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

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

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Streptococcal Toxic-Shock Syndrome: Spectrum of Disease, Pathogenesis, and New Concepts in Treatment

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Since the 1980s there has been a marked increase in the recognition and reporting of highly invasive group A streptococcal infections with or without necrotizing fasciitis associated with shock and organ failure. Such dramatic cases have been defined as streptococcal toxic-shock syndrome. Strains of group A streptococci isolated from patients with invasive disease have been predominantly M types 1 and 3 that produce pyrogenic exotoxin A or B or both. In this paper, the clinical and demographic features of streptococcal bacteremia, myositis, and necrotizing fasciitis are presented and compared to those of streptococcal toxic-shock syndrome. Current concepts in the pathogenesis of invasive streptococcal infection are also presented, with emphasis on the interaction between group A Streptococcus *virulence factors and host defense mechanisms. Finally, new concepts in the treatment of streptococcal toxic-shock syndrome are discussed.*

An emerging pathogen can be one that is totally new (e.g., human immunodeficiency virus), one that was known but has only recently been identified (e.g., *Helicobacter pylori*), or one that is old but has learned new tricks. The last type is, as Dr. Stanley Falkow contends, merely trying to "make a living" in a changing environment. Regardless of environmental pressures, many old pathogens have become major clinical problems because of increased virulence or antibiotic resistance (e.g., penicillin-resistant pneumococcus, multidrug resistant *Mycobacterium tuberculosis* , methicillin-resistant *Staphylococcus aureus* , and vancomycin-resistant *Enterococcus faecium*).

Arguably, group A *Streptococcus* (GAS) is the quintessence of an old organism that has become more virulent. In this manuscript, the epidemiology, clinical spectrum, and pathogenesis of GAS infection are discussed in relation to the streptococcal toxic-shock syndrome (TSS).

Current and Historical Perspectives on the Prevalence and Severity of Streptococcal Infections

The British tabloids have recently coined the term "flesh-eating bacteria" to describe invasive necrotizing infections caused by GAS and have suggested that epidemics of streptococcal infection are imminent. Such aggrandizement is unfounded, yet it has served to heighten public awareness of this sporadic, but serious, infectious disease. Strictly speaking, an epidemic is defined as an increase in the prevalence of disease over a baseline endemic rate. In this context, we are, in fact, experiencing an epidemic of severe invasive GAS infections; however, few concrete prospective population-based data support this notion. Estimates suggest that the incidence of these infections is 10 to 20 cases/100,000 population. Thus, the stimulus for such public interest has not been the incidence of the syndrome, but more likely, the dramatic nature of these infections.

Whether these types of group A streptococcal infections will decline, stay the same, or increase is not known. History is replete with descriptions of epidemics of GAS infections and their nonsuppurative sequelae. In the 1600s, epidemics of scarlet fever spread from Italy and Spain to Northern Europe (1), and in 1736, an outbreak occurred in the American colonies, killing 4,000 people (2). Major epidemics of rheumatic fever occurred in World War II in the U.S. military (3). Soon afterward post-streptococcal glomerulonephritis struck several regions of the United States (4,5).

Many of these epidemics waxed and waned before the advent of antibiotics, suggesting that either changes in socioeconomic conditions or variations in the expression of virulence factors by the pathogen were responsible. This concept is best exemplified by the extraordinary mortality rate of scarlet fever documented in the latter part of the 1880s in New York, Chicago, and Norway; 25% to 30% of children with scarlet fever died during that period (5,6). By 1900, the mortality rate had dropped to under 2% in all three locations. Since socioeconomic conditions

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likely did not change markedly during that time and antibiotics were not yet available, the decrease in mortality rates must have been caused by reduced expression of a streptococcal virulence factor or by the slow acquisition of herd immunity to that factor.

The epidemiology of GAS infection is complex. More than 80 different M types of *S. pyogenes* exist, and five separate and distinct scarlatina toxins, streptococcal pyrogenic exotoxins (SPEs) (5) have also been described; some of these can be transmitted to different M types by bacteriophage. Minor drifts in the antigenic or virulence properties of GAS could account for the 5- to 6-year cycles of scarlet fever documented by Kohler (9). In the same way as antigenic shifts in influenza virus cause pandemics, major alterations in GAS virulence properties could cause major changes in clinical disease. The recent increases in severe GAS infections, following a 50- to 60-year span of relatively benign clinical disease, support this notion.

Acute Life-Threatening Group A Streptococcal Infections

Streptococcal TSS

Recently, severe invasive GAS infections associated with shock and organ failure have been reported with increasing frequency, predominantly from North America and Europe (8-18). These infections have been termed streptococcal toxic-shock syndrome (TSS; Table 1) (19). Persons of all ages are affected; most do not have predisposing underlying diseases (11,20-25). This is in sharp contrast to previous reports of GAS bacteremia, in which patients were either under 10 or over 60 years of age, and most had underlying conditions such as cancer, renal failure, leukemia, or severe burns or were receiving corticosteroids or other immunosuppressing drugs (20-22). The complications of current GAS infections are severe; bacteremia associated with aggressive soft tissue infection, shock, adult respiratory distress syndrome and renal failure are common; 30% to 70% of patients die in spite of aggressive modern treatments (Table 2) (1,8,24-26).

Acquisition of Group A *Streptococcus*

The portal of entry of streptococci cannot be proven in at least half the cases (8) and can only be presumed in many others. Patients with symptomatic pharyngitis rarely develop streptococcal TSS, though such cases have been reported, especially in the last year. Procedures such as suction lipectomy, hysterectomy, vaginal delivery, bunionectomy and bone pinning have provided a portal of entry in many cases (author's unpublished observations). Most commonly, infection begins at a site of minor local trauma, which frequently does not result in a break in the skin (8). Numerous cases have developed within 24 to 72 hours of minor nonpenetrating trauma, resulting in hematoma, deep bruise to the calf, or even muscle strain. Virus infections, such as varicella and influenza, have provided a portal in other cases. In some cases the use of nonsteroidal antiinflammatory agents may have either masked the early symptoms or predisposed the patient to more severe streptococcal infection and shock (1). For the most part, these infections have occurred sporadically and have not been associated with clusters of cases or minor epidemics, though outbreaks of severe GAS infections have occurred in closed environments such as nursing homes (27,28).

Clinical Symptoms

Pain—the most common initial symptom of streptococcal TSS—is abrupt in onset and severe, and usually precedes tenderness or physical findings. The pain usually involves an extremity but may also mimic peritonitis, pelvic inflammatory disease, pneumonia, acute myocardial infarction, or pericarditis. Twenty percent of patients have an influenza-like syndrome characterized by fever, chills, myalgia, nausea, vomiting, and diarrhea (8).

Fever is the most common early sign, although hypothermia may be present in patients with shock. Confusion is present in 55% of patients, and in some, coma or combativeness is manifest (8). Eighty percent of patients have clinical signs of soft tissue infection, such as localized swelling and erythema, which in 70% of patients progressed to necrotizing fasciitis or myositis and required surgical debridement, fasciotomy or amputation (8). An ominous sign is the progression of soft tissue swelling to the formation of vesicles, then bullae, which appear violaceous or bluish. In such patients, emergent surgical exploration should be performed to establish the diagnosis and distinguish GAS infection from other necrotizing soft tissue infections. Among the 20% of patients without soft tissue findings, clinical symptoms include endophthalmitis, myositis, perihepatitis, peritonitis, myocarditis, and overwhelming sepsis. A diffuse, scarlatina-like erythema occurs in only 10% of patients. Nearly 50% of patients may have normal blood pressure (systolic pressure >110 mm Hg) on admission but develop hypotension within the subsequent 4 hours (8).

Laboratory Evaluation of Patients

On admission, renal involvement is indicated by the presence of hemoglobinuria and by serum creatinine values that are, on average, >2.5 times normal. Renal impairment precedes hypotension in 40% to 50% of patients (8). Hypoalbuminemia is associated with hypocalcemia on admission and throughout the hospital course. The serum creatinine kinase level is useful in detecting deeper soft-tissue infections;

when the level is elevated or rising, there is a good correlation with necrotizing fasciitis or myositis. Though the initial laboratory studies demonstrate only mild leukocytosis, the mean percentage of immature neutrophils (including band forms, metamyelocytes, and myelocytes) is striking, reaching 40% to 50%. Blood cultures are positive in 60% of cases (8).

