renal failure which required hemodialysis; upper gastrointestinal hemorrhage; severe central nervous system involvement; and systemic *Candida* infection.

State public health agencies and CDC demonstrated that many patients originally suspected to have Rocky Mountain spotted fever or another rickettsiosis (13) produced antibody to *E. canis*, a canine pathogen. In Oklahoma, the state with the highest incidence of Rocky Mountain spotted fever, serologic evidence suggested that human monocytic ehrlichiosis occurred at the same incidence as Rocky Mountain spotted fever (14). In a

prospective active surveillance study of febrile hospitalized patients in southeastern Georgia, ehrlichial disease was sixfold more prevalent than Rocky Mountain spotted fever and was associated with history of tick bite, anorexia, chills, weight loss, sweating, headache, nausea, myalgia, thrombocytopenia, and elevated serum concentrations of hepatic transaminases in 50% or more of the patients and by arthralgia, vomiting, diarrhea, abdominal pain, cough, rash, and leukopenia in fewer than half of the patients (15). Mild and asymptomatic seroconversion to ehrlichial antigens has also been associated with tick exposure (16).

A practical method for the cultivation of *E. canis* (the suspected cause of human ehrlichial infections), developed at CDC, used the cultivated organisms to establish a reliable indirect immunofluorescence assay (IFA) for the diagnostic demonstration of antibodies in serum (17). In retrospect, it is apparent that *E. canis* was a relatively sensitive surrogate antigen because it shares some, but by no means all, of the antigens of the etiologic *Ehrlichia*. The method of ehrlichial cultivation in a continuous canine histiocytoma cell line (DH82) was also successfully applied to the isolation of an *Ehrlichia* species from the blood of a febrile soldier at Fort Chaffee, Arkansas (3). Molecular methods were also applied to establish, on the basis of the DNA sequence of the 16S rRNA gene, that *E. chaffeensis* is a novel species (1).

Current Knowledge of *E. chaffeensis* and Human Monocytic Ehrlichiosis

Microbiology of the Etiologic Agent

Ultrastructural investigation of *E. chaffeensis* demonstrated that the inner and outer leaflets of the cell wall were equal in thickness. *Rickettsia* species have a thinner outer leaflet and thicker inner leaflet, and *Orientia* (*Rickettsia*) *tsutsugamushi* has a thicker outer leaflet and thinner inner leaflet (18-20) (Figures 2 and 3). Two distinct morphologic forms, larger reticulate cells with uniformly dispersed nucleoid filaments and ribosomes and smaller cells with central condensation of nucleoid filaments and ribosomes, were observed undergoing binary fission, evidence against a developmental cycle (Figure 3). Many forms were observed, including numerous intramorular vesicles and tubules originating from the

Figure 2. Human granulocytic ehrlichiae (BDS strain) in an equine peripheral blood neutrophil are located within four morulae. Reticulate (r) and dense-cored (d) cells are surrounded by two membranes: cell wall membrane and cytoplasmic membrane. Bar = 1 µm; magnification, x 27,000. (Courtesy of Vsevolod Popov, University of Texas Medical Branch at Galveston.)

ehrlichial cell wall and fibrillar material consisting of ehrlichial antigen. Abnormal forms included giant and multilobar ehrlichiae.

Western immunoblot technique has detected seven major proteins: 120, 66, 58, 44, 29, 28, and 22 kDa; the greatest number of antigens are shared with *E. canis* (21). Monoclonal antibodies and monospecific polyclonal antibodies demonstrated that the major, immunodominant 120-, 29-, 28-, and 22-kDa proteins (as well as a minor 30-kDa protein) are surface-exposed and that the 28- and 22-kDa proteins are related antigenically. Further human isolations of ehrlichiae have shown antigenic and genetic diversity among strains of *E. chaffeensis* (4).

DNA cloning has shown that 58- and 10-kDa proteins are genetically homologous to the

Escherichia coli GroEL and GroES heat shock proteins (22). The 120-kDa protein includes a region of identical 80 amino acid tandem repeat units, and preliminary evidence suggests that it mediates adhesion to the host cell. Establishment and study of tick isolates and identification of virulence factors are clearly important aims for future research.

Clinical Manifestations

Most cases of human ehrlichiosis have been diagnosed after a moderate-to-severe illness (23). Some patients have a life-threatening illness resembling toxic shock syndrome (24). Deaths have occurred in approximately 2% to 3% of patients, including previously healthy children (13,23-28). Among 237 cases between 1985 and 1990 investigated by CDC, 62% of patients were hospitalized (23). The median duration of illness, including that for treated patients, was 23 days. The signs and symptoms depict a systemic disease that has no clinically diagnostic features: fever (97%), headache (81%), myalgia (68%), anorexia (66%), nausea (48%), vomiting (37%), rash (6% at onset, 25% during the first week, and 36% overall), cough (26%), pharyngitis (26%), diarrhea (25%), lymphadenopathy (25%), abdominal pain (22%), and confusion (20%). Severe complications include respiratory and renal insufficiency and serious neurologic involvement. Of patients with chest radiographic examinations, nearly half have pulmonary infiltrates (13). Clinical laboratory findings include leukopenia (60%), thrombocytopenia (68%), and elevated hepatic transaminases (86%). Hepatic involvement has been described as severe in individual cases (29). Central nervous system involvement has been documented by the occurrence of seizures, coma, and cerebral lesions at autopsy as well as by cerebrospinal fluid (CSF) pleocytosis, increased CSF protein concentration, and the presence of *E. chaffeensis* in CSF demonstrated by immunocytology and polymerase chain reaction (PCR) (24,30,31). Recognition of serious myocardial involvement further emphasizes the potential gravity of this disease and the incompleteness of our knowledge of its clinical manifestations (32). Seroconversion to *E. chaffeensis* among 1.3% of 1,187 soldiers during training exercises with tick exposures suggests that human risk for infection with it or an antigenically related *Ehrlichia* is relatively high (16). However, the fact that two-thirds of the seroconverters remained asymptomatic emphasizes that the relationship between severity of illness and either host factors or ehrlichial strain differences in virulence remains unknown.

Epidemiology of Human Monocytic Ehrlichiosis and Ecology of *E. chaffeensis*

More than 400 cases of serologically confirmed *E. chaffeensis* infection have been documented at CDC, which has one of the few laboratories capable of performing diagnostic ehrlichial serology. That these cases most likely represent but the tip of the iceberg was confirmed when inquiry at MRL Diagnostics, a commercial reference laboratory that offers IFA serology for *E. chaffeensis*, reported 722 positive specimens between September 1992

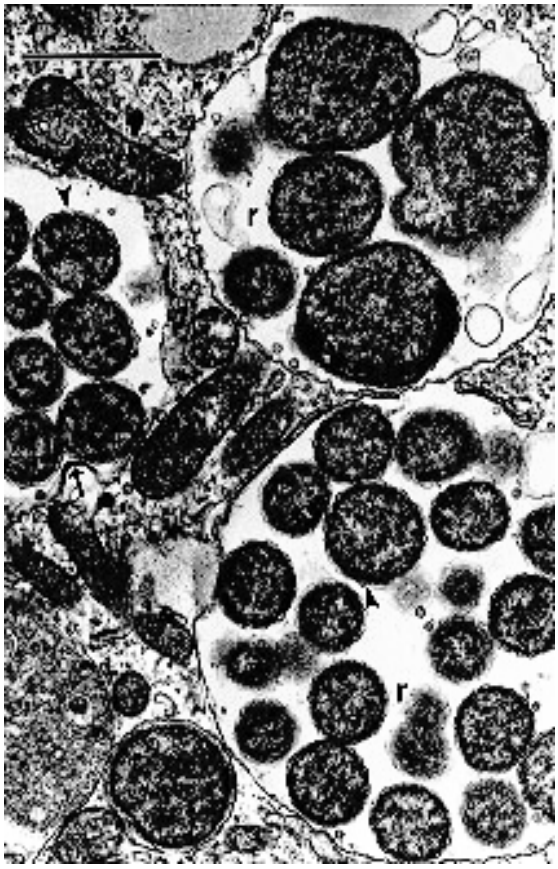

Figure 3. Human monocytic ehrlichiae (*Ehrlichia chaffeensis*, Sapulpa strain) in host-cell membrane-limited parasitophorous vacuoles (morulae) of a DH82 cell (canine macrophage cell line). Ehrlichial reticulate cells (r) are limited by two membranes. The outer one—the cell wall membrane—is usually wavy (arrowheads). One *Ehrlichia* is dividing by binary fission (arrow). Bar = 1 µm; magnification, x 18,000. (Courtesy of Vsevolod Popov, University of Texas Medical Branch at Galveston.)

and June 15, 1995. In fact, there is no system, required or otherwise, for notifying public health authorities of cases. Relatively few physicians even know that these diseases exist and, thus, most of them are not making the diagnosis. Even highly knowledgeable physicians find ehrlichiosis virtually impossible to diagnose on the basis of clinical signs and symptoms. Most patients (83%) report exposure to ticks or tick bite within the 3 weeks of onset of illness (13,23). Cases are predominantly rural (66%) and seasonal (68% during May-July). The median age of patients is 44 years, and three-quarters are male. Outbreaks of human monocytic ehrlichiosis among groups of golfers and campers emphasize the risk for infection during outdoor activities with tick exposure (16,33). Human monocytic ehrlichiosis occurs not only as an acute illness of apparently immunocompetent persons but also as an opportunistic infection of patients with compromised host defenses, including acquired immunodeficiency syndrome (AIDS) patients (24,26). The report of a fatal *E. chaffeensis* infection in an AIDS patient, in whom a diagnostic antibody response to *E. chaffeensis* never developed, suggests that such cases would not usually be diagnosed correctly.

E. chaffeensis has been detected in two tick species, *Amblyomma americanum* (the lone star tick) and *Dermacentor variabilis* (34,35) (the American dog tick). Human monocytic ehrlichiosis has been confirmed in 30 states. Most cases have occurred within the range of *A. americanum*, and a high proportion of the remainder have occurred within the range of *D. variabilis*. Ehrlichiae have been detected specifically by PCR in adult (but not in nymphs) *A. americanum* ticks in Missouri, North Carolina, Kentucky, and New Jersey; a single *E. chaffeensis* PCR-positive *D. variabilis* tick was found in Arkansas. On the other hand, cases reported in Wyoming, Utah, Washington, Europe, and Africa suggest the possibility of additional vectors (23,36,37) or other antigenically related ehrlichial organisms.

White-tailed deer (*Odocoileus virginianus*) in Alabama, Arkansas, Florida, Georgia, Illinois, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas, and Virginia have antibodies reactive with *E. chaffeensis*. These deer are susceptible to experimental infection with ehrlichiae that can circulate for weeks (38,39). The close association among *E. chaffeensis*, the lone star tick, and white-tailed deer was vividly illustrated by the sequential appearance of *A. americanum* ticks and antibodies to *E. chaffeensis* in a population of deer in Georgia (40). The prevalence of lone star ticks and seropositivity both rose from 1983 to 1990 when 100% of deer examined were infested with *A. americanum* and had serum antibodies to *E. chaffeensis*. The possibility that the immature ticks acquired the ehrlichiae from another mammalian host, such as a small rodent, before it was transmitted to deer cannot be excluded. Experimentally infected dogs have ehrlichemia for at least 26 days and, thus, could also serve as a reservoir host (41). Related ehrlichioses for which the ecology and transmission are known are maintained in a cycle involving a mammalian host and a tick vector (12). For example, *Rhipicephalus sanguineus* ticks acquire *E. canis* infection when feeding on infected dogs as larvae or nymphs (42,43). Although the ticks remain infected as they molt from stage to stage, transovarian transmission does not occur. It seems likely that deer, dogs, or small rodents serve as the reservoir hosts for *E. chaffeensis,* and that *A.americanum* ticks serve as the major vector. Indeed, larval and nymphal *A. americanum* acquire *E. chaffeensis* by feeding on infected deer, maintain the ehrlichiae transstadially, and transmit *E. chaffeensis* while feeding as nymphs and adults on naive deer (44).

Diagnosis and Treatment

The diagnosis of human monocytic ehrlichiosis is usually difficult to establish during the acute stage of the infection, even in severe cases. Ehrlichiosis should be considered in any febrile patient who has been exposed to ticks during the previous 3 weeks, particularly if leukopenia and thrombocytopenia are present.

Most cases have been confirmed by a humoral immune response generating antibodies reactive with *E. chaffeensis* or the surrogate antigen, *E. canis* (17,23). Use of *E. chaffeensis* antigens for IFA serology results in greater diagnostic sensitivity than use of *E. canis* antigens (3). Diagnostic rises in antibody titer usually occur by the third week after onset, and a precipitous decline in antibody occurs in most patients during the following year. A fourfold rise or fall in IFA titer with a peak titer of 64 or greater is considered diagnostic in a clinically compatible case. Only two cases have been documented by ehrlichial isolation (3,4), approximately 30 cases by *E. chaffeensis*-specific PCR

(31,34,45), and even fewer cases by immunohistology or immunocytology (25-28,30,46). Furthermore, antibodies to *E. chaffeensis* did not develop in six PCR-confirmed patients during convalescence, suggesting that serologic testing may be less sensitive than generally assumed (31,45). The sensitivity of PCR seems to be 80% to 87%; the specificity depends critically upon avoiding contamination with ehrlichial DNA. Search for morulae of *E. chaffeensis* in leukocytes is unrewarding. Even an exhaustive search of buffy coat smears seldom yields a diagnostic result.

Retrospective analysis showed that treatment with tetracycline was associated with reduced need for hospitalization of patients and with the shortest median duration of treatment to effect defervescence for hospitalized patients (2 days as compared with 3 days for chloramphenicol and 7 days for all other antibiotics) (23). *E. chaffeensis* is killed in cell culture in the presence of doxycycline or rifampin but is resistant to chloramphenicol, ciprofloxacin, erythromycin, cotrimoxazole gentamicin, and penicillin (47). *E. chaffeensis* can establish persistent infection even after tre atment with tetracycline and chloramphenicol (25). Persistent ehrlichial infection, in some instances even after treatment, is a well-documented aspect of the veterinary ehrlichioses, e.g., canine monocytic ehrlichiosis (*E. canis*) and tick-borne fever (*E. phagocytophila*). The long-term implications of persistent ehrlichiosis in humans are unclear but might include subsequent reactivation of infection or altered host defenses.

Pathology, Pathogenesis, and Immunity

E. chaffeensis is introduced into the dermis by the bite of an infected tick and spreads hematogenously throughout the body. Intracellular infection is established within phagosomes, most often in macrophages in the spleen, liver, lymph nodes, bone marrow, lung, kidney, and cerebrospinal fluid (25-28,30,46). Lesions potentially attributable to ehrlichial infection include focal necroses of the liver, spleen, and lymph nodes; multiorgan perivascular lymphohistocytic infiltrates; hemophagocytosis in the spleen, liver, lymph nodes, and bone marrow; interstitial pneumonitis; and pulmonary hemorrhage. In bone marrow specimens from 12 patients, the most important findings related to the hematopoietic response were myeloid hyperplasia (8 cases), myeloid hypoplasia (1 case), pancellular hypoplasia (1 case), and megakaryocytosis (7 cases) (46). The most striking discovery was the frequent occurrence of granulomas (in 8 cases) and marrow histiocytosis (in 1 case) as manifestations of the reaction of macrophages to this organism.

The pathogenic mechanisms of ehrlichial disease are poorly understood. *E. chaffeensis* directly causes necrosis of heavily infected cells in vitro and in immunocompromised patients (7,26); however; the role of host immune and inflammatory responses as disease mechanisms has yet to be determined. Observations of opportunistic fungal and viral infections in severe and fatal cases suggest the possibility of an ehrlichial role in the suppression or dysregulation of the immune response (6,25).

It is quite likely that *E. chaffeensis* is controlled by a combination of cell-mediated and humoral immune mechanisms. Interferon-activated human monocytes kill *E. chaffeensis* in vitro. The ehrlichicidal activity is reversed by holotransferrin, suggesting that *E. chaffeensis* is inhibited by intracellular iron depletion (48). The closely related *E. canis* organisms, which grow in canine macrophages in the presence of normal canine serum and cause macrophage necrosis, are killed by canine macrophages when the ehrlichiae are opsonized by immune serum (49,50). Among patients who are treated successfully with an anti-ehrlichial drug, the disease-associated lymphocytopenia is corrected and within 2 to 3 days lymphocytosis develops (51). The predominant (range, 41% to 97%) lymphocyte population comprises γ/δ T lymphocytes, cells that usually constitute only 3% to 8% of peripheral lymphocytes at the institution where the patients were studied. The function of these cells in this setting is unclear as are the consequences of the low α/β T-lymphocyte concentration.

Emergence of Human Granulocytic Ehrlichiosis

Human granulocytic ehrlichiosis was recognized originally in Duluth, Minnesota, by Johan Bakken as a clinical syndrome of a potentially fatal febrile illness in which the patient's neutrophils contained cytoplasmic inclusions (7). Upon reading an update on human ehrlichioses at the time of the index case, which had a dramatic fatal course, Bakken hypothesized that the infection might have been ehrlichiosis. Collaboration with the authors, who were working together at the

University of Texas Medical Branch at Galveston, resulted in the evaluation of a series of patients who were suspected by Bakken to have the disease. Cytoplasmic inclusions were observed in neutrophils, which differed from the findings in E. chaffeensis infections, in which the monocyte/macrophage is the principal target cell and the detection of circulating leukocytes with inclusions is a rare event. Moreover, IFA tests for antibodies to *E. chaffeensis* performed in the Texas laboratory and subsequently in Dumler's laboratory at the University of Maryland were uniformly negative (4).

In retrospect, June 18, 1992, was a significant day in the history of human granulocytic ehrlichiosis. It was Dumler's last day as a fellow in Galveston before moving to Baltimore to become an assistant professor and establish his own independent ehrlichial research laboratory, and it was Sheng-min Chen's first day as a fellow. Momentously, the blood of a 78-year-old Wisconsin man was collected and sent to Galveston by Bakken on the same day. Culture for ehrlichiae and acute- and convalescent-phase serologic assays for *E. chaffeensis, E. canis, E. sennetsu, and E. risticii* were all negative. PCR amplification of the 16S rDNA, the approach developed by Wilson (52) and applied by Relman and co-workers to identify the etiologic agent of bacillary angiomatosis (53), was performed successfully on the specimen by Chen 3 months later (2). Sequencing the gene was not a high priority compared with pursuit of the research aims of funded projects during the season when the ticks were expected to be less active. By April of 1993, DNA sequencing of half the 16S rDNA had been accomplished, and it was recognized that the organism was most closely related to *E. phagocytophila* and *E. equi*, closely related to *E. platys*, less closely related to *E. chaffeensis*, and distantly related to *E. sennetsu*. Completion and repeated confirmation of sequencing of both sense and antisense strands enabled genogroup-specific primers to be designed for nested PCR in July 1993.