Clinical Course

Shock is apparent at the time of admission or within 4 to 8 hours in virtually all patients (Table 2). In only 10% of patients does systolic blood pressure become normal 4 to 8 hours after administration of antibiotics, albumin, and electrolyte solutions containing salts or dopamine; in all other patients, shock persists. Similarly, renal dysfunction progresses or persists in all patients for 48 to 72 hours in spite of treatment, and many patients may require dialysis (8). In patients who survive, serum creatinine values return to normal within 4 to 6 weeks. Renal dysfunction precedes shock in many patients and is apparent early in the course of shock in all others. Acute respiratory distress syndrome

Table 1. Case definition of streptococcal toxic-shock syndrome (streptococcal TSS) and necrotizing fasciitis*

I. Streptococcal TSS
- A. Isolation of group A *Streptococcus*
  1. From a sterile site
  2. From a nonsterile body site
- B. Clinical signs of severity
  1. Hypotension
  2. Clinical and laboratory abnormalities (requires two or more of the following):
     a) Renal impairment
     b) Coagulopathy
     c) Liver abnormalities
     d) Acute respiratory distress syndrome
     e) Extensive tissue necrosis, i.e., necrotizing fasciitis
     f) Erythematous rash

Definite Case = A1 + B(1+2)
Probable Case = A2 + B(1+2)

II. Necrotizing fasciitis
- A. Definite case
  1. Necrosis of soft tissues with involvement of the fascia

PLUS
  2. Serious systemic disease, including one or more of the following:
     a) Death
     b) Shock (systolic blood pressure <90 mm of Hg).
     c) Disseminated intravascular coagulopathy
     d) Failure of organ systems
        a. respiratory failure
        b. liver failure
        c. renal failure
  3. Isolation of group A *Streptococcus* from a normally sterile body site
- B. Suspected case
  1. 1 + 2 and serologic confirmation of group A streptococcal infection by a 4-fold rise against:
     a) streptolysin O
     b) DNase B
  2. 1 + 2 and histologic confirmation:
     Gram-positive cocci in a necrotic soft tissue infection

* Streptococcal toxic-shock syndrome (streptococcal TSS) is defined as any group A streptococcal infection associated with the early onset of shock and organ failure. Definitions describing criteria for shock, organ failure, definite cases, and probable cases are included below.

Source: reference 61.

Table 2. Complications of group A streptococcal soft-tissue infection

Complication	Percentage of Patients
Shock	95
Acute respiratory distress syndrome	55
Renal impairment	80
Irreversible	10
Reversible	70
Bacteremia	60
Death	30

Source: reference 1.

occurs in 55% of patients and generally develops after the onset of hypotension (8). Supplemental oxygen, intubation, and mechanical ventilation are necessary in 90% of the patients in whom this syndrome develops. Mortality rates vary from 30% to 70% (1,8,24-26). Morbidity is also high; 13 of 20 patients in one series underwent major surgical procedures, which included fasciotomy, surgical debridement, exploratory laparotomy, intraocular aspiration, amputation, or hysterectomy (8).

Clinical Isolates

M types 1, 3, 12, and 28 have been the most common isolates from patients with shock and multiorgan failure (8,29). Recently, 80% of strains in Sweden from all types of GAS infection have been M type 1 (S. Holm, pers. comm.). Pyrogenic exotoxin A and/or B was found in most cases of severe infection. In the United States, pyrogenic exotoxin A is most frequently associated with these infections (8,23,29-33), while in Sweden and the United Kingdom, exotoxin B has been most common (12,25). Recently, streptococcal superantigen (SSA), a novel pyrogenic exotoxin, was isolated from an M 3 strain, albeit in small concentrations (34). In addition, mitogenic factor (MF) has been demonstrated in many different M types of GAS (35,36).

Necrotizing Fasciitis

Necrotizing fasciitis, a deep-seated infection of the subcutaneous tissue that progressively destroys fascia and fat but may spare the skin and muscle, can be caused by GAS, *Clostridium perfringens*, or *C. septicum.* Necrotizing fasciitis caused by mixed organisms such as aerobic gram-negative bacteria, anaerobes, and microaerophilic streptococci may develop in diabetic patients or patients with open wounds contaminated with bowel contents. Though Meleney called infections caused by hemolytic streptococci "streptococcal gangrene" (37), the process has been renamed necrotizing fasciitis. His patients' infections began at the site of trivial or inapparent trauma. Within 24 hours of the initial lesion—which frequently was only mild erythema—swelling, heat, erythema, and tenderness rapidly developed. During the next 24 to 48 hours, the erythema changed from red to purple and then to blue, and blisters and bullae, which contained clear yellow fluid, appeared. On days 4 and 5, the purple areas became gangrenous. From day 7 to day 10, the line of demarcation became sharply defined, and the dead skin began to separate at the margins or breaks in the center, revealing an extensive necrosis of the subcutaneous tissue. In more severe cases, the process advanced rapidly until several large areas of skin became gangrenous, and the intoxication rendered the patient dull, unresponsive, mentally cloudy, or even delirious. Meleney was the first to advocate aggressive "bear scratch" fasciotomy and debridement. With this treatment, together with irrigation with Dakains solution, the mortality rate dropped to 20% (37).

These older reports of necrotizing fasciitis (6) differ from reports of current necrotizing fasciitis cases associated with streptococcal TSS (8). First, recent cases have mainly occurred in young healthy persons who had no underlying disease but sustained minor trauma to an extremity. Earlier series describe older patients with multiple medical problems (6). Meleney's cases (reported from China) were probably among young healthy persons who sustained minor trauma, though the major difference between them and present cases is the low mortality rate (20% vs 20% to 60% in streptococcal TSS) (6,37) before antibiotics were available (37). Analysis of Meleney's reports also suggests that most of his patients did not have shock or organ failure, nor did they require amputation. In contrast, present cases of necrotizing fasciitis caused by GAS are invariably associated with severe manifestations of systemic illness and high morbidity despite the absence of underlying disease and the use of antibiotics, dialysis, ventilators, intravenous fluids, and improved surgical techniques. In summary, the high mortality rate among current cases of streptococcal necrotizing fasciitis could be due to the emergence of more virulent streptococci (8).

Streptococcal Myositis

Streptococcal myositis is an extremely uncommon GAS infection. Adams et al. (38) documented only 21 reported cases from 1900 to 1985, and Svane (39) found only four cases in more 20,000 autopsies. Severe pain may be the only early symptom, and swelling and erythema may be the only early physical findings, though muscle compartment syndromes may develop rapidly (8-10,38-41). Distinguishing streptococcal myositis from spontaneous gas gangrene caused by *C. perfringens* or *C. septicum* (42) may be difficult, though crepitus or demonstration of gas in the tissue favors clostridial infection (40). Patients with streptococcal TSS may

have both necrotizing fasciitis and myositis (8,38). In published series, the case-fatality rate for necrotizing fasciitis is 20% to 50%, whereas GAS myositis has a fatality rate of 80% to 100% (6). Aggressive surgical debridement is extremely important for establishing a diagnosis and removing devitalized tissue.

Bacteremia

Streptococcal bacteremia has occurred most commonly in the very young and in the elderly (5). Among children, predisposing factors (other than scarlet fever) include burns, varicella, malignant neoplasm, immunosuppression, and age less than 2 years (5). In patients with scarlet fever, the pharynx is the most common source of GAS. Frequently such patients have complications, such as extension of infection into the sinuses, peritonsillar tissue, or mastoids (septic scarlet fever or scarlet fever anginose); yet documented bacteremia occurs in only 0.3% of febrile patients (43). Among the children with varicella studied by Bullowa and Wischik (43), GAS bacteremia occurred in only approximately 0.5% of patients.

In elderly patients the source of GAS infection is invariably the skin and is associated with cellulitis or erysipelas (5). GAS sepsis in the elderly (mean age, 50 to 60 years) has also been associated with diabetes, peripheral vascular disease, malignancy, and corticosteroid use. Not surprising, mortality rates of 35% to 80% have been described in this patient population. In the past, GAS bacteremia was rare among persons 14 to 40 years of age; puerperal sepsis accounted for most bacteremia in this age group. Recently, intravenous drug abuse has emerged as a leading cause of GAS bacteremia in this age group (5). Martin and Hoiby have comprehensively demonstrated that the prevalence of GAS bacteremia in Norway in the late 1980s increased in all age groups, but the greatest increase (600% to 800%) was in adolescents and young adults (10). Thus, the demographics of invasive streptococcal infections have changed dramatically in the past 4 to 6 years.