What had progressed as a collaborative project became the major project in Dumler's laboratory as Chen and Walker refocused their efforts on funded *E. chaffeensis* research. The initial joint publications with Bakken documented 12 cases diagnosed by specific PCR and the identification of morulae in circulating neutrophils (7). Two patients had died. Necropsy performed on one patient showed ultrastructurally and immunohistologically identified ehrlichiae in neutrophil phagosomes in the spleen. Convalescent-phase IFA serology with surrogate antigens harvested from the blood of an *E. equi*-infected horse and *E. phagocytophila*-infected sheep demonstrated antibodies in 9 of the 10 survivors (7). The illness was characterized by chills, fever, myalgias, and headache; some patients also had nausea, confusion, cough, and arthralgias. Laboratory data included leukopenia in 50%, neutropenia in 17%, lymphopenia in 17%, anemia in 50%, thrombocytopenia in 92%, and elevated aspartate aminotransferase in 91%. Patients were predominantly older men (mean age, 68 years). Clinical history showed strong association with tick bite preceding the onset of illness.

Current Status of Human Granulocytic Ehrlichia and Human Granulocytic Ehrlichiosis

Microbiology of Human Granulocytic Ehrlichia and *E. equi*

Evidence is accumulating to support the premise that a single *Ehrlichia* species is the etiologic agent of a granulocytotropic ehrlichiosis of humans, horses, and dogs. The DNA sequences of the 16S rDNA from the peripheral blood of naturally infected horses and dogs in Sweden, dogs in Minnesota and Wisconsin, and horses in Connecticut are identical with the HGE and differ slightly from the published 16S rDNA sequence for *E. equi* (2,54,55, and J. E. Madigan, J. E. Barlough, J. S. Dumler, N. S. Schankman, E. DeRock, unpublished observations). Moreover, when infected human blood from HGE patients is injected into horses, HGE develops, can be serially transmitted to other horses, and induces protection against subsequent *E. equi* challenge (56). Also, naturally occurring canine *E. equi* infections have been transmitted to horses that had developed equine granulocytic ehrlichiosis, and *E. equi* has been transmitted experimentally by injecting equine blood into susceptible dogs (57,58). Reproducible, continuous in vitro cultivation of all species of granulocytotropic ehrlichiae, including *E. ewingii* and *E. equi*, is an achievable goal.

E. equi antigens harvested from the blood of horses with high levels of parasitemia have been examined for reactivity with convalescent-phase human (HGE), equine (*E. equi*), canine (*E. equi)*

and bovine (*E. phagocytophila*) sera by Western immunoblotting (59). The antigens judged most specific for *E. equi* were the 100-, 44-, 42-, and 25 kDa-bands. The most specific antigen, the 44-kDa band, was strongly reactive with the convalescent-phase human, equine, canine, and bovine sera, suggesting a specific antigenic relationship among these organisms from different animals and geographic origins. On the other hand, biologic studies of *E. phagocytophila* in Finland have shown apparent strain differences in virulence and lack of immunity to heterologous challenge (60). The molecular basis for these differences and the actual taxonomic relationships of HGE, *E. equi*, and *E. phagocytophila* remain to be determined.

Clinical Manifestations

A study of 41 cases of laboratory-confirmed human granulocytic ehrlichiosis from Minnesota and Wisconsin showed male predominance (78%), a median age of 59 years (range 6 to 91 years), median incubation period of 8 days, median period of fever and other symptoms before initiation of effective 5-day treatment, and year-round occurrence with a peak in June and July (61). Clinical manifestations included fever (100%), chills (98%), malaise (98%), myalgias (98%), headaches (85%), nausea (39%), vomiting (34%), cough (29%), confusion (17%), and rarely rash (2%). Four patients had pulmonary infiltrates visible on roentgenograms. The severity of illness is reflected in the rates of hospitalization (56%), admission to an intensive care unit (7%), and death (5%). The course of illness in patients who were not treated and yet survived included a 10-day febrile course in a child and 3- to 11-week remittent febrile course in adults (61).

Diagnosis and Treatment

A clinical diagnosis of human granulocytic ehrlichiosis should be considered in patients exposed to an *Ixodes scapularis* (*dammini*), *I. pacificus* or *I. ricinus* tick-infested environment who have a flulike febrile illness. Careful examination of a peripheral blood smear may show neutrophils that contain cytoplasmic vacuoles filled with ehrlichiae. Two different sets of PCR primers based upon the 16S rDNA sequence of HGE have been designed to amplify, detect, and identify HGE in the patient's blood during the acute stage of illness (2,62). Continued improvements in the technology are expected to yield highly sensitive and specific results in any well-managed clinical molecular diagnostics laboratory. Serologic diagnosis by IFA employing *E. equi*-infected neutrophils harvested from the blood of infected horses detects antibodies at a diagnostic titer of 80 or greater in the convalescent-phase sera of 100% of patients, but antibodies are usually not present in sera collected early in the illness.

Among 34 patients treated with doxycycline, 97% defervesced within 2 days. One patient who had not been treated with doxycycline had *E. equi* detectable by PCR in the blood 28 days after onset of symptoms.

Pathology, Pathogenesis, and Immunity

Following presumed injection of HGE into the patient's skin by the bite of *Ixodes* ssp. ticks, virtually none of the subsequent events are known. It is suspected that HGE infects a myeloid precursor in the bone marrow rather than mature neutrophils. Bone marrow examinations demonstrated hypercellularity in two patients and normocellular marrow in another patient. Autopsies of three patients who died of HGE showed opportunistic fungal pneumonia caused by a different agent (*Aspergillus fumigatus, Cryptococcus neoformans,* and *Candida albicans*) in each patient, suggesting altered host defenses (7,63). One of the patients who died had severe herpes esophagitis. Co-infection with HGE and *B. burgdorferi* is suspected to result in more severe disease. Examples from veterinary research indicating that *E. phagocytophila* and *E. equi* suppress the host defenses include opportunistic viral and fungal infections, decreased neutrophil adherence, emigration, phagocytosis, and bacterial killing, decreased production of antibodies, and decreased lymphocyte mitogenesis (64,65). The mechanisms by which ehrlichiae impair the host phagocytic and immune responses are not known.

Epidemiology and Ecology

Human granulocytic ehrlichiosis has been diagnosed in patients in Minnesota, Wisconsin, Massachusetts, Connecticut, New York, Rhode Island, Pennsylvania, Maryland, Florida, Arkansas, and California (7,63,66,67). Serologic evidence suggests that HGE or an antigenically related organism has also infected patients with Lyme borreliosis in Switzerland (68). An organism apparently identical to HGE is present in Sweden

(54). There is compelling evidence that *I. pacificus*, the vector of Lyme borreliosis in northern California, transmits *E. equi* to horses (69). Ten percent of *I. scapularis (dammini)* ticks collected from vegetation in northwestern Wisconsin in 1982 and 1991 were infected with HGE, including two specimens containing both HGE and *Borrelia burgdorferi*. An engorged *I. scapularis (dammini)* tick was removed from a patient with human granulocytic ehrlichiosis in the same geographic area. PCR showed that the tick's salivary glands contained DNA of HGE (62). Similarly, PCR-amplified HGE DNA was detected in 50% of *I. scapularis (dammini)* ticks collected in Connecticut; no *E. chaffeensis* was detected in these ticks (70). Although the demonstration by PCR that blood from a high proportion of deer in a study in Wisconsin contained HGE suggests that deer might be an important reservoir, the possibility of a rodent reservoir should also be investigated. Indeed, the illustrations in a 1938 Tyzzer article suggest that *Microtus pennsylvanicus* and *Peromyscus leucopus* are naturally infected with a granulocytotropic ehrlichia (71).

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Dr. Walker, professor and chair, Department of Pathology, and director, Center for Tropical Diseases, University of Texas Medical Branch at Galveston, has focused his biomedical research on rickettsial diseases during the last 22 years. Particular efforts have been expended in his laboratory since 1988 on the study of the agents and diseases of human ehrlichial infections. His efforts have contributed to the characterization of R. japonica, E. chaffeensis, *and* R. felis *as agents of emerging infectious diseases. The agent causing human granulocytic ehrlichiosis was first identified in his laboratory in Texas.*

Dr. Dumler, assistant professor of pathology at the Johns Hopkins Medical Institutions, is the key scientist and physician in the detection, identification, and characterization of human granulocytic ehrlichiosis and its etiologic agent. A major contributor to the understanding of E. chaffeensis, *the human* E. equi- *like agent, and the diseases that they cause, Dr. Dumler has authored or coauthored 25 articles on these topics.*

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Surveillance for Pneumonic Plague in the United States During an International Emergency: A Model for Control of Imported Emerging Diseases

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In September 1994, in response to a reported epidemic of plague in India, the Centers for Disease Control and Prevention (CDC) enhanced surveillance in the United States for imported pneumonic plague. Plague information materials were rapidly developed and distributed to U.S. public health officials by electronic mail, facsimile, and expedited publication. Information was also provided to medical practitioners and the public by recorded telephone messages and facsimile transmission. Existing quarantine protocols were modified to effect active surveillance for imported plague cases at U.S. airports. Private physicians and state and local health departments were relied on in a passive surveillance system to identify travelers with suspected plague not detected at airports. From September 27 to October 27, the surveillance system identified 13 persons with suspected plague; no case was confirmed. This coordinated response to an international health emergency may serve as a model for detecting other emerging diseases and preventing their importation.

In the past 50 years, the speed of international travel, as well as the number of travelers, has accelerated, providing a mechanism for the rapid dissemination of disease agents from one country to another. For this reason, vigilant surveillance is needed to prevent the importation and spread of emergent infections. The United States needs a response plan that involves international and domestic public health officials, physicians and hospitals, and the public and can be implemented at the first indication of an international health threat.

In 1994, in response to an epidemic of pneumonic plague in India, the Centers for Disease Control and Prevention (CDC) developed and implemented an enhanced surveillance system to supplement the existing regulations concerning imported plague. The protocol described here may serve as a model for detection and control of emerging diseases imported into the United States or other countries with frequent and diverse international traffic.

Background

In September 1994, India reported cases of plague for the first time in 28 years. Plague is caused by infection with the bacterium *Yersinia pestis* . Bubonic plague is typically acquired by the bite of fleas from infected rodents and is characterized by inguinal, axillary, and/or cervical lymphadenitis. Pneumonic plague may occur as a secondary development to the bubonic form or can be contracted by inhaling respiratory droplets from humans or other animals with plague pneumonia (1).

Bubonic plague cases were first identified by Indian health officials in the Beed District of Maharashtra State in late August. By September 24, more than 300 unconfirmed cases of pneumonic plague and 36 deaths had been reported from the city of Surat, Gujurat State, approximately 300 km west of the Beed District (2). After these reports, hundreds of thousands of Surat's two million residents fled, some to the major cities of Bombay, Calcutta, and New Delhi (3). Unconfirmed pneumonic plague cases and plague-related deaths were subsequently reported from several areas throughout India (4,5).

With the reported epidemic of plague in India, the potential for spread of the disease by infected travelers became a concern. Several countries closed their borders to Indian travelers and cargo and discontinued all flights of their air carriers to and from India (6). Because of its epidemic potential, plague is listed as a Class 1 internationally quarantinable disease in the International Health Regulations of the World Health Organization

(WHO) (7). These regulations authorize the detention and inspection of any vehicle or passenger originating in an area where a plague epidemic is in progress.

Response to the Epidemic

CDC's domestic response to the apparent plague epidemic in India involved two simultaneous and complementary components: 1) information dissemination and education, and 2) intensified active and passive surveillance to identify and treat suspected plague patients and their contacts.

Information Dissemination

After the initial reports from India, information on plague and the epidemic in India was urgently sought by the media, the public, medical practitioners, and public health officials throughout the United States. To meet this need, CDC circulated detailed and timely information to persons concerned with the potential plague crisis. From September 26 to 29, CDC produced six documents for distribution to public health officials and agencies: 1) a general plague outbreak notice, 2) a plague alert notice for international travelers from India, 3) a plague advisory for persons traveling to India, 4) plague treatment and prophylaxis guidelines for physicians, 5) guidelines for diagnosis and biosafety for persons handling samples from patients with suspected plague, and 6) an article on the Indian outbreak that appeared in CDC's widely circulated *Morbidity and Mortality Weekly Report (MMWR)* (8).

CDC pursued several avenues to convey information to medical practitioners and the public. Three articles on the epidemic were published in *MMWR* (September 30, October 7, and October 21) (8-10). Information on plague in general and the Indian epidemic in particular was made available on CDC's Voice Information Service, Fax Information Service, and a special plague hotline telephone number. A message intended for travelers to India concerning the perceived risks and appropriate prophylactic measures was added to the plague selections on the Voice Information Service menu. Finally, all airline passengers disembarking in the United States from India were given a plague alert notice that described the symptoms of plague and advised them to seek medical attention and notify state and federal public health authorities if they had any febrile illness within the next 7 days. The standard Health Alert Notice (yellow card) of the Division of Quarantine, CDC, was made available to all other international arriving passengers and advised them of appropriate measures in the event of illness.

Surveillance

The second component of CDC's response was to intensify active and passive surveillance for persons entering the United States who potentially had plague. Both the active (Figure 1) and passive (Figure 2) surveillance systems identified not only persons suspected of having plague but also those who might have been exposed to a patient with plague during the contagious period.

Active Surveillance System

CDC's Division of Quarantine maintains staff at major international airports in seven U.S. cities: Honolulu, Hawaii; Seattle, Washington; San Francisco and Los Angeles, California; Chicago, Illinois; Miami, Florida; and New York, New York. At airports where the division does not have staff, officials of the Immigration and Naturalization

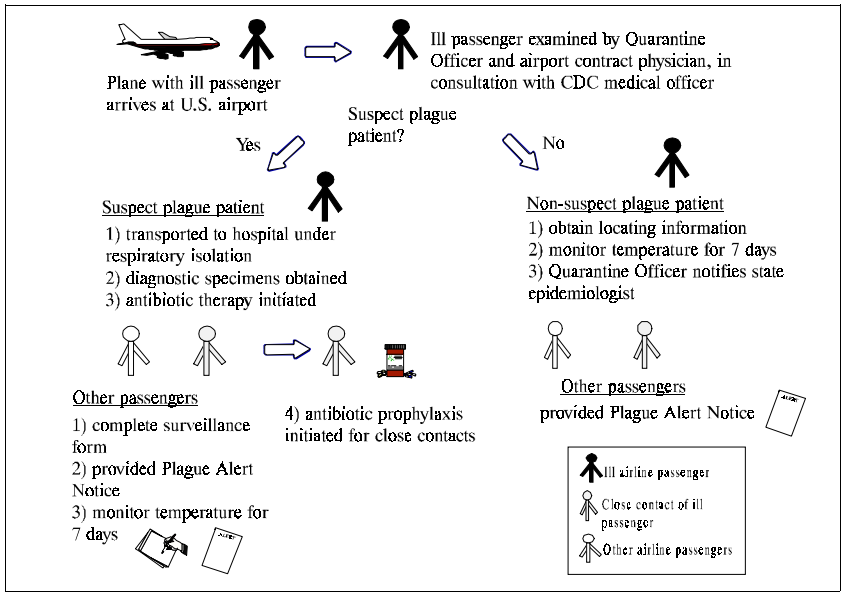

Figure 1. Active surveillance system: patient with suspected plague identified on arrival at U.S. international airport.

Service (INS), Division of Quarantine contract physicians, or both, serve as Quarantine Officers.

During the plague epidemic, crews on all commercial aircraft originating in or continuing from India were reminded of the regulations requiring them to notify the Quarantine Officer at the destination airport of any ill passengers and were instructed to be especially alert for passengers with fever, cough, or chills. When the aircraft landed, before passengers disembarked, a Quarantine Officer and a Division of Quarantine contract physician, in telephone consultation with the medical officer on call at CDC's Division of Vector-Borne Infectious Diseases, examined any passenger who reported illness and determined whether the suspicion of plague was sufficient to warrant the passenger's hospitalization and further evaluation. If deemed not likely to have plague, the passenger was placed under the surveillance of the local health department and released with instructions to consult a physician and to monitor his or her temperature for the next 7 days, the maximal incubation period for pneumonic plague after exposure (1). All other passengers were permitted to deplane and were given a copy of the plague alert notice.

If plague had not beeen ruled out as a possible cause of the passenger's illness, the passenger would have been considered a patient with suspected plague and would have been placed in isolation at the airport until he or she could be safely transported to a predetermined hospital. In the hospital, the patient would have been placed under respiratory isolation conditions, diagnostic specimens would have been obtained for testing in the CDC plague laboratory, and appropriate antibiotic treatment for plague would have been begun.

If the patient had been hospitalized, other passengers on the flight would have been informed that they were under surveillance in accordance with federal quarantine regulations. Locating information would have been obtained from all passengers, who would have been instructed to monitor their body temperature for 7 days and to report any illness to their county or state health department. Because pneumonic plague is transmitted from person to person through respiratory droplets (11) and air flow on passenger airlines is directed toward the floor (12), only passengers seated within 2 m of the patient (proximal passengers) and others with close personal contact would have been considered at reasonable risk for secondary transmission. Those proximal passengers would have been identified and advised to begin antibiotic prophylaxis and to continue it for 7 days. Had a suspected plague case been laboratory-confirmed, the state health departments and state epidemiologists would have contacted all proximal passengers and monitored completion of the antibiotic prophylaxis. All other passengers would have also been contacted to ensure that they continued to monitor themselves for febrile illness.

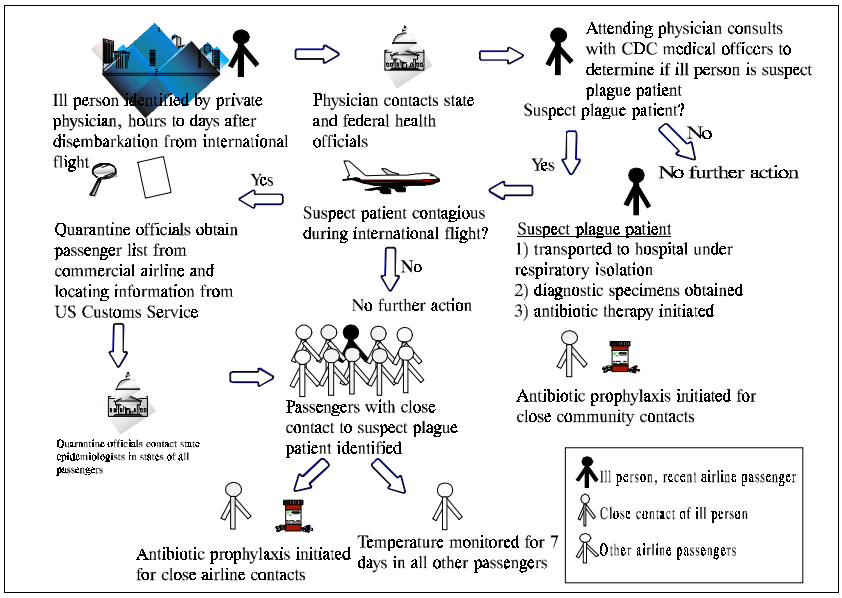

Figure 2. Passive surveillance system: patient with suspected plague identified a few hours to 7 days after arrival in the United States.

Passive Surveillance System

Private physicians, hospitals, and local public health officials were relied on to identify international air travelers from India who became ill within a short period (from hours to 7 days) after disembarkation and report the illness to the appropriate state and federal public health officials. The attending physician, in consultation with the CDC medical officer on call, then determined on the basis of clinical and epidemiologic evidence whether the ill person had a reasonable likelihood of having plague. If so, the patient would

have been placed under respiratory isolation in a hospital, diagnostic specimens would have been obtained, and antibiotic treatment would have been initiated. Close contacts of the suspected plague patient during the putative contagious period would have been identified and advised to begin antibiotic prophylaxis.

A concerted effort would have been made to determine the time the suspected plague patient became symptomatic, and thereby contagious (13), relative to the person's arrival in the United States. If the patient had been symptomatic at the time of the flight, a passenger list would have been obtained from the airline and the U. S. Customs Service. State epidemiologists in the states of residence of all passengers would have been informed of the need to contact and maintain surveillance of passengers within their jurisdiction who were possibly secondarily exposed. If seating assignments for the flight could be obtained, passengers seated within 2 m of the patient would have been advised to begin antibiotic prophylaxis; all other passengers would have been instructed to monitor their temperature for 7 days and to report any illness to state health officials.

Results

On September 29, plague information documents were sent by electronic mail or fax to four Executive Committee members and 50 members of the Council of State and Territorial Epidemiologists, 60 members of the Association of State and Territorial Public Health Laboratory Directors, 40 Executive Board members and 50 state representatives of the National Association of County and City Health Officials, 132 officers in CDC's Epidemic Intelligence Service (EIS), 15 field supervisors of CDC's Field Epidemiology Training Program, and one representative each in the U. S. Department of State and the Quarantine Health Services in Canada. Although an exact count is not available, more than 3,000 persons probably received these documents directly from CDC or secondarily through other agencies.

From September 27 to October 31, the CDC Voice Information Service received 6,665 calls accessing information about plague; 2,692 of these calls were received through the special plague hotline number. During this same period, 5,589 documents about plague were requested and sent by the CDC Fax Information Service.

On October 25, 1994, after an on-site investigation in India, a WHO team of scientists that included four CDC staff members, determined that the plague epidemic was of more limited scope than previously believed, and recommended the lifting of travel restrictions. On October 27, 1994, CDC authorized a stand-down of the heightened surveillance system at all ports of entry and a return to normal operations. During the 30 days that the surveillance system was in place, 13 airline travelers arriving in the United States were evaluated. Six patients with suspected plague were identified and evaluated in airports—JFK and La Guardia in New York City (four), Dallas-Fort Worth (one), Chicago-O'Hare (one)—and seven by private physicians in New York City (five), Albany, New York (one), and St Louis, Missouri (one). All 13 had a history of recent travel in India. None was found to have plague. Symptoms of illness included fever (eight), cough (six), vomiting (four), and malaise (three). The final diagnoses of persons evaluated were viral syndrome (four), malaria (two), concurrent malaria and dengue (one), typhoid (one), end-stage liver failure (one), and no illness (one) (14). The final diagnosis was unspecified in three patients.

Discussion

Plague pandemics have occurred throughout history (15). In the European epidemic known as Black Death, from 1345 to 1360, an estimated quarter of the world's known population of 24 million died. Originating in central Asia and carried by ship to Sicily, the disease spread east to China, south to Africa, north to Russia and Scandinavia, and west to Greenland in only a few years. Plague in North America can be traced historically to infected rats aboard ships from the Far East that docked in California during the early 20th century (16). Today, air travel that can transport a person anywhere in the world within 24 hours expands the opportunity for rapid spread of a transmissible disease like pneumonic plague. The potential for pneumonic plague to spread by air travel to the United States during the recent Indian epidemic elicited considerable public concern (7).

Although rare, plague is enzootic in the United States, and 10 to 15 human cases are reported each year; typically only one or two of these are pneumonic plague cases (17). Thus, most public

health officials and medical practitioners in this country have limited experience with plague (18). When the Indian epidemic began, detailed and reliable information from India was sparse; therefore, CDC disseminated factual and comprehensive information regarding pneumonic plague and the Indian epidemic to public health officials, physicians, and private citizens. The development and distribution of the e-mail, voice, fax, and printed documents were coordinated through a single branch within CDC, which ensured the accuracy and timeliness of the information conveyed. By serving as the central clearing house for international and domestic reports, CDC was able to gather and redistribute information rapidly and efficiently. Updates in *MMWR* contained data obtained within hours of publication. Most public health officials and agencies were accessible immediately by electronic mail or fax, and group mailing codes were constructed to facilitate simultaneous communication. These timely updates of information, which included periodic results of the enhanced surveillance system, heightened awareness of the public health threat and encouraged participation by health practitioners in the passive component of the surveillance system.

Like the information network, the surveillance system was centrally coordinated at CDC, but it relied on the contributions of many agencies and individuals to function effectively. Federal, state, and local health officials, the Immigration and Naturalization Service, the U.S. Customs Service, commercial businesses (passenger airlines), medical practitioners, hospital personnel, and the public played key roles in the successful implementation of the system. Many state and local health departments made additional efforts to alert the medical community to the potential for imported plague cases, to reiterate the surveillance protocol, and to emphasize the importance of obtaining a travel history from any patient with unexplained fever (14). This distribution of responsibility through the established public health network was essential to effective surveillance.

In 1992, the Institute of Medicine's Committee on Emerging Microbial Threats to Health recommended that surveillance of international infectious diseases be implemented and coordinated by a single government agency, ideally CDC (19); subsequently, CDC developed a comprehensive strategy for preventing emerging infectious diseases in the United States (20). In its response to the Indian plague epidemic, rather than constructing a new system specific to this emergency, CDC used a surveillance protocol that built on the existing quarantine framework to utilize trained staff in a position to readily respond. Future responses to the threat of importation of communicable diseases with epidemic potential will require a similar network of individuals and agencies, with specific roles and responsibilities but sufficiently flexible to adapt to the particular epidemiologic circumstances. A system similar to the one described here was put in place in response to the Ebola outbreak in Zaire in April and May 1995 (21).

A surveillance system must be effective without becoming overly burdensome to either those conducting the surveillance or those under surveillance; it must safeguard the public health without inhibiting commerce or interfering with individual freedoms. In the 1370s, during the latter years of Black Death, nautical travelers to the Republic of Ragusa, now part of Italy, were detained for 40 days (from which the word "quarantine" [*quaranti giorni*] derives) (15), a detention period inappropriately long in light of the current knowledge of plague's incubation period of 2 to 7 days (1). In the recent outbreak, closure of airports to all flights from India, compulsory quarantine of all international travelers, and an embargo of trade with India were extreme measures given the epidemiology of plague and the risk of importing a case (18). Primary surveillance efforts were focused at critical control points, i.e., international airports, where personnel resources for identification and control of imported plague cases are maximally efficient. The secondary system, utilizing private physicians and state and local health departments, permitted continued surveillance that was less intensive, but geographically expansive, without placing an unnecessary burden on international air travelers.

If a case of plague had been confirmed in an airline passenger, tracing passengers at risk would have been a substantial undertaking. Depending on the interval between disembarkation and diagnosis, hundreds of persons might have had to be located across the country. In addition to 39 of CDC's Epidemic Intelligence Service (EIS) Officers stationed in state and local health departments, 10 EIS Officers in CDC centers in Atlanta, Georgia, Cincinnati, Ohio, Washington D.C., and Fort Collins, Colorado, were recruited to assist

state and local health departments in tracing contacts if necessary. EIS Officers have often been called to assist in public health crises in which a large complement of epidemiologists was required; in 1993, 13 EIS Officers were among the scientists and public health officials assembled during the outbreak of hantavirus pulmonary syndrome in the southwestern United States (22). Because a rapid response to importation of a disease with epidemic potential often requires a national team of epidemiologists to assist local public health agencies, the Institute of Medicine and others have recommended the expansion and continued support of CDC's EIS program (19,20,23).

The surveillance system's first line of detection for plague cases depended on airline personnel, Immigration and Naturalization Service and U.S. Customs officials for the active component, and private physicians and health care providers for the passive component. Since the former are not trained medical personnel and may not detect an ill traveler in the absence of obvious signs and symptoms, and the latter may not be sufficiently alerted to the possibility of plague, diagnosis of some plague cases could have been delayed and not been efficiently detected by the surveillance system. It is unrealistic to expect any system to effectively screen all travelers returning from areas of recognized disease outbreaks. It is impossible to assess the sensitivity of the described surveillance system since no cases of pneumonic plague were identified either within or outside the system. In retrospect, the risk for an imported plague case was quite small, since the epidemic in India was limited in time and space and had far fewer cases than originally suspected (24). The WHO investigative team found no evidence of transmission in metropolitan areas other than Surat. Most of the patients with suspected plague in Surat came from poor neighborhoods, residents of which would be unlikely to travel internationally. In addition, the short incubation period and severe symptoms of pneumonic plague and the rapid deterioration of the patient's condition, substantially limited the contagious period and the opportunity for secondary transmission.

Although the epidemic potential for plague makes it a good model for developing emerging disease response capabilities, the direct applicability of this program for other emerging diseases may not be straightforward. The above protocol was developed in response to a regionally limited outbreak that occurred during a relatively brief period, similar to the recent Ebola outbreak in Zaire (21). To detect emerging diseases in the absence of a recognized outbreak, surveillance would need to be maintained at some baseline level for an indefinite period. Compliance with the enhanced plague surveillance protocol during the short period it was in effect appears to have been excellent, but how compliance might have waned over weeks to months is unknown. In addition, the protocol was specific to plague, a well-characterized disease with well-described pathogenesis and clinical features. The severe manifestations of pneumonic plague, the short incubation and contagion periods, and the availability of reliable diagnostic tests allowed for a focused protocol that could confidently identify cases. Other emerging diseases may be less well characterized, or even entirely unknown, and may require surveillance protocols of lesser specificity. Nevertheless, the plague surveillance system was broad enough (and consistent with the Institute of Medicine's recommendation that a global infectious disease surveillance system implement broad reporting criteria for detection of emerging diseases [19]) to identify four persons who had other potentially fatal notifiable infectious diseases.

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Changing Patterns of Autochthonous Malaria Transmission in the United States: A Review of Recent Outbreaks

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Three recent outbreaks of locally acquired malaria in densely populated areas of the United States demonstrate the continued risk for mosquitoborne transmission of this disease. Increased global travel, immigration, and the presence of competent anopheline vectors throughout the continental United States contribute to the ongoing threat of malaria transmission. The likelihood of mosquitoborne transmission in the United States is dependent on the interactions between the human host, anopheline vector, malaria parasite, and environmental conditions. Recent changes in the epidemiology of locally acquired malaria and possible factors contributing to these changes are discussed.

Malaria was endemic throughout much of the United States in the late 19th and early 20th centuries (1). Interrupted human-vector contact, decreased anopheline populations, and effective treatment contributed to a decline in transmission and to subsequent eradication. However, environmental changes, the spread of drug resistance, and increased air travel (2) could lead to the reemergence of malaria as a serious public health problem. The potential for the reintroduction of malaria into the United States has been demonstrated by recent outbreaks of mosquitoborne transmission in densely populated areas of New Jersey, New York, and Texas (3-5). A review of the malaria life cycle and recent outbreaks illustrates key elements that affect the risk for malaria transmission in the United States.

Life cycle and Entomologic Principles: Requirements for Transmission

The malaria parasites are protozoa of the genus *Plasmodium*. The four species of *Plasmodium* that cause human malaria, *P. falciparum, P. vivax, P. ovale, and P. malariae*, are transmitted by the bite of infective female mosquitoes of the genus *Anopheles*. The immature stages of the vector's life cycle (egg, larva, and pupa) are aquatic and develop in breeding sites, whereas the aerial adult stage is terrestrial. Anopheline species capable of transmitting malaria are found in all 48 states of the contiguous United States (1). The most important vectors are *An. quadrimaculatus* and *An. freeborni*, found east and west of the Rocky Mountains, respectively. However, other anopheline species have been implicated in local transmission, for example, *An. hermsi* in California (6).

Humans are the intermediate host and reservoir of the parasite, and the mosquito is the definitive host and vector. Female anophelines become infected only if they take a blood meal from a person whose blood contains mature male and female stages (gametocytes) of the parasite. A complex cycle of development and multiplication then begins with union of the male and female stages in the stomach of the vector and ends with parasites, called sporozoites, in its salivary glands, which are infective to humans (Figure 1). The time required for the complete maturation of the parasite (sporogonic cycle) in the mosquito varies and depends on the *Plasmodium* species and external temperature. At 27 ^{o}C, approximately 8 to 13 days are needed for the completion of this cycle for *P. vivax* and *P. falciparum* (7). At lower temperatures, the time for the sporogonic cycle is considerably longer: approximately 20 days at 20 ^{o}C and 30 days at 18 ^{o}C for *P. vivax*. Similarly, for *P. falciparum*, the sporogonic cycle takes 30 days at 20 ^{o}C. At a temperature below 16 ^{o}C or 18 ^{o}C, for these two species, respectively, the cycle cannot be completed and transmission cannot occur. On the other hand, 33 ^{o}C is the upper limit for completion of the sporogonic cycle.

Only anophelines surviving longer than the sporogonic cycle can transmit malaria, assuming they took an infective blood meal. Extrinsic factors that affect the lifespan of the female anopheline,

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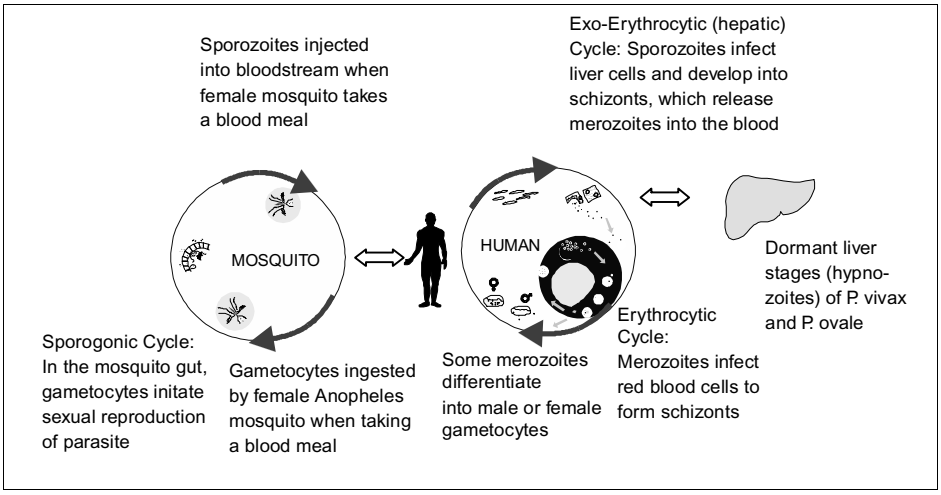

Figure 1. The malaria transmission life cycle.

and thus the completion of the sporogonic cycle, include ambient temperature, humidity, and rainfall. The efficiency and potential for transmission have been mathematically correlated to the survival of the mosquito population. Methods to determine the age range of mosquito populations are imprecise. Thus, determining the proportion of anophelines that have lived long enough to complete the sporogonic cycle is difficult.

Anophelines feed at night; therefore, transmission occurs primarily between dusk and dawn. When an infected mosquito takes a blood meal, it injects sporozoites from its salivary glands into the bloodstream (Figure 1). The sporozoites infect hepatocytes and begin a process of development and multiplication. The life cycle is completed when an anopheline takes a blood meal and ingests male and female gametocytes, allowing for sexual reproduction.

P. vivax gametocytes develop within the first few days of infection, and so a person may be infective early in the course of the illness. In contrast, *P. falciparum* gametocytes do not appear for a minimum of 10 to 14 days, by which time many people would have been symptomatic and received treatment. In addition, both *P. vivax* and *P. ovale* may form dormant liver stages, called hypnozoites, which may become active and cause a relapse of the infection and gametocytemia months to years after a person has left a malaria-endemic area. Hypnozoites are only formed at the time of the initial sporozoite inoculation.

This review of the malaria life cycle identifies the three factors essential for malaria transmission: adequate breeding sites and sufficient abundance of anophelines, weather conditions that allow completion of the sporogonic cycle, and gametocytemic persons. Historically, adequate housing, water management, and mosquito control activities acted to limit anopheline populations and prevented anopheline-human contact. In addition, conditions that promote mosquito survival and parasite development are not usually sustained; hence, the balance of these factors does not favor transmission. However, recent outbreaks demonstrate how tenuous the balance among these factors is. Changes that effect human-vector contact and increased density of gametocytemic persons during optimal weather conditions may be all that is necessary for transmission.

Malaria Surveillance

Historical Background

It is believed that malaria was introduced into the continental United States by European colonists (*P. vivax* and *P. malariae*) and African slaves (*P. falciparum*) in the 16th and 17th centuries. It became endemic in many areas of the country, paralleling the migration of people, with the exception of northern New England and mountainous and desert areas (Figure 2). The incidence of malaria probably peaked in approximately 1875, and it is estimated that more than 600,000 cases occurred in 1914 (1). Systematic reporting of malaria cases began in 1933; in 1934 125,556 cases were reported. The decline in transmission before the introduction of extensive mosquito control measures was attributed to a population shift from rural to urban areas, climatic conditions, increased drainage, improved housing and nutrition, better socioeconomic conditions and standards of living, greater access to medical services, and the availability of quinine for treatment (1). Additional activities, conducted in the 1940s, that led to the interruption of malaria transmission included larviciding, screening of houses, house

spraying (residual spray program with DDT), and use of DDT (for residual spray and larviciding), which removed breeding sites, decreased the density of anophelines, and interrupted anopheline-human contact. Improved surveillance allowed treatment of parasitemic persons, focused control activities geographically, and allowed accurate assessment of the problem.

Surveillance was conducted by CDC to evaluate the progress toward malaria eradication, and in the 1950s it was concluded that this goal had been achieved. At that time, it was recognized that because of international travel, presence of competent anopheline vectors, and environmental conditions that could favor transmission, malaria could be reintroduced into the United States. Surveillance activities have been maintained not only to identify outbreaks of local malaria transmission, but also to identify other cases acquired in the United States (for example, transfusion-induced cases) and to monitor trends in imported cases that guide CDC prevention recommendations.

Since 1957, nearly all cases of malaria diagnosed in the United States have been imported, i.e., have been acquired by mosquito transmission (autochthonous) in areas where malaria is known to occur (8). In general, approximately half the cases occur among U.S. civilians and half among foreign-born civilians. However, each year cases occur that are acquired congenitally or are induced, i.e., acquired through artificial means, such as blood transfusions. Rarely, cases occur that are classified as cryptic (an isolated case of malaria determined after an epidemiologic investigation not to be associated with secondary cases) or introduced (a case documented to be acquired by mosquito transmission from an imported case in an area where malaria does not normally occur) (8). In practice, the distinction between a cryptic and an introduced case may be difficult to ascertain. Frequently, epidemiologic investigations indicate that the infection must have been acquired in the United States and circumstantial evidence suggests it was mosquitoborne. Additional evidence to document mosquitoborne transmission in the United States, such as the presence of anopheline larvae or infective adults, or confirmation of secondary transmission is rarely obtained.Therefore, all locally acquired cases thought to be mosquitoborne will be included in the following discussion, regardless of whether the final classification was cryptic or introduced.