Current Hypotheses Regarding Mechanisms of Shock and Tissue Destruction Caused by Virulent Group A Streptococci

Pyrogenic exotoxins cause fever in humans and animals and also help induce shock by lowering the threshold to exogenous endotoxin (5). Streptococcal pyrogenic exotoxins A and B induce human mononuclear cells to synthesize not only tumor necrosis factor- α (TNF α) (44) but also interleukin-1 β (IL-1 β) (45) and interleukin-6 (IL-6) (45), suggesting that TNF could mediate the fever, shock, and tissue injury observed in patients with streptococcal TSS (8). Pyrogenic exotoxin C has been associated with mild cases of scarlet fever in the United States (author's observations) and in England (46). The roles of two newly described pyrogenic exotoxins, SSA and MF (see section on "Clinical Isolates"), in streptococcal TSS have not been elucidated.

M protein contributes to invasiveness through its ability to impede phagocytosis of streptococci by human polymorphonuclear leukocytes (47). Conversely, type-specific antibody against the M protein enhances phagocytosis (47). After infection with a particular M type, specific antibody confers resistance to challenge to viable GAS of that M type (47). While M types 1 and 3 strains have accounted for most strains isolated from cases of streptococcal TSS, many other M types, including some nontypable strains, have also been isolated from such cases. M types 1 and 3 are also commonly isolated from asymptomatic carriers, patients with pharyngitis, and patients with mild scarlet fever (7,29).

Could streptococcal TSS be related to the ability of pyrogenic exotoxin or M proteins type 1 or 3 to act as "super antigens" (48)? Data suggest that this exotoxin and a number of staphylococcal toxins (toxic shock syndrome toxin-1 [TSST-1] and staphylococcal enterotoxins A, B, and C) can stimulate T-cell responses through their ability to bind to both the Class II major histocompatibility ability complex of antigen-presenting cells and the V β region of the T-cell receptor (48). The net effect would be to induce T-cell stimulation with production of cytokines capable of mediating shock and tissue injury. Recently, Hackett and Stevens demonstrated that pyrogenic exotoxin A induced both TNF α and TNF β from mixed cultures of monocytes and lymphocytes (49), supporting the role of lymphokines (TNF β) in shock associated with strains producing that exotoxin. Kotb et al. (50) have shown that a digest of M protein type 6 can also stimulate T-cell responses by this mechanism; however, the role of specific superantigens in this or any other infectious disease has not been proven. Proof would require demonstration of massive expansion of T-cell subsets bearing a V β repertoire specific for the putative superantigen. However, quantitation of such T-cell subsets in patients with acute streptococcal TSS demonstrated deletion rather than expansion, suggesting that perhaps the life span of the expanded subset was shortened by a process of apoptosis (51). In addition, the subsets deleted were not specific for streptococcal pyrogenic exotoxins A, B, C, or mitogenic factor, suggesting that an as yet undefined superantigen may play a role (51).

Cytokine production by less exotic mechanisms likely contributes as well to the genesis of shock and organ failure. Peptidoglycan, lipoteichoic acid (52), and killed organisms (53,54) are capable of inducing TNF α production by mononuclear cells in vitro

(6,54,55). Exotoxins such as streptolysin O (SLO) are also potent inducers of TNF α and IL-1 β. Pyrogenic exotoxin B, a proteinase precursor, has the ability to cleave pre-IL-1 β to release preformed IL-1β (56). Finally, SLO and exotoxin A together have additive effects in the induction of IL-1 β by human mononuclear cells (49). Whatever the mechanisms, induction of cytokines in vivo is likely the cause of shock, and these two exotoxins, cell wall components, and the like, are potent inducers of TNF and IL-1.

The mere presence of virulence factors, such as M protein or pyrogenic exotoxins, may be less important in streptococcal TSS than the dynamics of their production in vivo. Recently, Cleary et al. proposed a regulon in GAS that controls the expression of a group of virulence genes coding for known virulence factors such as M protein and C5 peptidase (57). When DNA fingerprinting was used, differences were shown between M1 strains isolated from patients with invasive disease and strains from patients with noninvasive GAS infections (58). Finally, genetic information coding for exotoxins A or C may be introduced to strains of GAS by certain bacteriophage; after lysogenic conversion, synthesis of exotoxin A would occur during growth of the streptococcus (31,59,60). Multilocus enzyme electrophoresis demonstrates two patterns that correspond to the M1 and M3 type organisms that produce pyrogenic exotoxin A, a finding that supports epidemiologic studies implicating these strains in invasive GAS infections (33).

The interaction between these microbial virulence factors and an immune or nonimmune host determines the epidemiology, clinical syndrome, and outcome. Since horizontal transmission of GAS in general is well documented, the only explanation for the absence of a high attack rate of invasive infection is significant herd immunity against one or more of the virulence factors responsible for streptococcal TSS. This hypothetical model explains why epidemics have not materialized and why a particular strain of GAS can cause different clinical manifestations in the same community (8,61) (Figure 1).

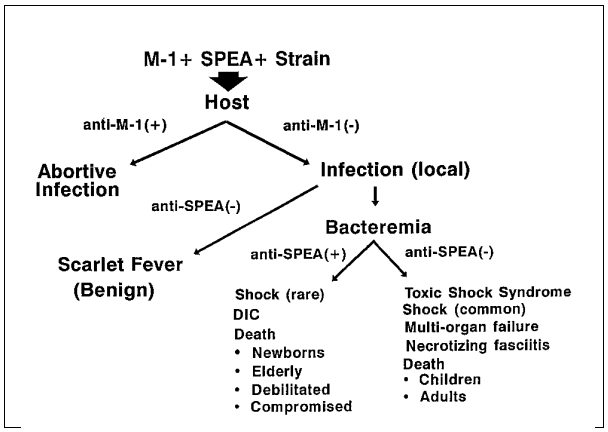

Figure 1. Pathogenesis of scarlet fever, bacteremia, and toxic shock syndrome. M-1 $^{+}$ SPEA $^{+}$ = a GAS strain that contains M protein type 1 and streptococcal pyrogenic exotoxin A (SPEA); +anti-M-1 = the presence of antibody to M protein type 1; -anti-M-1 = the absence of antibody to M protein type 1'; anti-SPEA+ = antibody to SPEA; and DIC - disseminated intravascular coagulation.

Treatment

Antibiotic Therapy – Cures and Failures with Penicillin

S. pyogenes continues to be exquisitely susceptible to ß-lactam antibiotics, and numerous studies have demonstrated the clinical efficacy of penicillin preparations for streptococcal pharyngitis. Similarly, penicillins and cephalosporins have proven efficacy in treating erysipelas, impetigo, and cellulitis, all of which are most frequently caused by *S. pyogenes* . In addition, Wannamaker et al. (6) demonstrated that penicillin therapy prevents the development of rheumatic fever following streptococcal pharyngitis if therapy is begun within 8 to 10 days of the onset of sore throat. Nonetheless, some clinical failures of penicillin treatment of streptococcal infection do occur. Penicillin treatment of *S. pyogenes* has failed to eradicate bacteria from the pharynx of 5% to 20% of patients with documented streptococcal pharyngitis (62-64). In addition, more aggressive GAS infections (such as, necrotizing fasciitis, empyema, burn wound sepsis, subcutaneous gangrene, and myositis) respond less well to penicillin and continue to be associated with high mortality rates and extensive morbidity (6,8,9,12,15,38,65). For example, in a recent report, 25 cases of streptococcal myositis had an overall mortality rate of 85% in spite of penicillin therapy (38). Finally, several studies in experimental infection suggest that penicillin fails when large numbers of organisms are present (66,67).

The Efficacy of Penicillin, Compared to Clindamycin, In Fulminant Experimental *S. pyogenes* Infection

In a mouse model of myositis caused by *S. pyogenes* , penicillin was ineffective when treatment was delayed ≥2 hours after initiation of infection (67). Survival of erythromycin-treated mice was greater than that of both penicillin-treated mice and untreated controls, but only if treatment was begun within 2 hours. Mice receiving clindamycin, however, had survival rates of 100%, 100%, 80%, and 70%, even if treatment was delayed 0, 2, 6, and 16.5 hours, respectively (67,68).

Eagle suggested that penicillin failed in this type of infection because of the "physiologic state of the

organism" (66). This phenomenon has recently been attributed to both in vitro and in vivo inoculum effects (69,70).