Overview of Locally Acquired Cases

From 1957, when the current surveillance system began, through 1994, 76 cases of introduced and cryptic malaria were reported (9-27). Single cases in Louisiana in 1983 and in Massachusetts in 1985 involved patients who had recently received blood transfusions (28, 29). The infections were likely induced by transfusion, although they were classified as cryptic because serologic testing of available donors did not implicate a source person. Apart from these two, 74 cases were reported from 21 states, including three northern states (Oregon, north-central New York, and New Hampshire), that were probably acquired by mosquitoborne transmission in the United States (Figure 3). The most common species identified was *P. vivax* , which accounted for 59 (80%) cases; *P. malariae* accounted for six (8%) cases, and *P. falciparum* for five (7%); the species was not identified for the remaining four (5%) cases. In 1992, *P. vivax* , *P. falciparum* , *P. malariae* , and *P. ovale* were identified in 51%, 33%, 4%, and 3% of

Figure 2. Areas of the United States where malaria was thought to be endemic in 1882 and 1912.

reported cases, respectively (30). The species was not identified in the remaining 9% of cases. The high proportion of locally acquired cases caused by *P. vivax* is not surprising for several reasons: vivax malaria is diagnosed most often among reported cases; the appearance of gametocytes early in the course of infection may allow for transmission to mosquitoes before treatment is received; relapse may occur months to years after leaving a malaria-endemic area when hypnozoites are reactivated; and the temperatures required for the completion of the sporogonic cycle are found in the United States.

The 74 cases represent 56 distinct episodes of probable transmission; 43 episodes involved one person without risk factors for malaria, nine involved two persons without risk factors, and four involved three or more persons. Before 1991, among cases with sufficient information, 41 (89%) of 46 outbreaks occurred in locations described as rural. Only three were in areas described as suburban, and two were in army barracks. Since then, the three episodes in New Jersey (1991), New York (1993), and Texas (1994) have all occurred in densely populated suburban or urban areas.

California

From 1980 through 1990, 13 outbreaks of presumed mosquitoborne transmission were reported from California. Most occurred in rural areas where medical services were limited and sanitary facilities and housing were often substandard, allowing for anopheline-human contact; they involved undocumented migrant workers from malaria-endemic areas who were implicated as the gametocytemic source. During an outbreak in Carlsbad, California, in 1986 (6), 28 cases (26 Mexican migrant workers and two Carlsbad residents) of *P. vivax* were documented during a 3-month period. The epidemic curve indicated secondary transmission, thus confirming mosquitoborne transmission. The principal risk factor for malaria was sleeping on a particular hillside outdoors during the evening. Adult female anophelines (*An. hermsi*) were captured from a marsh area below the hillside, and temperature and humidity were favorable for completion of the sporogonic cycle.

New Jersey

In 1991, two separate episodes of locally acquired *P. vivax* malaria were identified, occurring more than 70 miles apart (3); the first was consistent with the expected epidemiologic pattern, but the second occurred in a suburban and densely populated area. The index case-patient was an 8-year-old boy, without risk factors for malaria, (travel or exposure to blood or blood products). Few undocumented agriculture workers were living in this suburban area, but a large number of documented immigrants and undocumented factory workers were identified. U.S. census data from 1990 indicated that the population of immigrants from the Indian subcontinent,

Figure 3. Location of presumed mosquitoborne malaria cases reported from 1957-1994. Each point denotes the location of the episode, the species identified (V = *Plasmodium vivax*, F = *P. falciparum*, M = *P. malariae* and *S* = *P. sp* .), and year of occurrence.

where malaria is endemic, increased by 230% compared with census data from 1980. The weather was hotter and more humid than usual, and higher anopheline densities were reported from some regions of New Jersey. The second case-patient had no clear exposure to mosquitoes but may have been exposed during the early evening in a marshy area where he played ball.

New York City

In 1993, another outbreak of locally acquired malaria occurred in New York City (4). The index patient had no travel history or other means of acquiring malaria except local mosquitoborne transmission. The investigation identified two other cases of malaria; one in a person who had traveled internationally 2 years previously, and a third case which was initially unreported. This outbreak was unusual, not only because urban areas are poor habitats for anophelines, but also because the causative parasite was *P. falciparum*. The area where the cases were identified in northwest Queens had many immigrants; the 1990 census showed a 31% increase in the number of foreign-born persons, which accounted for 48% of all recent immigrants into Queens. Many of these immigrants were from malaria-endemic areas, including parts of South and Central America and Hispaniola (Dominican Republic and Haiti). In addition, more than 100 cases of imported malaria were reported in New York City during 1993 (Malaria Section/Division of Parasitic Diseases/CDC unpublished surveillance data). As seen with the earlier outbreaks, the weather that summer was hotter and more humid than usual. During the several weeks between the proposed dates of transmission and the investigation, the weather had changed, interfering with the identification of active anopheline breeding sites or adult anophelines.

Houston, Texas

Three cases of locally acquired malaria were identified in Houston in 1994 (5). This investigation had features similar to those seen in previous outbreaks in California. All three patients were homeless and lived in substandard housing, which provided an opportunity for exposure to anophelines at night. Two of the patients became ill, and malaria was diagnosed in July; the duration of illness was 11 days to 3 weeks. The third patient had symptoms in late July, but a diagnosis of malaria was not made until December when he had a relapse of *P. vivax*, which could only occur from mosquitoborne transmission. Results from an indirect immunofluorescence assay for malaria antibodies conducted on serum specimens obtained in August and December provided additional evidence that his illness during the summer was malaria. The infected persons lived in areas with large immigrant populations. Environmental investigation identified possible breeding sites, and adult female *An. quadrimaculatus* were captured in light traps. In addition, the average temperature and humidity favored mosquito survival and development.

The three outbreaks that occurred in the early 1990s in densely populated areas occurred in neighborhoods with many immigrants from countries with malaria transmission and weather that was hot and humid and, therefore, conducive to the completion of the sporogonic cycle and the survival of adult female anophelines. The delay between mosquito inoculation, diagnosis, and investigation often meant changes in weather and inability to confirm the presence of adult anophelines and active breeding sites.

Discussion

Understanding the factors that contributed to these outbreaks and improving case surveillance will facilitate detection of future outbreaks and development of appropriate prevention and control measures.

Two necessary criteria must be met for malaria transmission: anopheline vectors capable of transmitting malaria and gametocytemic persons. Both exist throughout the United States. Under current conditions, the average lifespan of anophelines in the United States is less than the duration of the sporogonic cycle. A common feature of all recent outbreaks has been weather that is hotter and more humid than usual, which may increase anopheline survival and decrease the duration of the sporogonic cycle enough to allow for the development of infective sporozoites. The possible effect of weather on malaria transmission has been cited in recent articles on the potential consequences of global environmental changes (31-33).

Detection of locally acquired cases depends on accurate diagnosis and reporting of cases. Prompt reporting is not universal as suggested by the Houston investigation (5). Delays in recognizing

cases are caused by not suspecting malaria in a person with a febrile illness who has not traveled internationally, by laboratories inexperienced with blood smear diagnosis, and by general lack of reporting of notifiable diseases. Prompt diagnosis, treatment, and notification are essential for proper treatment and evaluation of potentially gametocytemic persons.

Alternative hypotheses for explaining malaria infection acquired in areas without ongoing transmission have included importation of infective anophelines either on airplanes, ships, or in baggage (34-36). One recent report of two persons who acquired *P. falciparum* in Germany indicates that conditions supporting local mosquitoborne transmission were present in Germany, although the authors concluded that infected mosquitoes must have been imported in baggage (37). Like the United States, many parts of Europe, including regions of Germany, have had endemic malaria transmission and thus are at risk for introduced autochthonous transmission. These alternative hypotheses have been addressed in the U.S. investigations, but none of the episodes occurred close enough to international airports or harbors to support these hypotheses. The possibility of "baggage malaria" is intriguing but unlikely for reasons concerning mosquito survival during transport and expected host-seeking behavior once the mosquitoes are released from luggage.

Gametocytemic persons, both immigrants and native-born U.S. civilians, are present in the United States and can serve as reservoirs of infection. Water management, improved housing, and access to health care are critical for preventing transmission. Diligent malaria surveillance can detect outbreaks early and allow control measures to interrupt transmission.

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Cluster of Lyme Disease Cases at a Summer Camp in Kent County, Maryland

Lyme disease is the second most prevalent emerging infectious disease in the United States; more than 65,000 cases have been reported to the Centers for Disease Control and Prevention since the disease was first described by Steere and colleagues in 1977 (1).

In July 1994, a physician in Chestertown, Maryland, reported eight cases of Lyme disease to the Kent County Health Department. Five were from a summer camp 10 miles north of Rock Hall on the Chesapeake Bay. In one case-patient, a 9-year-old camper from Pennsylvania, erythema migrans (EM) rash and left facial nerve palsy developed the day after she arrived at the camp.

To determine whether Lyme disease was present at the camp, we interviewed the eight counselors who had EM or febrile illnesses during July and 43 of the remaining 91 camp employees. Clusters of cases of Lyme disease with a short and specific exposure period (i.e., 10–12 weeks for the 100 counselors and 2–4 weeks for the 1,600 campers) had not been investigated in recent years.

All 51 surveyed camp employees gave histories of tick exposure throughout the summer. Four counselors had EM of 5 cm in diameter without other symptoms or signs and were treated with amoxicillin by the camp physician. Four other counselors had recurrent fever of 102 °F to 104 °F, severe headaches, somnolence, malaise, fatigue, myalgia, and anorexia. All four described extensive fatigue, drowsiness, and difficulty in getting out of bed. Three described shaking chills, and one had watery diarrhea. The camp physician admitted them all to the camp dispensary; Lyme disease was not diagnosed in any of them; only the patient with diarrhea was given an antimicrobial agent, trimethoprim/sulfamethoxazole. All patients improved in 3 to 5 days.

Sera were obtained in mid-August from the 51 employees; for the eight patients described above, this was 4 to 7 weeks after the onset of illness. All sera were nonreactive in indirect fluorescence antibody (IFA) tests against antigens for *Rickettsia rickettsii* and *Ehrlichia equi* (used to screen for human granulocytic ehrlichiosis). One patient, who had an EM-like rash but no other symptoms, had an IFA titer of 512 for *E. chaffeensis* (used to screen for human monocytic ehrlichiosis). Serologic testing for *Borrelia burgdorferi* by enzyme immunoassay (EIA) (Lyme Stat, BioWhittker, Walkersville, Maryland) identified patients with positive or borderline results (Table 1).

Hard ticks were collected by dragging felt material at several sites within the camp on three occasions during August. Collected adult *Ixodes scapularis* were tested by an antigen capture EIA for outer surface protein A (2). Ten (16.9%) of 59 male ticks were positive for *B. burgdorferi* . Although the infection rate was higher in female ticks collected from the camp, the results cannot be interpreted because the female ticks were co-fed on rabbits; it is not certain whether this could cross-infect ticks feeding on the same animals.

We considered exposure to *B. burgdorferi* in this camp to be high (suspected acute Lyme disease-like illness incidence of 6% to 8%). The incidence rate depends on whether patients 6 and 7, who had flulike illnesses and positive EIAs and negative Western blot results (Marblot Strip Test System, Mardex Diagnostics, Carlsbad, California) are

Table. Results of WB antibody tests for *Borrelia burgdorferi* in summer camp residents with positive (titer ≥ 1.00) and borderline (titer = 0.80–0.99) EIA results

		Serology		
			WB	
Subject	Syndrome	EIA	IgM	IgG
1	EM	1.82	Pos	Pos
2	EM	1.40	Neg	Pos
3	EM	1.21	Neg	Neg
4	EM	3.21	Pos	Pos
5	Flulike	1.00	Neg	Pos
6	Flulike	1.04	Neg	Neg
7	Flulike	1.80	Neg	Neg
8	Flulike	2.46	Neg	Pos
9	None	0.96	Neg	Neg
10	None	0.96	ND	Pos
11	None	1.82	Neg	Pos
12	None	2.21	Neg	Neg
13	Sinusitis	1.11	Pos	Neg
14	Sinusitis	0.93	Neg	Neg
15	None	1.00	Neg	Neg
16	Rocky Mountain spotted fever, 1991	1.14	Neg	Neg

EIA = enzyme immunoassay; EM = erythema migrans; ND = no data; WB = Western blot.

considered to have had Lyme disease, and on assuming that the 49 unexamined counselors did not have Lyme disease. Also, Kent County has one of the highest incidences of Lyme disease in the state (3), many deer were present in the woods and fields in and around the camp, and all counselors reported frequent exposure to ticks.

The four patients who had an acute febrile illness without cutaneous lesions were not initially suspected to have acute Lyme disease. We believe that flulike illness without EM is a more common manifestation of acute Lyme disease than is generally appreciated since, as in patients 5 through 8 (Table), Lyme disease is often not considered in the differential diagnosis (4). Acutely febrile patients, who have been bitten by a tick in Lyme disease-endemic areas also should be considered for early antibiotic therapy. Doxycycline or another tetracycline is effective for Lyme disease as well as for infections with *E. chaffeensis* and *R. rickettsii*, which are also transmitted by ticks and may have a similar clinical syndrome (5). Serologic testing, although it can confirm the diagnosis during the convalescence phase, may not establish an early diagnosis in either case, since antibody responses to all three infections are usually delayed until 2 to 4 weeks after the onset of symptoms and may not occur in patients treated with antibiotics (5-8).

We interpreted the Western blots according to criteria proposed at the Second National Meeting on Serological Diagnosis of Lyme disease (6). Of the four patients with EM and with EIAs positive for *B. burgdorferi*, patient 3, who had antibodies to *E. chaffeensis*, did not have IgG and/or IgM evidence of *B. burgdorferi* infection by Western blot. He also had no symptoms compatible with monocytic ehrlichiosis. Two (patients 6 and 7) of the four with flulike illnesses and with EIAs positive for *B. burgdorferi* did not have *B. burgdorferi* infection confirmed by Western blot. Positive or borderline serologic results for *B. burgdorferi* infection in patients 9 through 16 (Table) who did not have a clinical history compatible with Lyme disease could have been caused by asymptomatic infection, antibody responses from prior infections, cross-reactions from other infections, or false-positive reactions (8). Many of the counselors had been at the camp during previous summers and could have had prior mild, nondiagnosed infections with *B. burgdorferi*. Another possibility is that the EIA titers in some of the patients were high normal values, which may have been the case for patients 9, 14, 15, and 16. Patient 13 who had IgM evidence of recent infection on Western blot may have had a mild infection with *B. burgdorferi* during the previous month. However, this is impossible to confirm without acute-phase and convalescent-phase (or preexposure and postexposure) serum samples. This is also pertinent to those with flulike symptoms and negative Western blot results (patients 6 and 7).

The usefulness of using EIA screening and Western blot confirmation in seroepidemiologic studies for Lyme disease has not been established. The positive predictive value of a diagnostic test is highly dependent on the prevalence of the disease being studied. If the prevalence of Lyme disease in the population screened is very low, the positive predictive value of testing may be too low to be diagnostically useful.

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Unexplained Deaths Due to Possibly Infectious Causes in the United States: Defining the Problem and Designing Surveillance and Laboratory Approaches

Many new infectious diseases have been identified in the United States during the last several decades (1). Among these are AIDS, Legionnaires' disease, toxic-shock syndrome, hepatitis C, and most recently, hantavirus pulmonary syndrome; all caused serious illness and death. In each instance, the disease was recognized through investigation of illness for which no cause had been identified. Retrospective studies of these and other newly recognized infectious diseases often identified cases that occurred before the recognition of the new agent; therefore, a more sensitive detection system may make the earlier recognition of new infectious agents possible.

Delays in recognizing new infectious agents have often been substantial. For instance, *Legionella pneumophila* was established as the cause of Legionnaires' disease in 1976 after an epidemic in Philadelphia, but sporadic cases in 1947 and an outbreak in 1957 were retrospectively identified (2, 3). Similarly, toxic shock syndrome was recognized in late 1979 and early 1980, but retrospective reporting and chart reviews documented cases as early as 1960 (4). HIV was identified in 1983 (5) yet retrospective investigations documented AIDS cases in the late 1970s and possibly as early as 1968 in the United States (6, 7).

The difficulty of identifying unknown etiologic agents is part of the reason for delays between the occurrence and recognition of new infectious diseases. Until recently, to identify new infectious agents we relied primarily on culture techniques. For fastidious bacteria such as *Legionella* sp., and new viruses, such as HIV, which have very specific growth requirements, successful isolation usually required numerous attempts with various culture systems, often extending over years. Advances in molecular techniques, including polymerase chain reaction (PCR) amplification and other DNA- (and RNA-) based techniques (e.g., representational difference analysis), allow identification and classification of unknown etiologic agents without having to culture them (8-10) and provide clues concerning appropriate conditions for subsequent isolation of the agent in culture (11,12).

A more systematic public health approach for the early detection of unknown infectious agents is needed. This need was acknowledged in *Addressing Emerging Infectious Diseases Threats: A Prevention Strategy for the United States*, a CDC publication about emerging infections (13). CDC has established an emerging infections program (EIP) network to conduct special population-based surveillance projects, develop surveillance methods, pilot and evaluate prevention strategies, and conduct other epidemiologic and laboratory studies. In late 1994, CDC funded four programs based at state health departments and academic institutions in California (Alameda, Contra Costa, Kern, and San Francisco counties), Connecticut, Minnesota, and Oregon. Some projects are conducted at all program sites and others, depending on local interest and expertise, at only one or two sites.

Surveillance for unexplained deaths due to possibly infectious causes (UDPIC) for early detection of new infectious diseases is one of the core activities being conducted at all sites. This paper estimates the number of UDPIC at the EIP programs and summarizes the surveillance and laboratory approaches that will be used to identify their cause. This is the first attempt to conduct surveillance for early detection of new infectious diseases in a large U.S. population.

To estimate the number of deaths that might be identified in surveillance for UDPIC, we used multiple cause-of-death data for the United States for 1992 from the National Center for Health Statistics (14). The year 1992 was the most recent for which national data were available at the time of this study. The analyses of death records were restricted to the EIP program populations and age group (1-49 years of age) in which surveillance for UDPIC was planned. Multiple cause-of-death data listed on the National Center for Health Statistics death record allow for analysis of mortality data based on the different causes (15). The International Classification of Diseases, 9th Revision (ICD-9) was used to define UDPIC (16). We selected 77 codes likely to represent UDPIC when listed on the death record (Table 1) (17).

Table 1. Selected codes from International Classification of Diseases, 9th revision (ICD-9) used to identify unexplained deaths due to possibly infectious causes (UDPIC)

007.9	unspecified protozoal intestinal disease
008.5	bacterial enteritis, unspecified
008.8	intestinal infectious due to other organisms: other organism, not classified elsewhere
009.0	infectious colitis, enteritis, and gastroenteritis
009.1	colitis, enteritis, and gastroenteritis of presumed infectious origin
009.2	infectious diarrhea
009.3	diarrhea of presumed infectious origin
027.9	unspecified zoonotic bacterial disease
038.9	unspecified septicemia
041.9	bacterial infection in conditions classified elsewhere and of unspecified site: bacterial infection, unspecified
046.9	unspecified slow virus infection of the central nervous system
047.9	unspecified viral meningitis
049.9	unspecified non-arthropod-borne viral diseases of central nervous system
057.9	viral exanthem, unspecified
079.9	viral infection in conditions classified elsewhere and of unspecified site: unspecified viral and chlamydial infection
099.0	venereal disease, unspecified
136.9	other and unspecified infectious and parasitic diseases: unspecified infectious and parasitic diseases
283.1	non-autoimmune hemolytic anemias
284.8	other specified aplastic anemias
286.6	defibrination syndrome
287.3	primary thrombocytopenia
287.5	thrombocytopenia, unspecified
320.9	meningitis due to unspecified bacterium
322.9	meningitis, unspecified
323.9	unspecified cause of encephalitis
357.0	acute infective polyneuritis
420.9	other and unspecified acute pericarditis
421.0	acute and subacute bacterial endocarditis
421.9	acute endocarditis, unspecified
422.9	other and unspecified myocarditis
424.9	endocarditis, valve unspecified
425.4	other primary cardiomyopathies
425.9	secondary cardiomyopathy, unspecified
446.6	thrombotic microangiopathy
465.0	acute laryngopharyngitis
465.8	acute upper respiratory infections of multiple or unspecified sites: other multiple sites
465.9	acute upper respiratory infections of multiple or unspecified sites: unspecified site
466.0	acute bronchitis
466.1	acute bronchiolitis
480.9	viral pneumonia, unspecified
482.9	bacterial pneumonia, unspecified
485	bronchopneumonia, organism unspecified
486	pneumonia, organism unspecified
511.9	unspecified pleural effusion
518.4	acute edema of lung, unspecified
518.8	other diseases of lung
519.9	unspecified disease of respiratory system
558	other and unspecified noninfectious gastroenteritis and colitis
780.6	pyrexia of unknown origin
782.1	rash and other nonspecific skin eruption
782.7	spontaneous ecchymoses
785.5	shock without mention of trauma
785.6	enlargement of lymph nodes
786.0	dyspnea and respiratory abnormalities
792	nonspecific abnormal findings in other body substances
792.0	cerebrospinal fluid
792.1	stool contents
792.2	semen
792.3	amniotic fluid
792.4	saliva
792.9	other nonspecific abnormal findings in body substances
795	nonspecific abnormal histologic and immunologic findings
795.3	nonspecific positive culture findings
795.4	other nonspecific abnormal histologic findings
795.7	other nonspecific immunologic findings
796.4	other nonspecific abnormal findings: other abnormal clinical findings
798	sudden death, cause unknown
798.1	instantaneous death
798.2	death occurring in less than 24 hours from the onset of symptoms, not otherwise explained
798.9	unattended death
799	other ill-defined and unknown causes of morbidity and mortality
799.0	asphyxia
799.1	respiratory failure
799.3	debility, unspecified
799.4	cachexia
799.8	other ill-defined conditions
799.9	other unknown and unspecified cause

Analyses for UDPIC were restricted to previously healthy persons 1 to 49 years of age by excluding persons outside this age-group and those who had any of the following ICD-9 codes as an underlying cause of death: 140 to 239.9, neoplasms; 250.0 to 250.9, diabetes mellitus; 279.0 to

279.9, disorders involving the immune mechanism; 295.5, other disease of spleen; 800 to 999.9, injury and poisoning; E800 to E998, supplementary classification of external causes of injury and poisoning. Patients with HIV disease listed anywhere on the death record were also excluded (codes 042, 042.0, 042.1, 042.2, 042.9, 043, 043.0, 043.1, 043.2, 043.3, 043.9, 044, 044.0, 044.9, and 795.8) (18).

Deaths meeting the study criteria were identified along with patient age, gender, race (black, white, and other), and autopsy status for the four EIPs (aggregate and by EIP program). To determine rates of UDPIC, we used 1992 census estimates for the four EIP programs (19).

In 1992, 744 UDPIC were identified among previously healthy persons 1 to 49 years of age in the four EIP sites. These deaths accounted for 14% of all deaths (n = 5,304) among persons 1 to 49 years of age in hospitals and emergency rooms. Most of the 744 UDPIC occurred among male patients (60%) and whites (72%) (Table 2). Overall rates among blacks were almost four times as high as those among whites (29.5 vs. 7.7 per 100,000). By site, overall rates ranged from 5.6 (in Minnesota) to 14.5 (in California) per 100,000 population. These geographic differences could be accounted for only in part by differences in the proportions of blacks by site. In Minnesota and Oregon the proportions of blacks were 2.8% and 1.9%, respectively, whereas in California and Connecticut the proportions were 14.7% and 12.4%, respectively.

Figure 1 shows the age-specific rates of UDPIC for persons 1 to 49 years of age. Persons 1 to 24 years of age accounted for only 19% of deaths, while persons 40 to 49 years of age accounted for 50%.

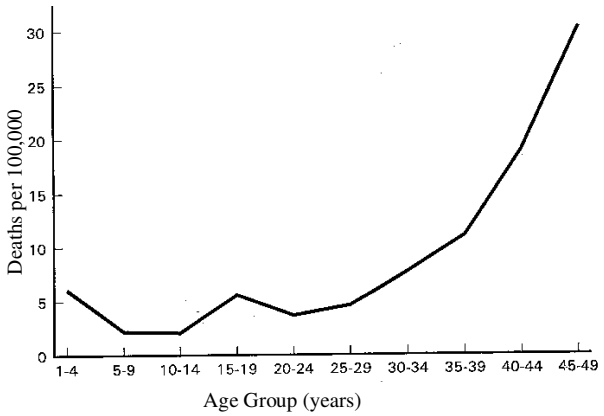

Figure 1. Age-specific rates of unexplained deaths due to possibly infectious causes (UDPIC) among previously healthy persons 1 to 49 years of age in the four emerging infections program sites, 1992.

Of selected ICD-9 codes (Table 1), the six disease classifications (and codes) accounting for the most of the UDPIC are shown in Table 3. A selected ICD-9 code was listed as the underlying cause of death in 253 (34%) of 744 UDPIC. Autopsies were performed in 293 (39%) of the 744 UDPIC.

Two approaches for surveillance were proposed as a basis for the EIP project. In the first, clinicians will be asked to report unexplained deaths and serious illnesses from possibly infectious causes. In the second, death certificate databases will be used to select patients with ICD-9 codes likely to represent UDPIC. The first approach allows prospective collection of data and specimens for deaths and serious illnesses. In the second approach, UDPIC will be identified retrospectively through information on death certificates.

Clinicians in the EIP areas have been asked to report by telephone to EIP program surveillance personnel all previously healthy persons 1 to 49 years of age who are hospitalized (or admitted to

Table 2. Unexplained deaths due to possibly infectious causes (UDPIC) among previously healthy persons by emerging infection program (EIP) site, 1992

		Rate (per 100,000 population aged 1-49 years)					
			Gender		Race		
EIP site	No. of UDPIC	Overall	Female	Male	Black	White	Other
California *	316	14.5	10.8	18.5	34.0	12.2	8.9
Connecticut †	83	14.2	10.5	18.5	37.9	11.4	-
Minnesota	189	5.6	4.8	6.6	11.0	5.4	9.5
Oregon	156	7.2	6.9	7.7	21.8	7.0	7.8
Total	744	8.9	7.4	10.9	29.5	7.7	8.7

*Alameda, Contra Costa, and San Francisco counties.
†New Haven County.

Table 3. Of selected ICD-9 codes, disease classifications accounting for most unexplained deaths due to possibly infectious causes (UDPIC) in the four study sites, 1992

	UDPIC with ICD-9 code included on death record by age group (%)			
Disease classification (ICD-9)*	1–49 yr; n = 744	1–14 yr; n = 75	15–39 yr; n = 295	40–49 yr; n = 374
Respiratory failure (799.1)	205 (28)	14 (19)	91 (31)	100 (27)
Unspecified septicemia (038.9)	108 (14)	8 (11)	42 (14)	58 (16)
Pneumonia, organism unspecified (486)	101 (14)	7 (9)	33 (11)	61 (16)
Other primary cardiomyopathy (425.4)	84 (11)	5 (7)	26 (9)	53 (14)
Shock without mention of trauma (785.5)	83 (11)	10 (13)	29 (10)	44 (12)
Other unknown or unspecified (799.9)	75 (10)	9 (12)	35 (12)	31 (8)
Totals †	505 (68)	39 (52)	193 (65)	273 (73)

*More than one of these disease classifications (ICD-9 code) may be listed on a death record.
†UDPIC with at least one of the six disease classifications included on the death record.

an emergency room) with a life-threatening illness with hallmarks of an infectious disease for which no cause is identified. Inclusion and exclusion criteria are shown below.

Inclusion criteria

1. 1 to 49 years of age
2. Admitted to a hospital or emergency room with life-threatening illness of potentially infectious etiology
3. No cause for illness identified by preliminary testing

Exclusion criteria

1. Preexisting chronic medical condition: malignancy; HIV infection; chronic cardiac, pulmonary, renal, hepatic or rheumatologic disease; or other known underlying chronic illness (e.g., diabetes mellitus)
2. Immunosuppressive therapy
3. Trauma
4. Toxic ingestion or exposure
5. Nosocomial infection

Clinicians and pathologists in the four EIP programs were informed of the surveillance system through a combination of mailings, oral presentations, and posters.

Classifying patients as having one or more infectious disease-related syndrome(s) as listed below should help identify groups of patients with similar illnesses for laboratory testing.

1. Acute abdominal symptoms (e.g, diarrhea, pain, nausea/vomiting) and history of (h/o) fever
2. Arthritis or osteomyelitis and h/o fever
3. Blood cell dyscrasia or coagulopathy and h/o fever
4. Conjunctivitis, keratitis, endophthalmitis, or periocular infection and h/o fever
5. Endocarditis, myocarditis, pericarditis and h/o fever
6. Hepatitis or hepatic insufficiency/failure and h/o fever
7. Meningitis, encephalitis, encephalopathy, dementia, or other neurologic syndrome with or without a h/o of fever
8. Rash, skin or mucosal membrane lesions, cellulitis, myositis, lymphadenitis, or lymphangitis and h/o of fever
9. Renal insufficiency/failure and h/o of fever
10. Respiratory failure, pulmonary infiltrates, or other pleuropulmonary manifestation and h/o of fever
11. Shock or sepsis and h/o of fever or hypothermia
12. Other

Information about exposures (e.g., travel or contact with animals or insects) resulting in infectious diseases will be collected. For patients who are still alive or have died recently, clinical and pathology laboratories will be asked to save clinical specimens (including biopsied tissues) obtained during clinical care and diagnostic evaluation. Range of specimens will vary but be appropriate for the given illness and organ systems affected. These specimens will be collected, divided into aliquots, and stored. Autopsies will be encouraged. With the exception of pathology specimens, specimens will be initially banked at the EIP sites. Fixed or frozen tissue specimens (premortem and postmortem) will be sent directly to CDC for examination. A CDC pathologist will be available to consult with the local pathologist and to discuss preparation and transport of tissues. Pathology results are expected to guide further laboratory testing on specimens.

Clinical and epidemiologic data will be periodically reviewed locally at each EIP and at CDC in aggregate. Each EIP will identify UDPIC not reported through the clinician-based system by using state-based (rather than national) electronic data systems to reduce delays in relaying information. When deaths not reported through the clinician-based system are identified, the medical chart will be reviewed, the patient's illness will be classified by syndrome and information available in the medical record concerning exposures will be collected. Samples of specimens will be obtained at autopsy. Deaths will be handled as in the clinician-based system with regard to periodic review and laboratory testing, although it is expected that fewer clinical specimens will be available from patients whose deaths were not reported through the clinician-based system.

Additional reference level laboratory tests for known pathogens will be done in state health laboratories and CDC. CDC will test for previously unrecognized infectious agents.

Initial identification of unrecognized etiologic agents at CDC will primarily rely on serology, immunohistochemistry, and nucleic acid probes. When a sufficient number of patients with similar illnesses are identified, a customized strategy for laboratory testing will be designed. Serology and immunohistochemistry will be used to narrow the scope of possible etiologies. Nucleic acid probes will be used with PCR to amplify from clinical specimens specific fragments of genetic material that can be sequenced and used for phylogenetic comparisons to known infectious agents. Clinicians who reported cases will be informed of laboratory results, but information will usually not be available in time to affect treatment of individual patients.

Until now, unexplained deaths and serious illnesses due to possibly infectious causes have not been addressed as a specific public health problem. The data obtained in the first phase of this project suggest that UDPIC in previously healthy persons account for 13% of hospitalized deaths among persons 1 to 49 years old in the EIP sites. Experience in recent years with new infectious diseases suggests that systematic study of UDPIC and similarly unexplained serious illnesses may allow earlier detection of emerging infections. This has been made more feasible by newly developed nucleic acid-based methods for identification of unknown etiologic agents.

Use of the 1992 National Center for Health Statistics multiple cause-of-death data to estimate the number of UDPIC has its limitations. The most important is in the selection of ICD-9 codes to identify these deaths. Even with codes such as 038.9 ("unspecified septicemia"), which seem relevant, without reviewing the medical record it is impossible to know if the cause of the septicemia was known by the clinician but not specified or was nosocomial. Codes representing potentially infectious deaths (e.g., 799 for "other ill-defined and unknown causes of morbidity and mortality") might also be assigned to noninfectious deaths. Another critical limitation is failure to identify deaths that are, in fact, unexplained but have been given an incorrect diagnosis.

For several reasons, our surveillance is limited to persons 1 to 49 years of age who have been healthy. The 1-year lower age limit was selected to avoid confusion with congenital problems in infants but include most children in day-care, where infectious diseases are common and a new infectious disease might spread rapidly. The upper age limit was set to exclude an expected increased proportion of unexplained deaths from noninfectious causes in persons 50 years and older. Many of the recently recognized life-threatening infectious diseases would have been detected among previously healthy persons in this age-group. Previously healthy persons might also be considered better sentinels for new infectious diseases because of their generally more vigorous interaction with people and higher likelihood of exposure to infections (e.g., travel or contact with animals or insects). However, restricting surveillance to previously healthy persons is likely to decrease the sensitivity of our system.

Patients who are immunocompromised—whether from HIV infection, malignancy, or immunosuppressive therapy—and many patients with other chronic illnesses, are more susceptible to known and unknown infectious diseases. New infectious diseases first identified in persons who are immunocompromised or have chronic illnesses have subsequently been found to also cause infection in persons with normal immune systems (20,21). Although sensitivity could be improved by including these populations in surveillance, available resources and a concern that laboratory evaluation would be complicated by the broader range of infectious possibilities compelled us to focus on previously healthy persons.

Clinician-based and death certificate–based systems for surveillance and laboratory evaluation are being used in combination because of their complementary strengths and weaknesses. The notable strengths of the clinician-based system are the contribution of clinicians and the timeliness of reporting. Because of their training and their relationship with patients, clinicians can recognize unusual and potentially new infections. This system also offers opportunities to collect and store clinical specimens (pre-mortem and post-mortem) that would not normally be saved, in addition to providing systematic and timely collection of exposure information that might not be available in the medical record. This system might also increase the likelihood of an autopsy. However, reporting is time-consuming and is not likely to affect the patient's care, which may lower the sensitivity of this approach.

The primary strengths of the death certificate–based system are its completeness and relative ease, once the data are electronically available. The completeness may make it sensitive for detection of new infections resulting in death (but assumes that the correct ICD-9 codes are selected and that they are coded accurately). Sensitivity is important because, to be effective, the combined approaches should detect relatively rare illnesses (e.g., in the range of one case per 100,000 to 1,000,000 population per year). The main disadvantages of this system are the vagaries of ICD-9 classification: codes are not designed to identify new infectious diseases and are assigned by persons not directly familiar with the case. The list of ICD-9 codes used to identify UDPIC is likely to be modified on the basis of information collected in this system and in the clinician-based system. Another problem is the delay in getting information on the death certificate into the database for review, which makes this system relatively slow. Further, the only clinical specimens likely to be available for laboratory evaluation are those collected at autopsy.

The goal of our project is early detection of new life-threatening infectious diseases. However, it is likely that in the process, we will identify cases in which known, but poorly recognized, infectious diseases are responsible, either because the diagnostic tests being used clinically are of poor sensitivity or because the diagnosis was unexpected by clinicians. Findings concerning such cases may be useful in identifying areas in which better diagnostic capabilities are needed and in improving estimates of infectious disease prevalence (22). A population-based bank of clinical specimens will be invaluable in current and future testing for newly recognized etiologic agents and for developing diagnostic tests. This project will better clarify surveillance strategies and help standardize nucleic acid-based techniques for identification of previously unknown etiologic agents. Through it, we expect to build U.S. capacity for detecting and responding to newly recognized infectious diseases not only at the EIP sites but elsewhere, nationally and internationally.

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Trends in Bacteremic Infection Due to *Streptococcus pyogenes* (Group A Streptococcus), 1986-1995

During the past 7 years, severe invasive infections caused by *Streptococcus pyogenes* (group A streptococcus [GAS]) have been reported with increasing frequency (1). It is not certain whether these reports reflect actual increase in the incidence of disease caused by this pathogenic bacterium or merely enhanced awareness and interest on the part of the medical community. This difficulty prevails whether the apparent resurgence of life-threatening infection is described within a region (2), a province (3), or in discrete outbreaks (4,5) and is heightened by the fact that streptococcal infection has not been a reportable disease in most locales.

An outbreak of infection may awaken interest in streptococcal disease, leading to publication bias (6,7). Continued study may show that what appears to be a general increase in the incidence of GAS infection may actually represent an outbreak, as has been demonstrated in Colorado (8,9), Sweden (10), and the United Kingdom (11). In a laboratory-based study, Burkert and Watanakunakorn (12) found that the frequency and apparent severity of bacteremic infection due to *S. pyogenes* did not change from 1980 to 1989. A meticulous, population-based study in Pima County, Arizona (13), found no change in the incidence of invasive GAS disease between 1985 and 1990 but did suggest the emergence of streptococcal toxic shock syndrome in the late 1980s, on the basis of the appearance of six such cases in 1987-1990 compared to zero cases in 1985-1987. In contrast, a preliminary, population-based report from Israel (14) found no such increase.

The Houston Veterans Affairs Medical Center (HVAMC) serves about 50,000 eligible veterans, most in Harris County, Texas, and surrounding counties, although a small number of these veterans live elsewhere in Texas or in bordering states. Most patients are middle-aged or elderly men, although approximately 20% are under the age of 40. Within the past decade, there have been broader medical options for some of our patients, especially those who are indigent and/or elderly, and narrower options for others, especially those whose medical insurance was initially marginal. Nevertheless, most persons who select the HVAMC for care of any problem tend to identify themselves with this medical center and look to it to provide complete medical care (15).

In contrast to that reported by some medical centers in the United States, our clinical experience did not suggest that the number or severity of infections due to *S. pyogenes* had increased during the past decade. Because our population was relatively stable and data were available in our Microbiology Laboratory for 1986 through 1995, we studied trends in bacteremic infection at the medical center during this period.

In the 9 years under study, the number of yearly inpatient admissions to the HVAMC decreased by 4.1%, while the number of outpatient visits increased by 33.9%. The number of patients who, in a given year, sought medical attention at the center (determined by social security numbers), increased by 18.7%, with a 5.2% decrease in those persons admitted to the hospital and a 19.4% increase in those who sought outpatient care. During this period, the number of blood specimens submitted for cultures each year remained essentially unchanged. We have noted no change in the clinical findings that prompt interns and residents to request blood cultures, although the possibility of unrecognized, subtle changes cannot be excluded.

The rate of isolation of GAS from blood cultures (Figure 1) or from all sterile sites (data not shown) remained unchanged between 1986 and 1995. The number of positive blood cultures tended to be lower in the summer and early fall and to rise in midwinter, but the average number has remained at approximately five to eight per year during the entire decade. The frequency with which GAS were isolated from all specimens declined significantly ($p = 0.003$) during the decade under observation.