Inoculum Size and the "Physiologic State of the Organism": Differential Expression of Penicillin-Binding Proteins

Penicillin and other ß-lactam antibiotics are most efficacious against rapidly growing bacteria. We hypothesized that large inocula reach the stationary phase of growth sooner than smaller inocula both in vitro and in vivo. That high concentrations of *S. pyogenes* accumulate in deep-seated infection is supported by data from Eagle et al. (66). We compared the penicillin-binding protein patterns from membrane proteins of group A streptococci isolated from different stages of growth, i.e., mid-log phase and stationary phase. Binding of radiolabeled penicillin by all penicillin-binding proteins was decreased in stationary cells; however, PBPs 1 and 4 were undetectable at 36 hours (69). Thus, the loss of certain penicillin-binding proteins during stationary-phase growth in vitro may be responsible for the inoculum effect observed in vivo and may account for the failure of penicillin in treatment of both experimental and human cases of severe streptococcal infection.

The Greater Efficacy of Clindamycin in Experimental *S. pyogenes* Infections: Mechanisms of Action

The greater efficacy of clindamycin is likely multifactorial: First, its efficacy is not affected by inoculum size or stage of growth (69,71); secondly, clindamycin is a potent suppressor of bacterial toxin synthesis (72,73); third, it facilitates phagocytosis of *S. pyogenes* by inhibiting M-protein synthesis (73); fourth, it suppresses synthesis of penicillin-binding proteins, which, in addition to being targets for penicillin, are also enzymes involved in cell wall synthesis and degradation (71); fifth, clindamycin has a longer postantibiotic effect than ß-lactams such as penicillin; and lastly, clindamycin causes suppression of LPS-induced monocyte synthesis of TNF (74). Thus, clindamycin's efficacy may also be related to its ability to modulate the immune response.

Other Treatment Measures

Though antibiotic selection is critically important, other measures, such as prompt and aggressive exploration and debridement of suspected deep-seated *S. pyogenes* infection, are mandatory. Frequently, the patient has fever and excruciating pain. Later, systemic toxicity develops, and definite evidence of necrotizing fasciitis and myositis appears. Surgical debridement may be too late at this point. Prompt surgical exploration through a small incision with visualization of muscle and fascia, and timely Gram stain of surgically obtained material may provide an early and definitive etiologic diagnosis. Surgical colleagues should be involved early in such cases, since later in the course surgical intervention may be impossible because of toxicity or because infection has extended to vital areas impossible to debride (i.e., the head and neck, thorax, or abdomen).

Anecdotal reports suggest that hyperbaric oxygen has been used in a handful of patients, though no controlled studies are under way, nor is it clear that this treatment is useful.

Because of intractable hypotension and diffuse capillary leak, massive amounts of intravenous fluids (10 to 20 liters/day) are often necessary. Pressors such as dopamine are used frequently, though no controlled trials have been performed in streptococcal TSS. In patients with intractable hypotension, vasoconstrictors such as epinephrine have been used, but symmetrical gangrene of digits seems to result frequently (author's unpublished observations), often with loss of limb. In these cases it is difficult to determine if symmetrical gangrene is due to pressors, infection, or both.

Neutralization of circulating toxins would be desirable; however, appropriate antibodies are not commercially available in the United States or Europe. Two reports describe the successful use of intravenous gamma globulin in treating streptococcal TSS in two patients (75,76).

In summary, if a wild "flesh-eating strain" has recently emerged, a major epidemic with a high attack rate would normally be expected. Clearly, epidemics of streptococcal infections, including impetigo, pharyngitis, scarlet fever, and rheumatic fever have occurred in the past. However, in the last decade, subsequent to early reports of streptococcal TSS, we have observed that the incidence has remained relatively low. I hypothesize that large outbreaks have not occurred because 1) most of the population probably has immunity to one or more streptococcal virulence factors (6,25); 2) predisposing conditions (e.g., varicella, and use of NSAIDs) are required in a given patient; and 3) only a small percentage of the population may have an inherent predisposition to severe streptococcal infection because of constitutional factors such as HLA Class II antigen type (77,78), B-cell (79), or specific V β regions on lymphocytes. This last hypothesis is further supported by the observation that secondary cases of streptococcal TSS, though reported (80), have been rare.

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Spiral Bacteria in the Human Stomach: The Gastric Helicobacters

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During the past decade, Helicobacter pylori *has become recognized as one of the most common human pathogens, colonizing the gastric mucosa of almost all persons exposed to poor hygienic conditions from childhood. It also is often found, albeit with a lower frequency, in groups of high socioeconomic status.* H. pylori *causes chronic active gastritis and is a major factor in the pathogenesis of duodenal ulcers and, to a lesser extent, gastric ulcers. In addition, the presence of this bacterium is now recognized as a risk factor for gastric adenocarcinoma and lymphoma. Nevertheless, most infections appear without clinical consequences. In this second decade of intensive research, it is important to understand why* H. pylori *is sometimes a dangerous pathogen, and to determine how it can be eradicated in those at highest risk for severe disease.*

At the end of the 19th century, several types of spirochetes and spirilla were observed for the first time in the stomach of animals (1,2). Beginning at the turn of the 20th century, similar spiral bacteria were found in gastrectomy specimens from patients with gastric cancer and peptic ulcer disease (3,4). In addition, gastroenterologists and surgeons noted—but could not explain—the almost universal presence of antral gastritis in patients with duodenal ulcers and the frequent presence of atrophic gastritis in patients with gastric ulcer and cancer. Nevertheless, the possibility that peptic ulcer disease or gastric cancer might be caused by an infectious agent was generally discounted. The observation made in 1975 that gram-negative bacteria were present in 80% of patients with gastric ulcer (5) was largely ignored by the scientific community which, at the time, was busily developing potent antiulcer agents (6). Skepticism remained the overwhelming reaction to the 1983 reports describing the frequent association between antral gastritis and the presence of *Campylobacter* -like bacteria (7), as well as of their culture and isolation from patients with gastritis (8). A similar reaction followed the subsequent demonstration that these *Campylobacter* -like bacteria were present in almost all patients with gastric and duodenal ulcers, and were generally associated with antral gastritis (9). In the past decade, however, a number of studies have confirmed and extended these early observations. A consensus regarding the major role of this bacterium, now named *Helicobacter pylori* , in causing gastroduodenal ulceration was formally presented in 1994 (10).

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Furthermore, in June 1994, the International Agency for Research on Cancer Working Group stated , " *H. pylori* plays a causal role in the chain of events leading to cancer," referring to adenocarcinoma and lymphoma of the stomach as well as to the more benign mucosal-associated lymphoid tissues (MALT) (11-13).

An important consequence of the considerable interest generated by these clinical observations is that extensive bacteriologic and molecular studies have been performed on this bacterium and similar organisms. 16S rRNA gene sequence analysis has revealed important differences between *H. pylori* and the closely related *Campylobacter* , *Flexispira,* and *Wolinella* genuses. These differences have necessitated the creation of the genus *Helicobacter* , which, to date, includes eight gastric, three intestinal, and two hepatic species (14). Each of these *Helicobacter* species colonizes different, or a spectrum of, mammalian species.

This review summarizes our current knowledge of the two *Helicobacter* species that have been observed in the human stomach and reported on extensively in the literature: *H. pylori* , the type strain, and *H. heilmannii* , also known as *Gastrospirillum hominis* (15,16).

Characteristics of Gastric Helicobacters Observed in Humans

H. pylori , a gram-negative bacterium with a curved, spiral, or gull-wing shape, is 2.5 to 3.5 µm long and 0.5 to 1.0 µm in diameter and has a periodicity of 1 to 2 µm. It has smooth surfaces, and one to six polar-sheathed flagellae emerge from one of its rounded ends. Since it is morphologically similar to *C. jejuni* , it was initially named "pyloric *Campy-*

lobacter " and subsequently *C. pyloridis* and *C. pylori* before finally being named *H. pylori* . This organism colonizes only the non-acid-secreting mucosa of the stomach and is not found where parietal cells are numerous. Thus, it may be observed in the gastric antrum and the cardia, but also in the corpus, when atrophic gastritis is present, and attached to the gastric epithelial cells found in the duodenum, when gastric metaplasia is present.

G. hominis (*H. heilmannii*) is tightly spiraled, and is 3.5 to 7.5 µm in length and 0.9 µm in diameter; it has a periodicity of 0.8 to 1 µm and up to 12 flagellae at each pole. 16S rRNA indicates that this organism belongs to the genus *Helicobacter* , and is more closely related to a *Helicobacter* sp. isolated from the stomach of cats (*H. felis*) than to *H. pylori* (17). The name *H. heilmannii* was proposed in honor of the late German pathologist Heilmann. However, the subsequent examination of the rRNA of different clinical isolates indicates that there is enough heterogeneity among isolates tentatively identified as *H. heilmannii* that it is premature to propose an official name (17) . This bacterium colonizes only the parietal cell area of the gastric mucosa and may be found within parietal cells (18,19).

Diagnosis

H. pylori infection may be diagnosed by harvesting gastric biopsy specimens during endoscopy, by culturing and isolating the bacterium under microaerobic conditions (90% N_2, 5% O_2, and 5% CO_2), and by characterizing the enzymes (urease, catalase, and oxidase) it produces. Visualization of the bacterium by light microscopy on slides stained with hematoxylin and eosin, Gram, Giemsa, Genta, or Warthin-Starry stain is also of great benefit since it allows the concurrent diagnosis of the extent of the antral chronic-active gastritis that *H. pylori* causes. However, because *H. pylori* colonization is focal, negative biopsy results do not exclude the possibility of infection in areas not sampled. Infection also may be diagnosed by determining plasma and salivary immunoglobulin (Ig) G or IgA levels with enzyme-linked immunosorbent assays (20,21). This latter technique is noninvasive, specific, and sensitive and is believed to reflect the mucosal and systemic immunity induced by *H. pylori* infection.

Two other tests, which rely on the production of urease, also can be used to identify *H. pylori* . One is the CLO (for *Campylobacter* -like organisms) test, which is performed by placing a mucosal biopsy specimen in medium containing urea and a pH-sensitive dye that changes color in the presence of OH^- ions. The second test is the noninvasive ^{14}C or ^{13}C breath test following the oral administration of ^{14}C- or ^{13}C-urea. Neither of these tests is specific for *H. pylori* since *G. hominis* , which generates urease, also gives a positive reaction. Until specific methods based on the polymerase chain reaction (PCR) amplification of 16S rRNA (17) become widely available, the diagnosis of *G. hominis* infection must rely on histologic morphologic characteristics; histologic identification must be confirmed by transmission electron microscopy since other spiral organisms, e.g., *Flexispira rappini* , also may be present in the stomach of humans (22).

Epidemiology

The seroepidemiology of *H. pylori* has been extensively studied in the United States and in other countries (23). The high frequency of seropositivity (up to 100% in some age groups in Albania) and acquisition of the infection during infancy are characteristic of disadvantaged socioeconomic groups living in crowded or poor hygienic conditions and appears to be independent of gender and ethnic origin. In adults of higher socioeconomic groups, the rate of seroconversion is estimated at 0.5% per year, although the frequency of seropositivity increases with age and may be as high as 40%. A longitudinal study has indicated that the high frequency of seropositivity in older adults might be due to a higher rate of *H. pylori* infection in Western countries in the years between the two world wars than during recent years (cohort effect) (24). Alternatively, the increase in frequency of infection in older adults might be due to years of low but cumulative risk for infection. Although the route of transmission for this infection is not known, the contamination of drinking water may play a role in certain developing countries (25). In the United States and in other regions, direct contact and/or consumption of food or water contaminated by saliva (26), gastric contents, or feces (27) may be major factors. The recent observation that *H. pylori* can be isolated from cats (28) suggests that transmission from pets to humans (or humans to pets) is also possible.

The epidemiology and route of transmission of *G. hominis* are largely unknown. The frequency of this infection appears to range from less than 1% of the population in industrialized countries (29) to 3% to 8% in developing countries (30). Although the detection of spirilla in the stomach of cats and dogs suggests possible transmission from pets, marked morphologic differences exist between these spirilla and the organism found in the stomach of humans.

Pathogenicity

H. pylori is considered a pathogen because its presence is always associated with chronic active gastritis, and eradication of the bacterium is always followed by resolution of gastritis. In addition,

nearly all patients with duodenal ulcer disease have *H. pylori* gastritis, and ulcer relapse is exceptional after *H. pylori* eradication. Thus, the presence of *H. pylori* seems necessary for the production of duodenal ulcers, with the exception of ulcers attributed to the use of nonsteroidal antiinflammatory agents or to the Zollinger-Ellison syndrome (10). The association with gastric ulcers is not as strong, although *H. pylori* infection is present in 80% of patients with gastric ulcers who do not consume nonsteroidal antiinflammatory agents (10). However, most *H. pylori*-infected persons do not report any clinical symptoms. This may be because these persons are colonized by less virulent strains or because other host or bacterial cofactors are required for overt disease.

In addition, three prospective cohort studies have demonstrated that *H. pylori*-infected persons have an increased risk of developing intestinal-type, but not undifferentiated, gastric adenocarcinoma (10). In fact, the association of *H. pylori* with either gastric ulcer or gastric cancer may be underestimated in these studies: the atrophic gastritis that follows long-term infection makes the gastric niche less hospitable for the bacterium, which may either eliminate *H. pylori* or make it difficult to detect. Nevertheless, atrophic gastritis per se is believed to be a precancerous lesion that leads to carcinogenesis without the presence of *H. pylori*.

The pathogenicity of *G. hominis* is unclear. The organism has been associated with upper gastrointestinal complaints, and its carriage is generally accompanied by gastritis, although the inflammation and gastric atrophy are less than noted with *H. pylori* (31,32). In addition, *G. hominis* was observed in gastric cancer patients (3) as well as in patients with only minimal gastritis (29). In this relatively small number of cases, the frequent concurrent infection with *H. Pylori* makes interpreting the respective pathogenic role of either bacterium difficult. It is probable that *G. hominis* will turn out to be at least somewhat pathogenic, as it makes urease and products of urease action that have been implicated in inflammation.

Colonization and Virulence Factors

H. pylori multiplies with great efficiency in the hostile environment within the stomach but survives poorly in the gastric lumen; it is mainly found where the pH ranges between 4 and 7, i.e., under the mucous layer and in close proximity, or even attached, to gastric superficial epithelial cells. The virulence and the ecologic niche of *G. hominis* are unknown, although its presence within parietal cells of patients with gastrointestinal complaints (18,19) suggests that it is even more resistant to acid than *H. pylori*.

The production of urease was the first putative colonization or virulence factor studied. The production of this enzyme is shared by the two organisms, and it may explain their extraordinary ability to survive in an environment previously considered sterile because of the presence of proteolytic enzymes, as well as the low pH of gastric contents. Because the ecologic niches of these bacteria are rich in urea, urease generates OH^- ions that neutralize gastric acid. Although the neutralization of gastric acid benefits the two bacteria, the production of hydroxide ions also is toxic to gastric epithelial cells in vivo, as indicated by in vitro experiments (33).

Two other important virulence factors shared by *H. pylori* and *G. hominis* are their spiral shape and the motility of their flagellae, which render them resistant to peristaltic flushing of the gastric contents and enable them to persist in the mucous layer. Because *G. hominis* appears to infect fewer persons than *H. pylori*, a more important role might be attributable to characteristics that are unique to *H. pylori*; these include the production of other enzymes (catalase, oxidase, protease, and phospholipase), as well as the synthesis of specific adhesin proteins that enable them to adhere to mucous and epithelial cells, both in vivo and in vitro (34-36).

The putative virulence factor of *H. pylori* that has commanded the most attention during the past few years has been its vacuolating cytotoxin (*vacA* gene product). Intragastric administration of the toxin to mice causes some (but not all) of the tissue damage seen in *H. pylori*-infected persons (37). In addition, cytotoxin production is highly correlated with the production of a high molecular weight (120 to 128 kilodaltons) major protein antigen that is called cytotoxin-associated protein (*cagA*) and is not the toxin itself (38).

Diversity of *H. pylori*

H. pylori isolates may differ with respect to each of the virulence factors described above; this diversity is likely to contribute to variation in colonization or disease. For example, urease-negative strains have been isolated, and the vacuolating cytotoxin is produced by only a subset of *H. pylori* strains (*vacA* + or tox+ strains) (39-41). This observation is probably clinically relevant because most or all strains from duodenal ulcer patients, and many strains from gastric cancer patients, produce cytotoxin, whereas only a fraction of strains from patients with gastritis alone produce the cytotoxin (42,43). This phenotypic diversity is mirrored in great diversity on the DNA level. Thus, only cytotoxin-producing strains contain the gene for this cytotoxin-associated protein (*cagA*) (38,42), although genetic tests have shown that *cagA* protein is not needed for toxin production (44). Strains that

do not produce the 128-kDa *cagA* protein generally lack the entire *cagA* gene and additional neighboring genes. Although the function of the *cagA* region is unknown, its presence or absence is easily scored by hybridization or PCR and thus serves as an easy marker for probable cytotoxin production and possible virulence of *H. pylori* strains. Additional virulence factors are likely to be present. For example, another recently discovered region constitutes at least 21 kilobases of the *H. pylori* genome in hybridization experiments, and its presence is highly correlated with the presence of *cagA*: 39 of 40 strains lacking *cagA* also lacked this region, and 50 of 52 strains containing *cagA* contained this region. This newly discovered region is being called *cagII*, and the effort to sequence it is nearly complete (D. E. Berg, pers. comm.). Preliminary searches have identified several open reading frames with strong homologies to virulence functions from other microbes (45).