These data suggest that the frequency of bacteremic infection due to *S. pyogenes* has not increased in a population of veterans residing in and around Houston, Texas. The number of isolates of *S. pyogenes* from blood and from all sterile sites has remained unchanged, whereas the number of persons served in inpatient and outpatient encounters actually increased. The slight, but significant decrease in the total number of isolates from all sources might suggest that an

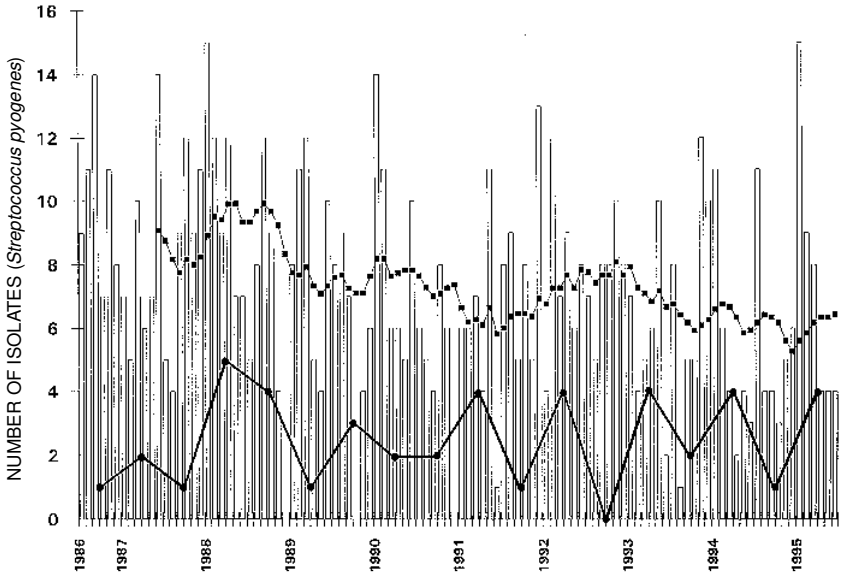

Figure 1. The bars show the number of isolates of *Streptococcus pyogenes* (GAS) each month at the VAMC, Houston. The upper line connecting solid squares indicates the running monthly average (average of the preceding 12 months). The lower line connecting solid circles indicates the number of blood cultures positive for *S. pyogenes* during each 6-month period.

unchanging number of blood isolates represents an increased prevalence of virulent strains, but the large number of variables involved in collecting all specimens makes these data difficult to interpret.

By showing that the incidence of *S. pyogenes* bacteremia has not changed, these results appear to support our anecdotal observation that the incidence of life-threatening GAS infection has not increased. Without reviewing every single case, which is best done prospectively rather than retrospectively, we cannot exclude the possibility that the number of cases associated with streptococcal shock syndrome has increased, as was suggested in Pima County (13). Even that study, however, showed only six cases of shock (two per year) in 1987 through 1990 compared to none in 1985 through 1987; one wonders whether bias in recording data might have been partially responsible, and whether this increase will persist. We also do not know what proportion of our cases is associated with necrotizing fasciitis, although it must be noted that Hoge, et al. (13) specifically did not document the association between this clinical syndrome and streptococcal toxic shock. Our findings cannot exclude the possibility that serious GAS has increased in younger adults, as has been reported by others (1,13), since our population tends to be (but is not exclusively) middle-aged or older. Also, the effect of increases may not have been observed in Houston. In any case, reporting bias is difficult to avoid, and prospective population-based studies must be carried out over an adequate number of years before they can provide valid conclusions.

If the frequency of severe GAS infection has, in fact, increased, a virulent clone or the mutation of a clone that has antigens to which most adults lack antibody could be responsible. Serotype M1 has predominated in reports of streptococcal toxic shock syndrome (16-18). Some investigators (19) have proposed that allelic variation in the streptococcal pyrogenic exotoxin A gene (speA) may contribute to disease severity; however, others have not found a high prevalence of isolates that produce pyrogenic exotoxin A among organisms that cause streptococcal toxic shock syndrome. Although the gene that encodes pyrogenic exotoxin B, a cysteine protease, has been universally present in virulent isolates, this gene is also present in nearly all other GAS isolates; therefore, its importance in toxic shock-producing strains remains unclear. Finally, changes in herd immunity to certain exotoxin B variants and/or M protein serotypes may result in an increased susceptibility to newly emerging mutant strains (20).

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PHLS Surveillance of Antibiotic Resistance, England and Wales: Emerging Resistance in *Streptococcus pneumoniae*

To the Editor: The commentary, by Dr. M. S. Cetron and colleagues, on the action plan for drug-resistant *Streptococcus pneumoniae* (1) prompts us to describe the main system for surveillance of antibiotic resistance in use by the Public Health Laboratory Service (PHLS) in England and Wales and our recent results for resistance in *S. pneumoniae*.

Since 1974, the diagnostic laboratories in the PHLS network (53 in 1993-94) and increasing numbers of National Health Service and private laboratories have reported, on a voluntary basis, all bacterial isolations from blood or cerebrospinal fluid to the PHLS Communicable Disease Surveillance Centre. Since 1989, they have been asked to include their antimicrobial susceptibility test results on all isolates. In 1993, for example, antimicrobial susceptibility test results were received from 195 laboratories, on >28,000 nonduplicate isolates. Some data are sent as written entries on the report forms, but increasingly they are being transmitted electronically. Results may be analyzed according to region or reporting laboratory or by patient characteristics, such as age.

All laboratories do not test the same antimicrobial agents, but a nucleus set is tested by most laboratories for each species. Results are reported as "susceptible" (S), "resistant" (R) or "intermediate" (I). Although the methods are not standardized, external quality assurance is provided by the UK National External Quality Assessment Scheme (2), to which almost all laboratories subscribe. In addition, many laboratories refer isolates that show particularly critical resistance traits (such as ß-lactam resistance in *S. pneumoniae*, or glycopeptide resistance in *Enterococcus* species) to the PHLS Antibiotic Reference Unit (ARU) for determination of minimum inhibitory concentrations (MICs); these can often be matched against the submitted results. Occasional prevalence surveys in the PHLS network, with testing of isolates in the ARU, act as a further monitoring measure.

The application of the system can be seen in the recently published results of 6 years' surveillance of resistance amongst isolates of *S. pneumoniae* causing bacteremia or meningitis (3,4). There has been a statistically significant trend to increased resistance to penicillin (from 0.3% in 1989 to 2.5% in 1994), although these are low percentages in comparison with those seen in many other countries (5). Moreover, a considerable increase has been observed in resistance to erythromycin (from 3.3% in 1989 to 11.2% in 1994). These figures are based on susceptibility testing of more than 2,500 isolates in each of the 6 years. In 1993, resistance to penicillin and erythromycin was significantly more common amongst pneumococci from bacteremia and meningitis in the younger age groups (≤ 9 years). A significant rise during the 6 years was also seen in trimethoprim resistance, but no significant change was observed in resistance to tetracycline or chloramphenicol.

The resistance totals include isolates reported as resistant (R), and as intermediate (I), as we cannot be sure of the basis for this discrimination in the diagnostic laboratories. The proportion of isolates reported as I is very small, with the exception of antimicrobial agents for which m any str ains have MICs near the breakpoint defined to separate sensitive and resistant strains. In the case of *S. pneumoniae*, this applied to trimethoprim (6.3% of isolates reported as I in 1993); < 0.5% of isolates were reported as I to other antimicrobial agents.

The results of the National External Quality Assessment Scheme exercises have shown acceptable proficiency. For example, in the detection of penicillin resistance, in five distributions of pneumococcal strains that require an MIC of 0.25 mg/l, 74% to 90% of laboratories obtained correct results; in six distributions of strains that require an MIC of 1.0 mg/l. 95% to 99% of laboratories did so (J.J.S. Snell, pers. comm.). The isolates included in the analysis that were also tested in the ARU gave closely similar results: for example, of 86 pneumococcal isolates tested for susceptibility to penicillin in early 1995, 82 (95%) gave the same result in the sender's laboratory and in the ARU. A survey of resistance in pneumococci (from all sites) conducted in March l995 with MIC determination by the ARU (unpublished) showed similar proportions of resistant strains.

These observations demonstrate that the results of susceptibility tests undertaken for the management of individual patients may be compiled and analyzed for surveillance purposes. Duplicate isolates from the same infection episode should not be included, and satisfactory quality assurance should be undertaken. Increasing use of computers and networking among the clinical laboratories should facilitate the process of data collection.

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Infectious Diseases in Latin America and the Caribbean: Are They Really Emerging and Increasing?

During 1995, infectious disease epidemics in Latin America and the Caribbean received wide publicity: dengue throughout the region, Venezuelan equine encephalitis (VEE) in Venezuela and Colombia, and hemorrhagic fever in Nicaragua. Increased awareness of these diseases followed extensive reports in the scientific community about the threat of emerging infections (1,2). Are infectious diseases increasing in the region or are we simply seeing the results of better reporting of persistent problems? Analysis suggests that both factors are at work.

Dengue and Dengue Hemorrhagic Fever

During the 1950s and 1960s, under the leadership of the Pan American Health Organization (PAHO), most countries in the Americas successfully reduced or eliminated infestation with *Aedes aegypti*, the principal vector of dengue and urban yellow fever. As a result, much of the Americas became free of dengue. Dengue transmission, however, persisted in many Caribbean islands and in some countries of northern South America that failed to control the vector; therefore, several outbreaks occurred during the 1960s and in subsequent decades (3).

Ae. aegypti eradication programs, however, were not sustained and the mosquito reinfested all Latin American countries except Chile and Uruguay. As a consequence, dengue spread throughout the region, causing severe epidemics or even pandemics during the 1970s and 1980s. Currently, dengue is endemic in virtually all countries with *Ae. aegypti*, and epidemics occur periodically.

Between 1968 and 1980, only 60 suspected or confirmed cases of dengue hemorrhagic fever (DHF) were reported, all by five countries in and around the Caribbean. After the 1981 DHF outbreak in Cuba, reports of DHF in the Americas markedly increased. The Cuban epidemic was the most notable event in the history of dengue in the Americas: almost 400,000 cases of dengue, over 10,000 cases of DHF, and 158 deaths were reported. The Cuban authorities implemented a successful vector control program and the country is still virtually free of *Ae. aegypti*. After this outbreak, cases of DHF continued to occur in the Americas, although at relatively low levels, until 1989 when another large epidemic with 2,500 cases of DHF occurred in Venezuela. Since then, Venezuela has reported large numbers of DHF cases every year, and in 1995 the country reported the largest outbreak of dengue/DHF in its history: almost 30,000 dengue cases and 5,000 DHF cases. Since 1968, 25 countries of the Americas have reported more than 35,000 confirmed or suspected DHF cases and approximately 500 deaths.

In 1995, dengue and DHF activity in the region was higher than in any year except 1981. As of November, countries in the Americas had reported more than 200,000 dengue cases and 6,000 DHF cases, and approximately 90 deaths. Brazil has had the largest number of dengue cases, but more than 80% of the DHF cases occurred in Venezuela. The reinvasion of the Americas by dengue virus type 3, which had been absent for 16 years, has increased the threat of large epidemics and consequent risk for DHF (4). This serotype was isolated in Panama and Nicaragua at the end of 1994, and in 1995 it spread to other Central American countries (except Belize) and Mexico, causing severe outbreaks. High levels of infestation with *Ae. aegypti* are common from the United States to Argentina, making it likely that dengue epidemics will increase in frequency and severity.

Cholera

Another disease reemerging in the Americas is epidemic cholera, which had been absent from this hemisphere for approximately 90 years before it was introduced into Peru in January 1991 (5). Since then more than 1 million cases of cholera have been reported in 20 countries in the region. Only Uruguay and the islands of the Caribbean have been spared. Though the annual total of reported cases has decreased since 1991, the disease is persistent and problematic in several Latin American countries.

Venezuelan Equine Encephalitis

An outbreak of human infection with VEE virus associated with a large number of equine cases and deaths was detected in northwestern Venezuela in April 1995. The disease spread to the adjacent Colombian state of La Guajira in September (6). Unusually heavy rains during 1994 and 1995 contributed to the epidemic by

increasing breeding sites for the mosquito vectors *Ae. taeniorrhynchus* and *Psorophora confinnis*. Viral strains with epizootic and epidemic potential appear to have emerged from enzootic strains maintained in enzootic rodent-mosquito cycles (7). In addition, failure to immunize wild and domestic equine populations allowed the virus to amplify and spread. By mid-October 1995, reported human cases totaled 26,500 in Venezuela and 22,300 in Colombia, with 24 deaths in the latter. Attack rates in severely affected communities were 18% to 57%.

This epidemic of VEE is the largest since that of 1962-1971, when the disease extended from northern South America through Central America and Mexico to the United States (8). Intensified vector control (including application of adulticides and larvicides), equine vaccination, and restriction of equine movement appear to have at least temporarily controlled the epidemic.

Leptospirosis

In late October 1995, Nicaragua reported several hundred cases of a hemorrhagic febrile illness in and near the community of Achuapa, approximately 110 km northwest of Managua; eight patients died. The affected communities had experienced unusually heavy rains and flooding during the 2 weeks before the cases were noted. Although dengue was occurring elsewhere in Nicaragua at the same time, that diagnosis was excluded by negative laboratory tests and the absence of *Ae. aegypti* in the local area. In addition, some of the patients had frank pulmonary hemorrhage [not typical of dengue]. By the end of October, the Centers for Disease Control and Prevention had ruled out dengue, other arboviruses, and hemorrhagic fever viruses as causes but had identified leptospira by immunohistochemistry in tissues from four patients with fatal cases. By mid-November, the Ministry of Health reported that 2,480 persons had been ill, 750 were hospitalized, and at least 16 died. (Investigation to define the extent of illness and the responsible serovars was still in progress at this writing.)

Of the examples discussed here, dengue and DHF are certainly reemerging. Dengue has been signaling its return for more than 15 years and DHF since 1989; these diseases will likely persist as epidemic problems unless drastic changes in vector control are achieved. Enzootic VEE has persisted in northern Venezuela and Colombia since the previous major epidemic of 1962-1971. PAHO had urged the countries to increase vaccination coverage of equines because of increased viral activity in 1993 and 1994. The appearance in 1995 of strains similar to these of the 1962-1971 epidemics, with locally intense transmission, raised the possibility that VEE would reemerge as a major epidemic disease. Whether vigorous vector control measures and immunization programs have contained that threat is not yet known, but we must continue to regard the threat as real. Leptospirosis is a persistent, often under-recognized, problem to which the international community has paid relatively little attention. In Nicaragua, public health interest was sparked by concern that the epidemic of a new disease would pose a threat to other communities and countries, but attention waned as that threat diminished. Yellow fever, which is usually present in relatively low numbers in remote areas of South America, reemerged with force in Peru during 1995. At least seven departments of that country have been affected (470 cases and a 40% case-fatality rate by September 1995).

Several factors have contributed to the reemergence of infectious diseases in the Americas. Investments in public health have been decreasing because of economic recession and a shrinking public sector or have been diverted from infectious disease programs to other pressing problems (9). Human populations throughout the region have grown and become increasingly urban, with many living in inadequate housing without sanitation or potable water. At the same time, population and commercial pressures have led to the invasion of forests, exposing people to exotic agents and enzootic diseases, including yellow fever, rabies transmitted by vampire bats, aren aviruses, and others. Human behavior has contributed to epidemic plague in Peru and the rapid spread of diseases such as cholera. To this list can be added the effects of deforestation and habitat and climate change. Unusually heavy rainfall contributed to at least three of the epidemics considered in this commentary (10).

Infectious diseases (whether new or reemerging) are a real and serious problem in Latin America and the Caribbean. To combat the threat of these diseases, PAHO, with the participation of other institutions in the region, has prepared a regional plan to improve surveillance for emerging disease and enhance countries' ability to respond effectively by strengthening laboratory capacity, training, and research and by implementing prevention and control strategies. Ministers of health from countries throughout the region discussed and endorsed the plan at a meeting of PAHO's Directing Council in September 1995. Successful implementation of the plan will require committed action by public health authorities and collaboration and cooperation by many institutions and experts throughout the region.

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Microbial Threats and the Global Society

The public health threat of microbial organisms living in what can be regarded as one large global society was the subject of a recent interactive workshop sponsored by Tufts University's Education for Public Inquiry and International Citizenship 10th anniversary celebration, held at Tufts University in Medford, Massachusetts. The participants* discussed microbes as the harbingers of disease and society as both potential victim and guardian of health. Microbial threats were identified as new, reemerging, and not yet known.

The forum examined the many unanswered questions regarding the origin and causes of infectious disease agents. The failure of traditional treatments due to antibiotic resistance and the ineffective control and continued spread of infectious agents were also discussed. Participants addressed environmental and behavior factors that foster the "amplification" and "spread" of disease organisms: bathhouses conducive to the spread of HIV infection, homelessness and crowded living promoting the spread of tuberculosis, day-care centers that are ideal environments for the spread of drug-resistant pneumococcus.

In the context of the workshop at Tufts, analyses of emerging infectious disease issues generated insights about the political and social framework within which to address these threats to health: A minority group may be particularly affected by a new or reemerging disease, as was the case, for example, of AIDS in the gay population or tuberculosis in the immigrant and homeless population. These groups become valuable resources for understanding the factors leading to the emergence or reemergence of the disease and should be the focus of public health efforts for curtailing its spread. However, as the history of AIDS demonstrates, because of political concerns, investigative efforts are often delayed or inadequate to stop the spread of the disease.

An emerging or reemerging organism, however, propagates and spreads unhindered by the social concerns of its potentially infectable host. To microorganisms, the world is a single entity without borders. Microorganisms have more freedom than we do and also more genetic flexibility. Thus, in the contest between humans and microbes, we are at a disadvantage. We can neither easily acquire resistance mechanisms *against* the organ isms, nor rapidly respond to an infectious disease problem in another country. The recent difficulties in dealing with a possible plague epidemic in India are just one example. Moreover, antibiotics which have been a front-line weapon against diseases are becoming increasingly ineffective, and new antibiotics to treat and contain drug-resistant bacterial strains are not available.

Inadequate microbiologic diagnostic capability—also the result of the national and international political climate—works to the advantage of emerging microbes. During the plague outbreak in India, laboratory facilities that could confirm the diagnosis were lacking. In the United States, similar inadequacies in laboratory diagnostic capacity interfere with rapid reporting of common community-acquired infections and their susceptibility to antibiotics. If physicians promptly knew what they were treating, the need for use of an antibiotic as well as the proper kind of antibiotic would be based on data, not guesswork.

The emergence of antibiotic resistance was not factored into strategic planning by public health authorities. If it had been, perhaps conditions could have been in place to handle it, as well as AIDS, tuberculosis, and other emerging pathogens. Insurance against devastating happenings in infectious disease has never been given the attention it deserves. Such insurance would have been helpful, not just in money, but also in expertise to forfend and then cope with the calamity, like insurance for earthquake damage to structure and other unexpected disasters. Should we not consider insuring our future by putting more money and expertise into basic research, into systems for surveillance, and into ways to curtail the spread of a disease once it has emerged?

To meet the demands of increased public health activity and to implement an "insurance policy" for the future, we need to be able to *communicate* the problem to a broad audience that sometimes has little understanding of the science. To some public health officials, recognition that an infectious disease problem exists is sufficient to address the problem. However, to those not trained in the field who may be making important policy decisions, the "public safety" aspect of the problem can be emphasized. Health, like crime and traffic, should become once again a major society issue.