In addition to these extensively studied genes, genetic diversity of various *H. pylori* strains can be demonstrated by the use of two sensitive, efficient, and reliable PCR-based methods (46,47). This approach is particularly useful because it allows tracing of strains in epidemiologic studies.

Infection and Immune Response

One of the most puzzling aspects of gastric infection with *H. pylori* is its persistence despite intense local and systemic immune responses. These immune responses are extremely complex and vary among infected humans. The systemic response is characterized by a marked increase in plasma IgG, which remains present for months after the infection has been cured. The local response includes the production of IgA, which binds to the surface antigens of *H. pylori* in vitro and coats the bacterium in vivo. In addition, infection is consistently associated with an intense inflammatory response and the infiltration of cells into the gastric mucosa. Although polymorphonuclear cells are often present, most cells in such infiltrates are mononuclear cells. Both B and T cells are present, and recent studies have indicated that the natural killer activity of peripheral blood lymphocytes can be increased by *H. pylori*, possibly by its stimulating the production of interferon and other cytokines (48). Thus, the long-term carriage of the infection may be related to the ability of the bacterium to influence the T-cell response. Fragmentary evidence also suggests that this infection can be abortive and cure spontaneously without the use of antibiotics (A. Dubois and D. E. Berg, unpublished).

On the other hand, the mucosal response may promote colonization, as indicated by the observation that patients with acquired immunodeficiency syndrome (AIDS) tend to have a lower rate of infection than aged-matched subjects who are negative for human immunodeficiency virus (49,50). The latter study (50) also demonstrated that AIDS patients had a different pattern of gastritis, characterized by greater mononuclear cell responses, fewer lymphoid follicles, and a greater prevalence of intestinal metaplasia. The immune response may also prevent the invasiveness of *H. pylori*, as suggested by the anecdotal but puzzling observation of invasive *H. pylori* infection in a patient with AIDS (51).

Treatment

Although *H. pylori* is sensitive to many antimicrobial drugs in vitro, it is difficult to eradicate from the stomach. This may be ascribed to antibiotic breakdown by gastric acid, clearance by gastric emptying, and the difficult-to-penetrate mucous layer in which the bacterium resides. Resistance of *H. pylori* to specific antibiotics, especially metronidazole, is also frequent. Therefore, it is generally accepted that a combination of at least two, and possibly three, antimicrobial agents should be given for a minimum of 1 week. The regimen found to be most effective is the administration of amoxicillin (or tetracycline) plus metronidazole and bismuth subsalicylate 2 to 4 times a day for 2 to 3 weeks (52). The use of one antibiotic associated with an antisecretory agent, such as a histamine H_2 receptor antagonist, has given disappointing results. In contrast, the combination of a proton pump inhibitor (H^+-K^+ ATPase antagonist) with amoxicillin or acid-stable macrolides (clarythromycin or roxithromycin) appears more promising; a number of studies are being conducted to determine the optimal dose, duration, concomitant therapy, and cost-effectiveness of these compounds (53,54). Recently, it was shown that at least a 7-day course of any of these regimens is required to obtain a high (90%) cure rate, but that continuing treatment for more than 10 days does not significantly improve its efficacy. Finally, topical therapy for 1 h was recently tried with excellent results, albeit in only one center at this time (55). This treatment involves a 2-day administration of a mucolytic agent to dissolve the mucous layer and of a proton pump inhibitor. On the third day, a balloon is introduced into the second portion of the duodenum under fluoroscopic control, and a solution of pronase, amoxicillin, metronidazole, and bismuth subsalicylate is injected into the stomach, where it is left for 1 h. The presence of the duodenal balloon appears to prevent emptying of the antibiotics and the mucolytic agent, thus ensuring maximum efficacy of the therapy.

Future Research

The past 12 years have seen extensive progress in research on *H. pylori* as a cause of chronic active gastritis, duodenal ulcer disease, and gastric cancer. This has been largely due to an unusual collaboration among gastroenterologists, pathologists, molecular geneticists, bacteriologists, and immunologists. However, our understanding of how *H. pylori* colonizes and causes diseases is far from complete, and it will benefit from studies performed in animal models that can be experimentally infected with *H. pylori* (56-59). In addition, no easily administered treatment leading to eradication of this bacterium in all patients is yet available, although a better knowledge of its physiology may lead to the development of such a "silver bullet." Studies in animals that are not naturally infected with *H. pylori* suggest possibilities for vaccines (56,57), and ongoing trials in nonhuman primates are exploring the possibility of immunizing hosts that can be naturally infected with this organism. Although the elimination of peptic ulcer disease and of certain forms of gastric cancer will require extensive and coordinated efforts from public health authorities, this goal now appears to be within the reach of the scientific and medical community.

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HIV-1 Patients May Harbor Viruses of Different Phylogenetic Subtypes: Implications for the Evolution of the HIV/AIDS Pandemic

The virus variants isolated from HIV-infected persons worldwide share remarkable diversity, especially in the envelope glycoprotein, gp120. Phylogenetic studies have clustered HIV-1 isolates into eight subtypes (A-H). Nevertheless, even within a single infected person, HIV is present as a "quasispecies," or a swarm of closely related variants. This genetic diversity, which in the case of HIV-1 accumulates at a rate of approximately one nucleotide substitution per genome per replication cycle, gives the virus an enormous flexibility to respond to a wide array of in vivo selection pressures. As a consequence, drug-resistant and immunologic escape mutants are rapidly generated in infected persons through all stages of infection. On a global scale, the HIV pandemic is recognized as consisting of many separate epidemics, each with characteristic geography, affected populations, and predominant viral strain type. With an estimated 15 million infected persons, the geographic distribution of viral subtypes is becoming more dispersed, and these demarcations are further confounded by growing evidence of mixed infections.

The epidemic emergence of mixed heterotypic infections with HIV-1 and HIV-2 variants has been recognized for some time in the geographic areas where both types of viruses are present. We reported these infections in Côte d'Ivoire and Brazil (1, 2); they have also been reported from India (3). In contrast, homotypic mixed infections of distinct HIV-1 variants have only recently been suggested by the presence of broadly reactive sera and evidence of HIV recombinants from geographic regions in which multiple HIV-1 subtypes are circulating. Dual HIV-1 infection in two patients from Thailand has been demonstrated by viral DNA sequence analysis (4).

As the HIV-1 pandemic has grown, the simultaneous presence of multiple subtypes in a region has become common. As a consequence, an increased frequency of HIV-1 mixed infections could be expected. Thus, there is a need to estimate the prevalence and geographic distribution of this type of infection. Sequence analysis of HIV proviral DNA has been the method of choice to characterize HIV genetic diversity. However, because even relatively limited sequence determinations of small polymerase chain reaction (PCR) fragments are time consuming and very labor-intensive, this method is not particularly practical for large-scale molecular epidemiologic studies. To address this problem, we have developed a genetic method based on restriction site polymorphism to screen for homotypic HIV-1 mixed infections within infected populations. The concept of this assay is based on the observed correlation between the restriction maps of HIV-1 isolates with their phylogenetic classification, which is based on the sequence data. Thus, certain restriction enzymes may be used to predict the phylogroup of HIV-1 infected samples. The differences in electrophoretic mobility of endonuclease digestion products result from restriction site polymorphisms in the selected region of the HIV-1 genome and allow for quick recognition of the distinct phylogenetic subtypes. A 297 bp pol fragment spanning the entire viral protease gene is used for our analysis. The viral gene is amplified by nested PCR using DNA templates from uncultured peripheral blood mononuclear cells (PBMC) or virus culture. Preliminary classification of HIV-1 strains to well defined subtypes A, B, C, D, and F is done by sequential endonuclease restriction analysis. AluI restriction polymorphism in a PCR-amplified protease gene segregates viral strains into two groups: subtypes B and D belong to one group, and subtypes A, C, and F to another (Figure 1A). Further differentiation of HIV-1 subtypes within those two groups is accomplished by analysis of HinfI, BclI, MaeI, SpeI, and ScaI restriction enzyme digestion patterns of the protease gene (Pieniazek et al., manuscript in preparation). The electrophoretic migration patterns visualized by ethidium bromide staining or by radiolabeled probes are then determined on a 10% acrylamide gel. In single infections, a single restriction pattern is detected, whereas in multiple infections involving HIV-1 strains of distinct subtypes, complex digestion patterns are observed in infected persons. As an example, in Figure 1A, we present three distinct AluI restriction patterns of the protease gene that are characteristic for single infections by viruses of subtypes A, C, and F (pattern #1) and by subtypes B and D (patterns #2 and #3). In Figure 1B, we show a typical combination of two distinct AluI restriction patterns (#1 and #2) found in a patient infected with two viral strains of subtypes F and B. Basing our analysis on the conserved protease gene region, we should detect most HIV-1 strains; however, some highly divergent isolates could escape PCR amplification as a result of primer mismatches. Moreover, since a single nucleotide substitution could either generate or destroy a restriction site, sequence analysis remains the ultimate tool in identifying variants of multiple infections. Nevertheless,

this assay can be conveniently applied to screen a large number of samples.