Requests for increasing support for surveillance, education, and research must take into account current political and social priorities and

emphasize direct benefits to the U.S. population; international efforts should involve the collaboration of other countries.

Nongovernment agencies need to be enlisted in this public health effort; thus a larger portion of society will be involved in the fight against the ever-increasing threat of infectious diseases.

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Xenotransplantation: Risks, Clinical Potential, and Future Prospects

The reemergence of xenotransplantation as a therapeutic option for the hundreds of thousands of people dying each year of heart, kidney, lung, and liver failure has raised ethical, social, and scientific questions. End-stage organ failure is one of the most important public health problems facing Americans today. Heart failure, for example, kills four times as many people as does HIV infection and three times as many people as does breast cancer; it is a disease with an increasing incidence, and the cost of taking care of affected patients is 8 to 35 billion dollars each year. The single most effective therapy for it is transplantation. Preventive therapies have had little impact on diseases due to end-stage organ failure and are unlikely to have an impact at least in the next decade. In the meantime, demand for organs, which far outstrips the supply, continues to grow. It has been estimated that approximately 45,000 Americans under age 65 could benefit each year from heart transplantation, yet only 2,000 human hearts are available annually. Patients are more likely to die waiting for a human donor heart than in the first 2 years after transplantation.

Although clearly an experimental procedure, xenotransplantation between closely related species, such as baboons and humans, offers an alternative to allotransplantation as a source of human organ replacement. Alternatives to allograft donors, such as baboon or pig xenografts, require serious investigation if clinical transplantation is ever to meet the current demand and continue the explosive growth pattern it has established over the past quarter century.

Biologic cardiac replacement poses the immunologic problems of rejection and infection associated with transplantation. Increasing clinical experience worldwide has shown that rejection and infection can be managed successfully in most patients who receive human cardiac allografts. Further, the introduction of cyclosporine as the primary immunosuppressive agent for cardiac transplant recipients has resulted in excellent survival rates (85% 1-year survival at most centers) and has decreased illness associated with infection and rejection. Although considerable advances have been made in the field of cardiac xenotransplantation since its first clinical application by Hardy in 1964 (1), it remains uncertain whether xenotransplantation as destination therapy can be successfully applied to humans. However, heart, kidney, and liver xenografts have been able to support human life for an extended period. It is this fact that investigators wish to exploit in clinical bridging studies. By providing temporary heart, kidney, or liver support as a bridge-to-transplantation, these biological devices may allow patients to recover end-organ function and await allograft transplantation in a more stable clinical state, thus improving their chances of survival. Bridging strategies cannot alleviate the human organ donor shortage. However, if one views bridging strategies as a first feasibility test, then cross-species transplantation does offer the possibility of eventual long-term organ replacement. Success in this more ambitious goal would help alleviate the human organ donor shortage.

Nonhuman primate organ donors have been favored by those wishing to minimize the genetic disparity between donors and human recipients. Chimpanzees, although most compatible with standard selection criteria (e.g., compatibility of size and blood types), are unavailable as an acceptable source of clinical xenotransplantation. Another choice is the baboon, which is not endangered, has an anatomy and physiology similar to those of humans, and grows to a weight of approximately 70 pounds. Baboon size would limit the clinical application of xenotransplantation with baboon organs to pediatric patients and small adults. Small body size, the infrequency of blood group O (universal donor) animals, and the limited number of colony-bred animals are distinct disadvantages to the baboon as a donor.

Extended graft survival is possible, but ABO blood group compatibility is mandatory before xenotransplantation (2). The distribution of ABO blood groups found in baboons indicates that approximately one third are group A, one-third group B, and one-third group AB. Universal donor group O, however, is exceedingly rare. In Americans of Western European descent, the relative frequency of blood types is approximately 45% group A, 8% group B, 4% group AB, and 43% group O (2).

Although available in large numbers, wild baboons are not suitable from an infectious disease perspective. Most experts have suggested that colony-bred animals represent a more suitable donor pool. However, these animals number only in the hundreds and are, therefore, only likely to

partially meet the epidemiologic demands of the pediatric population with end-stage organ failure.

Xenotransplantation between baboons and humans raises the issue of xenozoonoses (3,4). The organisms of greatest concern are the herpesviruses and retroviruses, which can be screened for and eliminated from the donor pool. Others include *Toxoplasma gondii* , *Mycobacterium tuberculosis* , and encephalomyocarditis virus. Less likely to be found in animals raised in captivity in the United States are the filoviruses (Marburg and Ebola), monkeypox, and Simian hemorrhagic fever virus. Organisms that are unlikely to be transmitted with an organ transplant (but should be screened for) include lymphocytic choriomeningitis virus, gastrointestinal parasites, and GI bacterial pathogens.

The risk for xenozoonoses is likely to be restricted to the xenogeneic tissue recipient. Nevertheless, one must consider and anticipate the potential for xenozoonotic transmission through the human population, constituting a public health concern. The risk for recognized zoonotic pathogens can be reduced, if not eliminated, by controlling the donor animal vendor source and the individual donor animal by employing described screening tests and strict sterile procedures during organ harvesting and donor autopsy for tissue and blood. The risk for unrecog nized pathogens is present but ill defined.

Surveillance for the transmission of known or unknown pathogens among health care workers must be conducted by monitoring for unexpected or unexplained adverse health events. It is difficult to monitor for the unknown; therefore, surveillance should include notifying the principal investigator's office of any unexplained illness in exposed health care workers, as well as telephone interviews of these personnel every 6 months by the principal investigator's office.

Concurrent with scientific advances in xenotransplantation have been the necessary ethical debates concerning the appropriateness of this endeavor (5). Disputes regarding animal experimentation notwithstanding, the ethical issues raised by many of these debates are strikingly similar to those put forth 25 years ago in reference to the (then new) field of human heart transplantation. Indeed, the timeless nature of these queries itself attests to their essence, for such ethical concerns are appropriate in the appraisal of *any* new therapeutic procedure in medicine.

Can one ever hope to determine if or when the clinical application of xenotransplantation is justified? The assessment of any experimental therapy, as Fox and Swazey (6) have suggested, should encourage the investigator to address three critical questions: 1) in the laboratory, what defines "success" sufficient to warrant advancement to the clinical arena? 2) under what clinical conditions should this advancement proceed? and 3) in the clinical arena, what d efines "success" sufficient to warrant further evaluation (6)? Providing answers to this threefold inquiry requires a reliance upon defined "success," itself an appraisal of judgment that can only confidently be made in retrospect.

Because human heart transplantation is now considered by most justifiable for the treatment of end-stage heart disease, I would first like to review the history of cardiac allotransplantation in light of its ability to address the above threefold inquiry. I will also discuss the history of cardiac xenotransplantation with reference to scientific advances made in the field throughout the past quarter century. Finally, in light of these analyses, I hope to illustrate the role of baboon heart xenotransplantation as an alternative to allotransplantation for permanent cardiac replacement in the treatment of end-stage heart disease.

After the first human cardiac allograft procedure performed by Barnard in 1967 (7) the field of cardiac transplantation witnessed a surge in both enthusiasm and attempted trials, which was followed by a marked drop in procedures throughout the 1970s because of poor survival rates. During the initial peak, 21 human heart allotransplants were performed in the 6-month interval between December 1967 and June 1968 (with a cumulative 1-year survival of 22%), and 105 cardiac allotransplantations were performed in 1968 alone (8-10). However, these early clinical trials were marred by numerous failures, as 65% of persons undergoing the procedure before June 1970 died within 3 months of transplantation (6).

Few centers continued animal research and human procedures during the so-called black years of cardiac transplantation. The initial explosion in clinical trials accordingly elicited numerous responses suggesting that too much was being attempted too soon.

Some would propose that this was the price of eventual "success," and that further experimental studies at the time could not have avoided early

losses. And yet, there has been, and may always be, a tacit recognition by medical innovators that the ultimate experiment must be performed in humans, for no animal model can truly reflect the human condition. Proponents of allotransplantation at the time of the first heart transplantation cited the more than 60-year history of experimental cardiac transplantation, beginning with Carrel's original work in 1905. Although most of this work began in the 1930s, subsequent investigations regarding the experimental transplantation of mammalian hearts showed that cardiac transplantation was technically feasible and suggested the possibility of clinically relevant survival rates. During the decade before Barnard's first clinical application, cardiac allograft survival had been shown to exceed 250 days (mean 103 days) in adult dogs treated with an immunosuppressive regimen that included azathioprine and methylprednisone used intermittently. The mean survival in untreated dogs used as controls was 7 days (11).

Since that time, with further expansion of knowledge in virtually all areas of clinical cardiac transplantation, 1-year survival has increased from 67% in 1976, to approximately 85% reported currently at most hospital centers (3). Human recipients have survived for as long as 20 years after transplantation, and the 10-year posttransplant survival rate is now approximately 45% (12). While these figures depict a clear improvement in raw survival, cardiac transplantation is still not a cure for end-stage heart disease. Recipients must take immunosuppressive medication for life and be monitored for infection, rejection, and graft arteriopathy. However, these results are impressive considering that the recipient population today is considerably sicker than earlier allograft candidates. In light of these findings, few would deny cardiac allotransplantation its present claim to "success." To further understand the evolution of this achievement, however, we may now look back upon the early years of cardiac allotransplantation and try to address the proposed threefold inquiry.

First, for Barnard and co-workers what can we presume as "success" warranting advancement to the clinical arena? They performed the first human adult cardiac allotransplantation when the maximum survival in immunosuppressed adult dogs had been 250 days (average survival 103 days) (11) and suggested that "against the background of this research . . . the time arrived when a cardiac transplant could be contemplated with hope of success" (7). Indeed, in their report of this case, they further described the scientific basis of their clinical advancement by explaining that "this achievement did not come as a surprise to the medical world. Steady progress toward this goal had been made by immunologists, biochemists, surgeons, and specialists in other branches of medical science all over the world during the past decades to ensure that this, the ultimate in cardiac surgery, would be a success" (7). Although we may, in retrospect, consider them justified in their declaration, in fact, at that time the endeavor was highly controversial and came as a surprise to much of the medical world.

Second, under what con ditions did they proceed with this clinical trial? Given the "hope of success," Barnard and colleagues selected a patient "considered to have heart disease of such severity that no method of therapy short of cardiac transplantation could succeed" (7). The patient, a 54-year-old man, had remained in intractable congestive heart failure (following multiple myocardial infarctions) despite all medical management (13).

Finally, in this clinical arena, what defined for Barnard and colleagues "success" warranting further investigation? A concurrent editorial in the *South African Medical Journal* may provide some insight into their thinking: "The claim 'successful' can be used even at this early stage because to-date it is a feat which makes medical history, no matter how short the further survival of the patient might be (4). "Success," by such an analysis, was thus not targeted posttransplant survival time, but rather *any* posttransplant survival time (given the ground-breaking nature of the endeavor). Further editorials regarding the ethics of cardiac transplantation viewed the procedure as a legitimate experiment but not a treatment (15), while in 1968, the American College of Cardiology suggested (with regard to the "success" of allotransplantation) that results varied: ". . . the spectrum of success ranges from short-term restoration of circulation to complete physical recovery" (16).

Indeed, "success" did vary along a spectrum of results. Barnard and colleagues' first allotransplant recipient lived for 18 days and ultimately died of pneumonia. However, their second recipient, 1 month later, survived more than 19 months before dying of chronic rejection (17). Their third

patient also lived more than 20 months after allotransplantation and ultimately died of carcinoma of the stomach without signs of acute or chronic rejection (18). One can only speculate how different the world reception to allotransplantation would have been had the latter two patients represented the first and second recipients of cardiac allotransplants. Would these survival data be considered "success," or would they still pale in comparison with the theoretical goal of obtaining a graft that could function normally indefinitely?

Clinical cross-species transplantation dates to the early twentieth century, with kidney xenografts from rabbit, pig, goat, non-human primate and lamb donors (19). After these early failures, the scientific literature was largely devoid of reports of clinical xenotransplantation for nearly 40 years. In 1963, Reemtsma and colleagues described six human recipients of chimpanzee kidneys, the longest survivor of whom died of causes unrelated to rejection 9 months after xenotransplantation (20).

The first cardiac xenotransplantation, performed by Hardy in 1964, also represented the first attempt at cardiac transplantation in humans, predating Barnard's report by nearly 4 years (1). Since 1964, when Hardy and colleagues at the University of Mississippi performed the world's first heart xenotransplant using a chimpanzee as a donor, there have been eight documented attempts at clinical heart xenotransplantation. Five of these donors were nonhuman primates (2 baboons, 3 chimpanzees) and three were domesticated farm animals (1 sheep, 2 pigs) (21-25). The longest survivor was a newborn infant with hypoplastic left heart syndrome. "Baby Fae" was the recipient of an ABO-blood group mismatched baboon heart that functioned for 20 days (26). However, by the time the first human neonatal cardiac xenotransplantation was performed by Bailey in 1984 (the so-called "Baby Fae" case), there had been only limited experimental experience with prolonged graft survival in the newborn xenotransplant recipient. Studies presented by Bailey and co-workers shortly before the Baby Fae case described a mean survival time of 72 days in newborn lamb-to-goat xenotransplants, with one survivor living to 165 days (27).

This advancement of xenotransplantation into the clinical forum was met with r esistance in the medical community because of a perception that research with acceptable survival "success" had not been achieved experimentally. As Losman in an editorial regarding the Baby Fae experience stated, "It appears that this baboon-to-infant transplantation did not rest on such a [scientific] basis [as did Barnard's earlier operation in 1967] "(28).

During the past 3 years, investigators at the University of Pittsburgh reported two cases in which they transplanted a baboon liver into a human recipient, obtaining a 70-day survival in their first reported case, and a 26-day survival in the second (29; J.J. Fung, pers. comm.) The investigators' overwhelming effort to prevent rejection led them to use a harsh immunosuppressive regimen that permitted multiple life-threatening infections. Rejection was not the major clinical obstacle they encountered; therefore, they recommended a more directed and less arduous immunosuppressive regimen for future patients.

More alarming have been the atte mpts to apply xenotransplantation of distantly related species to the clinical arena. In 1968, both Cooley and Ross transplanted sheep and pig hearts, respectively, into dying human recipients (30,31). Both grafts failed upon reperfusion, presumably because of hyperacute rejection.

More recently, Czaplicki and co-workers in 1992 described a case in which they attempted the xenotransplantation of a pig heart into a human recipient with Marfan's syndrome (32). By their report, no evidence of hyperacute rejection was present at the time of death nearly 24 hours after xenotransplantation. Their protocol used an unusual immunosuppressive regimen in which both donor and recipient received, in addition to conventional immunosuppression, both thymic tissue extracts and fetal calf sera. This regimen also included the extracorporeal perfusion of two pig hearts with the recipient's blood in an attempt to remove human anti-pig antibodies before the orthotopic transplantation of the functional pig heart (33). As astonishing as this case may be in its extension to the clinical arena of a technique not yet shown to be effective in the experimental laboratory, it is not unique. Also in 1992, Makowka and colleagues transplanted a pig liver into a 26-year-old woman dying of acute liver failure from autoimmune hepatitis (pers. comm.). Despite the fact that, at present, it appears unlikely that sufficient "success" has been achieved in the laboratory regarding xenotransplantation between distantly related species to warrant

advancement to the clinical arena, these investigators were able to obtain approval from their hospital's ethics committee and institutional review board to proceed with the clinical trial. Most experts in the field of xenotransplantation share the opinion that pig-to-human organ transplantation remains at least 3 to 5 years from clinical trials.

Considerable advances in the field of cardiac xenotransplantation have subsequently emerged worldwide since Hardy's first clinical attempt in 1964, with a better understanding of the xenorejection process and a more sophisticated insight into mechanisms for its control. Extended graft survival has been achieved in a number of different experimental models, including a greater than tenfold graft survival in non-human primates treated with conventional cyclosporine-based immunosuppression (34,35) a more than thirtyfold increase in survival over controls described by Celli and colleagues in a rodent model (36), and survival beyond 1 year reported by Kawauchi and colleagues in a non-human primate model (37). These findings support the potential for achieving clinically relevant graft survival in humans.

The question is whether we have reached a stage in laboratory experimentation to justify further attempts at advancing cardiac xenotransplantation to the clinical arena. If we view the current status of experimental accomplishments in xenotransplantation with the same scrutiny as that of allotransplantation at the time of Barnard's endeavor, we are left with similar conclusions; first, comparable graft survival time has been achieved in animal models of xenotransplantation as was evident for allotransplantation before 1967. Second, with our current understanding of cardiac allotransplantation has also come a greater awareness of its limitations. Thus, the conditions for the advancement of xenotransplantation arguably could be fulfilled by a patient with end-stage heart disease who is a candidate for allotransplantation, but for whom a donor cannot be identified in time. Finally, the clinical "success" of xenotransplantation might also be considered (as was the case for allotransplantation) *any* graft survival, and the goal of xenotransplantation to strive for extended graft survival.

However, political and scientific sensibilities today clearly differ from those of the 1960s, and so the critical assessment of xenotransplantation must be more rigorous than our previous discussion. Indeed, the above comparison was put forth largely to underscore the more humble origins of the (now) successful therapy (allotransplantation) to which xenotransplantation is currently compared.

What then defines "success" in the laboratory warranting advancement from the laboratory to the operating room? Having demonstrated dramatic prolongation of cardiac xenograft survival through experiments in rodent and non-human primate models (27,34-37), which model most closely approximates the human condition (and thus which therapy will be most successful in avoiding clinical rejection) remains to be established. Therefore, it is reasonable to suggest both that we have reached a formidable limitation for precisely predicting the applicability of experimental laboratory evidence and that answers may only be sought from experiment in humans. This concept was realized by the American Medical Association with regard to allotransplantation, in reference to which it released an official statement acknowledging this notion in 1969 (38).

Concerns most commonly voiced with respect to the clinical application of xenotransplantation, however, pertain to a larger ethical controversy regarding human experimentation. Reemtsma, in a related comment concerning the Baby Fae case, suggested the following: "There is a widespread misperception that medical treatments and surgical procedures are easily classif ied as either experimental or accepted. In fact, all treatments have an element of experimentation, and new surgical procedures are based on extrapolations from prior work. . . . When does a surgeon decide to apply a new operation to a patient? . . . the decision is based on balancing, on the one hand, the experimental evidence suggesting that the procedure may succeed, and, on the other, the clinical urgency. . . (39).

Under what conditions will the clinical advancement of xenotransplantation proceed? For those initial patients in whom clinical xenotransplantation will first be applied, clinical urgency, in the complete absence of other suitable alternatives, undoubtedly will represent the motivating factor to proceed. Who will comprise this initial cohort? As Caplan has pointed out: "There would appear to exist a pool of terminally ill persons, both children and adults for whom no therapeutic alternatives exist or are likely to exist in the near

future. . . . It would [thus] appear ethically defensible to allow research involving xenografting in human subjects to proceed in those areas where no reasonable alternative to therapy exists (40). In this context, innumerable reservations have been voiced regarding the ethics of proposing alternative experimental therapies to such patients for whom therapy has either failed or is non-existent. However, with regard to clinical experimentation under these circumstances, one must also recall (as Shimkin has suggested): "To do nothing, or to prevent others from doing anything, is itself a type of experiment, for the prevention of experimentation is tantamount to the assumption of responsibility for an experiment different from the one proposed" (41).