By using this method, we have screened HIV-1 proviral DNA from 208 specimens collected from countries in South America, Africa, and Asia where HIV-1 strains of distinct subtypes are found. We observed the simultaneous presence of two distinct digestion patterns in PCR amplified protease gene (Figure 1B) in 31 samples; our observation suggests superinfection with HIV-1 strains of distinct origin. To eliminate the potential for laboratory cross-contamination, we analyzed the restriction patterns of the protease gene from multiple aliquots of the patient's PBMC. In addition, the analysis was repeated on DNA from a second collected blood sample from each of the patients. The analyses for the first five of 31 patients were completed, and data are summarized here (details are in Janini et al., manuscript in preparation). Sequence and phylogenetic analysis of the viral protease gene (Figure 2) in PBMC from those five patients confirmed dual infections caused by HIV-1 strains of subtypes B and F in one person (Br5), subtypes F and D in another patient (Br22), and subtypes C and D in a married couple (Br19 and 20). Moreover, in the child (Br30) of this couple, two distinct AluI digestion patterns were also found; the major HIV-1 strain clustered among subtype C viruses of the parents. The minor strain of this child is likely to represent subtype D, but there was not sufficient material for cloning and further sequencing of this strain.

Detection of naturally occurring heterotypic and homotypic multiple infections may have important implications for immunotherapies because infection with one HIV subtype may not fully protect against subsequent superinfections with distinct HIV strains. However, we do not know if the acquisition of viruses in the dually infected adult patients was sequential or simultaneous. Nevertheless, the consequences of mixed infections may profoundly affect the ability of the virus to change and may modify the direction of the pandemic through altered patterns of viral pathogenesis, increased genetic variation through recombination, and the generation of

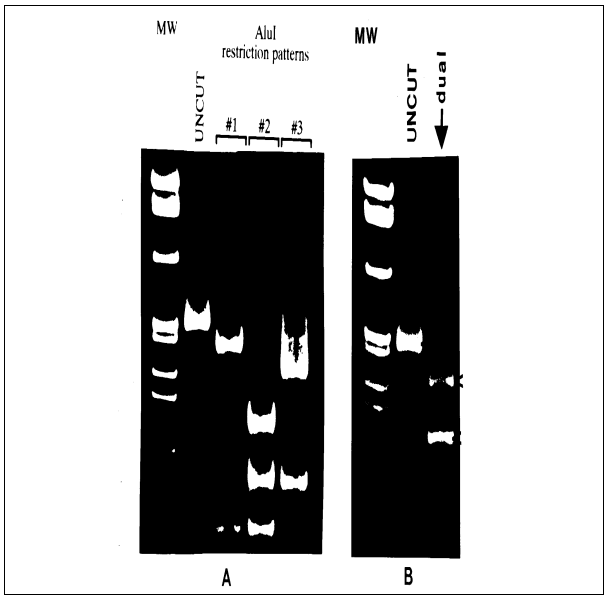

Figure 1 .
A. Three distinct AluI digestion patterns of PCR amplified protease gene representing single HIV-1 infections by viral strains of subtypes A, C, and F (pattern #1), and subtypes B and D (patterns #2 and #3).
B. The presence of two distinct AluI digestion patterns (#1 and #2) of the protease gene in PBMC of the patient dually infected by viral strains of subtypes F and B (lane 3). Arrows indicate diagnostic fragments detected by hybridization with the radioactive probe (2). MW represents molecular weight markers— ΦX174 RF DNA, HaeIII digest.

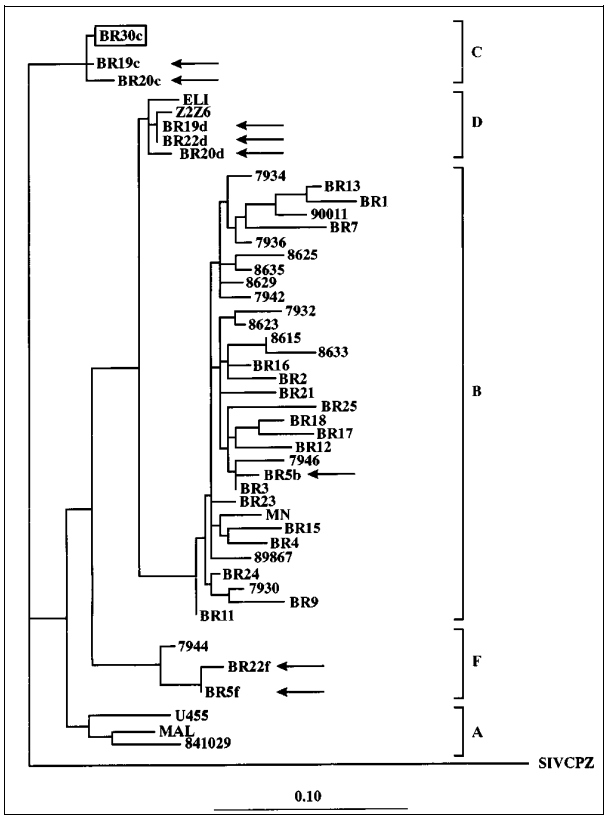

Figure 2.
Phylogenetic classification of HIV-1 strains in dually infected patients. HIV-1 sequences from dual infections (Br5, 19, 20 and 22) are indicated by arrows, and the major strain in the infected child (Br30) is boxed. The tree was constructed on the basis of the DNA sequences of the protease gene by using the maximum likelihood method with the fastDNAml program (6). SIV-cpz protease sequence was used as an outgroup. The distinct HIV-1 subtypes are delineated. The scale bar shows the ratio of nucleotide substitutions for given horizontal branch length. Vertical distances are for clarity only.

pseudotype virions, including phenotypically mixed virus particles. It is to be anticipated that such events would ultimately broaden the cellular tropism for HIV and mandate the designed polyvalent immunotherapies. Finally, our data together with recently published genetic analysis for HIV-1 and HIV-2 (5) suggest that multiple homotypic infections with divergent HIV strains may be more common than previously thought. The screening assay described here will be useful in estimating incidences of such HIV-1 infections. We believe that this information is crucial for both evaluating the pandemic and developing intervention strategies.

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Simultaneous Infection of *Ixodes ricinus* Nymphs by Two *Borrelia burgdorferi* Sensu Lato Species: Possible Implications for Clinical Manifestations

Data from European studies indicate that in humans, particular *Borrelia burgdorferi* genospecies may be associated with specific clinical manifestations of Lyme disease. Infections by *B. burgdorferi* sensu stricto tend to lead to arthritic symptoms, whereas infections by *B. garinii* appear to cause neurologic complications. Late cutaneous manifestations (acrodermatitis) appear to be associated with *B. afzelii* (1). Mixed clinical manifestations have also been described (2). Recently it has been demonstrated, by using polymerase chain reaction (PCR), that DNA from more than one of the three *Borrelia* species associated with Lyme disease in Europe was present in the biological fluids of Lyme disease patients (3). These data raise questions concerning the relative growth of the *Borrelia* species after a bite by a dually infected tick, the clinical significance of human infection caused by more than one species of *Borrelia* , and the origin of these multiple infections. This last point evokes the following question: do they result from successive bites by two infected ticks or from a single bite by a tick infected by more than one species?

To investigate whether ticks are infected by different species of the *B. burgdorferi* complex at the same time, we carried out a survey of the vector *Ixodes ricinus* during the spring of 1994, in Rambouillet Forest near Paris. A total of 249 unfed nymphs, collected from vegetation, were analyzed by PCR. The ticks were then crushed in phosphate-buffered saline, solubilized in 0.5% Tween 20, and boiled for 10 min. The resulting lysate was used as a template for the amplification reactions by either the universal ospA-based primers SL or the three pairs of genospecific-based primers (3). These last primers distinguish the three Lyme disease-associated *B. burgdorferi* sensu lato species, i.e., *B. burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii* . In some cases, amplified DNA products were digested with specific restriction enzymes to confirm the typing of the *Borrelia* strain.