What is the goal of the clinical application of xenotransplantation? The need for donor organs irrefutably outweighs the resources available, and mechanical devices and xenotransplantation have emerged as the two most promising alternatives to allograft cardiac replacement. Mechanical left ventricular assist devices (LVADs) have witnessed relative success as "bridges" in carefully selected patients with heart failure. (A "bridge" is a temporary method of life support designed to carry a patient indefinitely until a human heart can be found and transplanted. It is not a "destination" therapy.)

Criteria for LVADs exclude patients with biventricular failure, and (because of the relatively large size of the device) patients with a total body surface area less than 1.5 square meters (~120 lb). Thus, many women and virtually all children are not candidates for mechanical left ventricular assistance. As has been the case for Food and Drug Administration protocols using LVADs, proposed investigations involving biologic assist devices (xenografts), have sought to evaluate a short-term alternative to allotransplantation in patients for whom a donor heart is not immediately available, and death is imminent. Only candidates who meet criteria for heart transplantation, but do not meet criteria for LVAD insertion, would be considered for a heart xenobridge. Similar clinical scenarios have been proposed for other solid organ transplants. Since they were first introduced by Cooley in 1969, temporary mechanical circulatory support devices have become critically useful tools in the therapeutic armamentarium available to patients awaiting transplantation (42). Nevertheless, at present, the widespread application of mechanical circulatory support is limited both by patient selection criteria and by the temporary nature of the device. For excluded patients, as well as many adult male candidates. For excluded patients, cardiac xenotransplantation may be the only reasonable alternative to cardiac allograft replacement.

Investigations in clinical xenotransplantation have been accused of using "the guise of [being a] bridge-to-transplantation" to appear acceptable to Institutional Research/Ethical Boards (5). However, the use of xenografts (or mechanical devices) solely as bridges to allotransplantation will not increase the donor pool, and, therefore, successful permanent xenotransplantation must itself be seen as the target for future clinical investigations. The goal of these studies is thus not to engage, as Hastillo and Hess (5) would suggest, in the "premature use of unproven procedures in fellow humans," but rather to impact positively on the current shortage of human donor organs (6). In 1996, the clinical picture is no less bleak and the conclusions no less valid. The question that remains is not how but rather *when* xenotransplantation should advance to the clinical arena. Most of the uncertainties surrounding its advancement will only be answered by its undertaking.

In the foreseeable future, clinical xenotransplantation may achieve its targeted goal of extended graft survival. As was the case during the early years of allotransplantation, clinical xenotransplantation must persevere under the consideration of and often in spite of scrutiny by its most demanding critics, for while "success has a hundred fathers, failure is an orphan" (43).

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Another Human Case of Equine Morbillivirus Disease in Australia

Another human case of equine morbillivirus (EMV) disease has occurred in Australia. The patient was a 35-year-old farmer, who lived near Mackay, in northern Queensland. He died in the Royal Brisbane Hospital on October 21, 1995 (1). The patient was probably infected with the novel virus 12 months before his death—approximately the time of the first reported outbreak of EMV.

A hitherto unknown infectious disease, EMV was first observed in Brisbane, Queensland, in September 1994, when an outbreak of acute respiratory disease in horses at three stables in Hendra, a Brisbane suburb, was reported (2). In a 2-week period, 14 racehorses and two persons at the stable contracted the disease. One of the human cases and some of the equine cases were fatal. A total of 21 horses were infected with the virus. Fourteen horses died as a result of clinical illness (they either died from the infection or were euthanized). The remaining horses had either symptomatic or asymptomatic infection and were euthanized.

The cause of infection in the recent case has been confirmed as EMV at the Australian Animal Health Laboratory in Geelong through the testing of samples taken from the patient before he died (I. Douglas, Australian Communicable Disease Service; PROMED).

The Mackay patient was married to a veterinary surgeon. The couple bred horses and grew sugar cane. In August 1994 (a month before the outbreak of EMV in southern Queensland), two horses died on the couple's property. The veterinary surgeon, assisted by her husband, performed autopsies on the two animals. The diagnoses, based on these autopsies, were "avocado poisoning" and "brown snake bite," respectively (1).

In August-September 1994, soon after the death of the horses, the husband became ill with a mild meningoencephalitis, which improved with antibiotics. Cerebrospinal fluid examination showed a neutrophilic pleocytosis suggestive of a viral infection (1). Serum collected at the time of the examination and stored was found to contain a low but significant titer of antibody to EMV.

The patient appeared to have recovered; however, he was admitted to the hospital 5 weeks before his death with signs of encephalitis. Evidence of EMV infection included a high serum neutralizing antibody titer against the virus and a positive polymerase chain reaction (PCR) test of cerebrospinal fluid collected before his death. Tests of autopsy specimens confirmed the infection.

Direct fluorescence antibody and PCR tests of fixed tissue blocks from one of the horses at the Australian Animal Health Laboratory have confirmed that it was infected with EMV (I. Douglas, PROMED). However, it is likely that both horses were infected.

The Mackay patient's symptoms were predominantly neurologic. He displayed no respiratory symptoms until aspiration pneumonia developed. By contrast, the major clinical symptoms of EMV in Hendra were respiratory.

No recent outbreaks of clinical illness have been reported in the horses on the Mackay property—or elsewhere in Queensland—since the 1994 outbreak. Also, investigation has not shown a link between the horses on the Mackay property and those in the Hendra stables. A serologic survey of over 2,000 horses, undertaken in 1994 after the Hendra outbreak, yielded negative results. That survey included more than 200 horses in the Mackay/Rockhampton/Townsville areas. Similarly, the Queensland veterinary authorities have obtained samples from more than 3,000 animals from 294 populations (including farms, race meetings, and horse events) since October 23, 1995; 2,349 of the samples have been tested, all with negative results. Moreover, blood samples recently taken from all the domestic animals on the Mackay property, including approximately 90 horses, have been tested for virus by the Animal Health Bureau, Queensland Department of Primary Industry and Energy. All results were negative.

An extensive epidemiologic investigation is being conducted by the Queensland Department of Health and the Department of Primary Industry and Energy. All persons who may have had exposure to the virus in either episode have been tested for EMV infection and had negative results (1). No serologic evidence of further human infection has been found.

No human-to-human transmission of EMV has been reported. It is believed that the disease is spread through contact with the body fluids of infected sick or dying animals.

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Social Science and the Study of Emerging Infectious Diseases

Topics related to emerging and reemerging infectious diseases attracted a considerable audience at the annual meeting of the American Anthropological Association, November 15–19, 1995, in Washington, D.C. The meeting had a separate session entitled "Emerging and Reemerging Infectious Diseases: Biocultural and Sociocultural Approaches."

The session brought together anthropologists interested in and working on emerging infectious diseases from various subdisciplinary perspectives. Presentations were made on the following subjects: outline of a research agenda, deforestation and the emergence of infectious diseases in the rain forests of Papua-New Guinea, the cholera epidemic in Latin America, evolutionary aspects of emergent infections, societal impacts of the test for acquired immunodeficiency syndrome, compliance and iatrogenesis in tuberculosis treatment in the United States, patchwork policies that affect long-term treatment of tuberculosis in Nepal and Uganda, the reemergence of schistosomiasis in Egypt, dengue control in Latin America, cultural and political ecologic models of emergent infections, and the politics of leprosy eradication. Abstracts are available from the conference organizers, listed below.

Anthropologists interested in international health and the social science aspects of infectious diseases are organized in a working group called the International Health and Infectious Disease Study Group of the Society of Medical Anthropology (American Anthropological Association). Requests to subscribe to this group's newsletter can be sent to

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WHO Establishes New Rapid-Response Unit for Emerging Infectious Diseases

The World Health Organization (WHO) has established a new rapid-response unit to control and prevent the growing incidence of new and reemerging diseases worldwide. The unit's focus will be improved containment of disease outbreaks, such as that caused by the deadly Ebola virus, which struck Zaire in 1995.

The WHO unit will be called the Division of Emerging Viral and Bacterial Diseases Surveillance and Control (EMC). It will be capable of mobilizing staff from WHO headquarters in Geneva and from the organization's regional offices.

In addition to mobilizing WHO's own technical staff and expertise, EMC will coordinate the activities of the agency's traditional partners, for example, its international network of collaborating centers, bilateral donors, expert advisers, and nongovernmental organizations.

Teams equipped to implement epidemic control measures will be placed on-site within 24 hours' notification of an outbreak. This strategy, when implemented in Zaire, not only rapidly contained the recent Ebola outbreak but also prevented its spread to Kinshasa, the capital city of 2 million.

Among EMC's goals are 1) to strengthen local surveillance and disease control so that countries can develop the early warning systems needed to detect emerging or reemerging diseases through innovative field epidemiology and public health laboratory training programs and 2) to continue WHO's activities in developing a network of public health laboratories to strengthen regional and international collaboration in outbreak detection and control.

EMC will continue to expand WHO's network—termed WHONET—that detects and monitors antibiotic resistance worldwide. WHO will use the information collected to continue to advocate research and development of new antibiotics to replace those that are no longer effective.

For further information on WHO's rapid-response unit, contact

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Rotavirus Vaccine Workshop Held

More than 125 participants from at least 15 countries attended the Fifth Rotavirus Vaccine Workshop at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, October 16-17, 1995.

Rotavirus has emerged as the most important cause of severe diarrhea in children worldwide. It is a problem not only in developing countries, where it kills an estimated 870,000 children each year, but also in the United States, where it remains the most important single cause of hospitalization or clinic visits for childhood diarrhea.

Moreover, although studies from many countries indicate that only four serotypes are predominant worldwide, some strains at every site studied cannot be serotyped. In some countries such as India, the diversity of strains is extensive. Further studies are needed to define the extent of cross-protection against these strains that is induced by the vaccine to determine whether additional antigens need to be included in vaccines for such areas.

This workshop included sessions on epidemiology, virology, pathogenesis and immunity, and vaccines currently being tested. Each session had numerous presentations by leaders in the field of rotavirus research. Researchers reported that several live oral rotavirus vaccines, based on animal strains of rotavirus combined with reassortant strains, have been tested in field trials in children. These appear to protect American children against rotavirus and are more efficacious against severe disease. These vaccines like natural protection, are not 100% protective so many investigators are exploring alternative approaches to vaccines such as the use of virus-like particles, native DNA, and microencapsulation of antigens.

No published volume of proceedings from the workshop is planned, but a supplemental issue of the *Journal of Infectious Diseases* scheduled for early 1996 will contain papers from the meeting.

The workshop was held under the auspices of the National Institutes of Health, Emory University School of Medicine, and the World Health Organization.

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International Conference Addresses Preparedness for Emerging Strains of Pandemic Influenza

An international meeting on pertinent issues related to recognizing, identifying, and controlling newly emerging strains of pandemic influenza was held in Bethesda, Maryland, December 11–13, 1995. The conference, "Pandemic Influenza: Confronting a Reemergent Threat," was sponsored by the National Institutes of Health, the University of Michigan, the Centers for Disease Control and Prevention, the Food and Drug Administration, the U.S.-Japan Cooperative Medical Science Program, and the World Health Organization.

Epidemic strains of influenza cause infections almost every year throughout the world because of continuous minor genetic changes in the virus. However, periodically a major change occurs, such as reassortment between mammalian and avian strains of the virus. These pandemic strains are novel to the human immune system and, therefore, can cause substantial disease worldwide. The conference concentrated on issues that would be crucial to controlling an influenza pandemic.

Plenary and workshop sessions examined the following topics: Can pandemics be predicted? What are the specific approaches for pandemic control? What are the advantages and limitations of vaccines and antiviral agents? The workshops also focused on factors contributing to the emergence of pandemic strains and various aspects of surveillance, such as the adequacy of current global surveillance structure for early identification of a pandemic strain, the use of virologic and

epidemiologic surveillance once a strain is identified, and the rapid exchange of information globally. The following immunologic and molecular questions were addressed as well: What basic research advances would allow us to respond more rapidly after the next human pandemic strain is detected? Is the presence of novel influenza A virus in pigs a predictor of the next influenza pandemic? Is an H2 influenza virus the next human pandemic subtype or are H7 viruses equally possible? Also discussed were the practical issues of vaccine needs, production, and distribution.

Conference participants then reviewed international pandemic plans and the U.S. pandemic plan being prepared by the Federal Interagency Group on Pandemic Preparedness.

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Course Offered on Clinical and Pathologic Features of Emerging Infections

The Armed Forces Institute of Pathology (AFIP), Emory University, and the Centers for Disease Control and Prevention (CDC) are cosponsoring a course on emerging and reemerging pathogens. The course will be taught in Atlanta, Georgia from April 27 to May 1 and will discuss the epidemiology, clinical features, pathology, and pathogenesis of such diseases as plague, Lyme disease, Kaposi sarcoma, microsporidiosis, Buruli ulcer, ehrlichi osis, hantavirus pulmonary syndrome, and Ebola virus infection. Emerging drug resistance in pneumococci and other streptococcal infections will also be discussed.

The course is designed for pathologists, epidemiologists, infectious disease physicians, veterinarians, microbiologists, parasitologists, and others interested in the pathology as well as the emergence of infectious diseases. The course, to be held at the Emory Conference Center Hotel, will provide 38 hours of Category I CME credit and will consist of 32 hours of lectures with open discussion periods, 6 hours of glass and color slide review, and a visit to CDC laboratories. For more information, contact the course director, Center for Advanced Medical Education, AFIP, Washington, D.C. 20306-6000 (phone: 800 -577-3749 or 301-295-7921; fax: 301-427-5001).

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NASA Sponsors Symposium on Remote Sensing and Control of Insect-Transmitted Diseases

Health officials and disease control experts met November 28-30 in Baltimore, Maryland, for a symposium on the use of satellites to monitor and control insect-transmitted diseases.

Sponsored by the National Aeronautics and Space Administration (NASA) and the Third World Foundation of North America, the symposium was held to inform government officials from various countries of NASA's scientific and technologic capabilities for detecting, monitoring, and improving the control of diseases. Health ministers and medical directors from more than 20 countries, including Bangladesh, Belize, China, Ghana, Indonesia, Kenya, Malaysia, Nigeria, Peru, and Rwanda, attended.

The symposium featured discussions on the economics of disease surveillance, deforestation, and urbanization. The keynote address, "The resurgence of vector-borne infectious diseases as major public health problems in the 1990s," was given by Duane Gubler, director, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). Participants also discussed possible joint activities between NASA and interested countries. Further information can be obtained from NASA's Office of Life and Microgravity Sciences, Washington, D.C., which manages the agency's global monitoring and human health research program in conjunction with the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and CDC.

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CDC Convenes Meeting to Discuss Strategies for Preventing Invasive Group A Streptococcal Infections

Since the mid-1980s, the epidemiology of invasive group A streptococcal (GAS) infections in the United States and worldwide has changed, and the incidence of invasive infections, streptococcal toxic shock syndrome (strep TSS), and necrotizing fasciitis has increased. These changes may be the result of a shift in GAS M-types and a corresponding increase in strains that produce certain pyrogenic exotoxins. Recognizing the importance of monitoring changes in the occurrence of severe group A streptococcal disease, the Council of State and Territorial Epidemiologists recommended in April 1995 that invasive GAS infections and strep TSS be added to the National Public Health Surveillance System.

Most invasive GAS infections occur sporadically and are acquired in the community. For these cases, preventing illness and death depends on improving recognition and treatment. Primary prevention of invasive GAS disease may be more feasible for infections that are acquired in institutions (such as hospitals and nursing homes) and for secondary cases that occur among contacts of persons with invasive disease. Most nosocomial infections (for example, wound infections, postpartum endometritis, and sepsis) occur in surgical or obstetric settings, or are associated with intravenous catheters. Secondary invasive disease in the community is uncommon, although studies of household contacts of those with GAS infectionshave found a substantially increased risk for infection in this group. GAS infections spread easily from person to person after contact with respiratory secretions of an infected person and have traditionally caused epidemics of pharyngitis, scarlet fever, and rheumatic fever. Recently, clusters of invasive infections have been reported in families, hospitals, and nursing homes; community-wide outbreaks have also been reported.

As state health departments initiate surveillance for invasive GAS disease and strep TSS, guidelines for prevention will help in interpreting these data and in formulating a public health response. CDC convened a meeting of experts from academia and public health (October 10-11, 1995), to discuss existing data and strategies for preventing invasive GAS disease in institutions and the community. Discussions centered on the magnitude of risk for secondary disease among close contacts of persons with invasive infection and the potential for preventing disease by chemoprophylaxis, and on approaches for investigating and preventing infections in institutions. Recommendations are being developed, and the conclusions of the participants will be presented at a later date.

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*The members of the Working Group on Prevention of Severe Group A Streptococcal Infections are Gus Birkhead, New York State Department of Health; John Brundage, U.S. Army; Matt Cartter, Connecticut State Department of Health; Mike Gerber, University of Connecticut; Walter Hierholzer, Yale University; Ed Kaplan, University of Minnesota; Kris MacDonald, Minnesota Department of Health; Dennis Stevens, V.A. Medical Center, Boise, Idaho; Karen Green and Allison McGeer, Princess Margaret Hospital, Toronto, Ontario, Canada; Stan Shulman and Ram Yogev, Children's Memorial Hospital, Chicago, Illinois; Richard Facklam, Julia Garner, William Jarvis, Orin Levine, Benjamin Schwartz and Jay Wenger, CDC.

Regional Conference on Emerging Infectious Diseases Sparks Plan for Increased Collaboration

The World Health Organization (WHO), the Naval Medical Research Unit Three (NAMRU-3), and the Centers for Disease Control and Prevention (CDC) jointly sponsored the first conference in the region on issues of emerging and reemerging infectious diseases for members of WHO's Eastern Mediterranean Regional Office (EMRO). The meeting was held in Cairo, Egypt, November 26-29, 1995. Delegates from the WHO South-East Asia Regional Office and African Regional Office also participated.

The meeting brought together persons representing key resources that have begun working together to organize a regional program of laboratory assistance and enhanced surveillance communications for infectious diseases. Participants included WHO infectious disease program officers and key personnel from WHO collaborating centers, national reference laboratories, national infectious disease programs, ministries of health, and university public health programs.

Thirty countries were represented by more than 200 participants. On the final day of the conference, the participants adopted a regional plan of action for strengthening surveillance, laboratory capabilities, and communications.

To begin this cooperative effort, a 1-day meeting of WHO-EMRO collaborating centers was convened on November 30. Representatives from six EMRO collaborating centers attended as well as representatives from WHO-EMRO, WHO headquarters, CDC, the Naval Medical Research Institute in Washington, D.C., and the Battelle Foundation.

Participants noted that WHO collaborating centers are an excellent resource for the implementation of programs to address emerging infections because they represent some of the most competent and experienced diagnostic and reference capacities in the region. However, the representatives agreed that the relationships among the collaborating centers themselves are not well developed. Many were unaware of the existence or capabilities of most other regional collaborating centers and most had no regular communications or interactions with other collaborating centers. All welcomed closer ties with an increase in communication, collaboration, and interaction.

The participants unanimously approved a recommendation to establish an Association of EMRO collaborating centers composed of representatives from each WHO collaborating center within the region. Member organizations of the association will work together, in coordination with EMRO, to address the complex needs of surveillance, laboratory diagnostics, and disease reporting of emerging infections at the local and regional level.

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