Thirty of the 249 nymphs were positive for *B. burgdorferi* when SL universal primers were used. Further testing of 5 of 30 nymphs by PCR, using genospecific primer sets and restriction analysis, did not confirm the preliminary results with the universal primers. This may have been due either to the genotypic variability of *B. burgdorferi* sensu lato or to the existence of other distinct subgroups or genomic species included in *B. burgdorferi* sensu lato, as other data appear to indicate (4). Of the 25 other nymphs, 22 were analyzed by both restriction analyses and the specific primers, and three by restriction analysis alone. (The available tick material was not sufficient to perform PCR with genospecific primers.) Nineteen nymphs were infected by a single species of *Borrelia* (four by *B. garinii,* 15 by *B. afzelii*), and six were infected by more than one (two by both *B. burgdorferi* sensu stricto and *B. garinii* , three by *B. garinii* and *B. afzelii* , one by *B. burgdorferi* sensu stricto and *B. afzelii*).

From these results, it appears that when nymphs are infected with one species, *B. afzelii* is the most prevalent. This species may actually be prevalent in this study area or may have a greater tropism for dermal tissue and/or for the peripheral circulatory system of the vertebrate than the other two species. In infected nymphs, the simultaneous presence of more than one genospecies in unfed nymphs of *I. ricinus* was not exceptional (24%), and all combinations of two species were observed. The association of three genospecies has not yet been detected. Simultaneous infections in unfed nymphs could have different explanations. The first is a larval meal on a host infected by more than one species. Recently, *Apodemus speciosus* (field mice) infected by two different species have been found (5). A second possibility is successive infectious interrupted larval meals. A third possibility is an infectious larval meal by a previously transovarially infected larva. The fourth possibility is a mixed infection acquired transovarially.

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Epidemiologic and Evolutionary Relationships between Romanian and Brazilian HIV-1 Subtype F Strains

The initial classification of HIV-1 viruses as Western or African strains has been replaced by phylogenetic subtyping that uses nucleotide sequence data. Eight distinct phylogenetic HIV-1 lineages or subtypes, A to H, have been defined (1). Considering that the rate of HIV-1 genome evolution is estimated at 0.5% to 1% per year and that the average genetic distance between the HIV-1 subtypes is approximately 20%, it is likely that these subtypes originated before the HIV-1 pandemic (1). The global mosaic of HIV-1 subtypes is consistent with the hypothesis that most regional epidemics started with the introduction of one or a few variants that diversified locally rather than through radiant waves of already diversified HIV-1 subtypes that spread from the place of origin. In this report, we address the evolutionary and epidemiologic relationships between the HIV-1 subtype F viruses recently identified in two geographically distinct regions, Romania and Brazil.

The HIV-1 epidemic among Romanian children living in orphanages was recognized during 1989-1990 (2). Epidemiologic studies have shown that most children became infected by horizontal transmission of HIV-1 through blood transfusions or through the use of unsterilized medical equipment. We have shown by nucleotide sequence analysis that all HIV-1 isolates from children in southeastern Romania are highly related genetically (3). The average interperson nucleotide distance within the C2-V3 region of the *env* gene was 0.9% to 3.6%. Phylogenetic analysis of these sequences showed that the Romanian HIV-1 strains clustered together with a single Brazilian HIV-1 strain in a previously unrecognized evolutionary clade later designated as the F subtype. We now have the opportunity to augment this comparison with additional F subtype sequences from the two countries and to further address the relationship between Romanian and Brazilian viruses.

In the phylogenetic analysis of the envelope C2-V3 nucleotide sequences (Figure 1), we included eight HIV-1 F subtype viruses isolated from children in southeastern Romania (L19570-L119579) (3) as well as two representative sequences of the HIV-1 viruses found in children living in northcentral Romania (R18586 and R18598) (Bandea et al., manuscript in preparation). From Brazil, we included, in addition to the sequence of the first identified F subtype strain (BRA7944)(4), three recently reported F sequences (BZ126A, BZ162A, and BZ163A) (5) and four F strains (BR46, BR57, BR58, and BR59) isolated in our laboratory from patients in Rio de Janeiro. We also included two HIV-1 strains from Cameroon, CA4 and CA20, that have been tentatively classified as F subtype viruses (1,6) and reference nucleotide sequences representing the other HIV-1 subtypes.

The results of this phylogenetic analysis show that the Brazilian and Romanian sequences cluster in two highly related but separate groups. The genetic distance between Brazilian subtype F sequences was 5% to 13.8% , which is within the limits of the established intrasubtype distance values (1). Among Romanian C2-V3 nucleotide sequences this distance was 0.9% to 6.5%, and between the two groups it was 7.5% to 12.9%. These values support the inclusion of the Romanian and Brazilian groups within the same F HIV-1 subtype. The reliability of these phylogenetic results was verified by bootstrap analysis (100 data sets) and by pruning, which consists of sequential removing of different strains and rerunning the analysis.

The two sequences from Cameroon associated only weakly with Romanian and Brazilian groups, and this association was not stable. The genetic distance between the Romanian and Brazilian sequences and sequences from Cameroon was 16.5% to 24.1%, which is typical of intersubtype rather than intrasubtype genetic distances. Our analysis does not support a strong linkage between the Cameroonian strains and the other F subtype viruses; however, in evolutionary terms, these viruses may be closer to each other than to the other subtypes, or they may have undergone convergent evolution within the envelope region that we analyzed. Analysis of additional sequences from other regions of the HIV-1 genome may clarify the relationship between the Cameroonian strains and the other subtypes.

The amino acid GPGR motif at the tip of the V3 protein loop has been considered a signature sequence for the Brazilian F subtype viruses (5). This relatively conserved motif was noticeable because it is characteristic among B subtype viruses, whereas the GPGQ sequence predominates among all the other HIV-1 strains, including all the initial Romanian F viruses. Two of the newly identified Brazilian strains (BR58 and BR59), however, contain the GPGQ motif, and the Romanian strain R18598 contains the GPGH motif (Figure 2). Phylogenetically, these sequences group with their respective geographic clusters (Figure 1), which indicates that independent mutations may have occurred at this locus.

As indicated earlier, the genetic distance between Romanian HIV-1 nucleotide sequences is very small, which suggests a direct epidemiologic link among

these strains and a short period of evolution. The exclusive presence of highly related viruses in two geographically distinct provinces of Romania strongly suggests that the initial pediatric HIV-1 epidemic in this country started from a single infectious source. This suggestion is also supported by the fact that F subtype viruses are uncommon and, therefore, the chances for independent multiple introduction of highly related F subtype strains in Romanian children are very low. Although no nucleotide sequence data are available about HIV-1 strains circulating in the adult population in Romania, the epidemiologic and serologic studies indicate that the number of infections is small, and probably most HIV-1-infected adults were infected though sexual contact with foreign visitors or with Romanians that traveled outside the country (2). It is expected, therefore, that most of the HIV-1 strains infecting the adults represent internationally prevalent subtypes. It is remotely possible, however, that adults were infected with F subtype viruses and could have served as the original or intermediary carriers for HIV-1 transmission among the groups of children living in distinct geographic regions of Romania.

In Brazil, the relatively long genetic distance between F subtype viruses could indicate that a single ancestor was introduced during the early phases of the HIV-1 epidemic and diverged locally, or that multiple different F strains were introduced to this country. The low prevalence of F subtype viruses worldwide makes the latter alternative less likely. However, no information is available about the HIV-1 strains present during the early phases of the epidemic in Brazil. Our ongoing studies and the published data (4,5) indicate that the HIV-1 F subtype infections represent roughly 10% of the estimated number of cases. This relatively large number of F subtype infections and the estimated rate of HIV-1 divergence suggest an early introduction of the F subtype viruses in Brazil.

Because of the geographic position and the sparsity of socioeconomic relations between Romania and Brazil, the potential for an epidemiologic link between HIV-1 F subtype viruses is small. A more compelling argument against a direct epidemiologic link between F subtype viruses from these two countries can be made on the basis of the topology of the phylogenetic branches that set apart the two groups of viruses (Figure 1). If a direct epidemiologic link existed between the two groups, the clustering would be integral with one group branching from within the other. The tree topology shows the two groups of viruses on separate branches with a relatively distant common ancestor. Although limited in scope, our findings support an evolutionary relationship between the Romanian and Brazilian F subtype

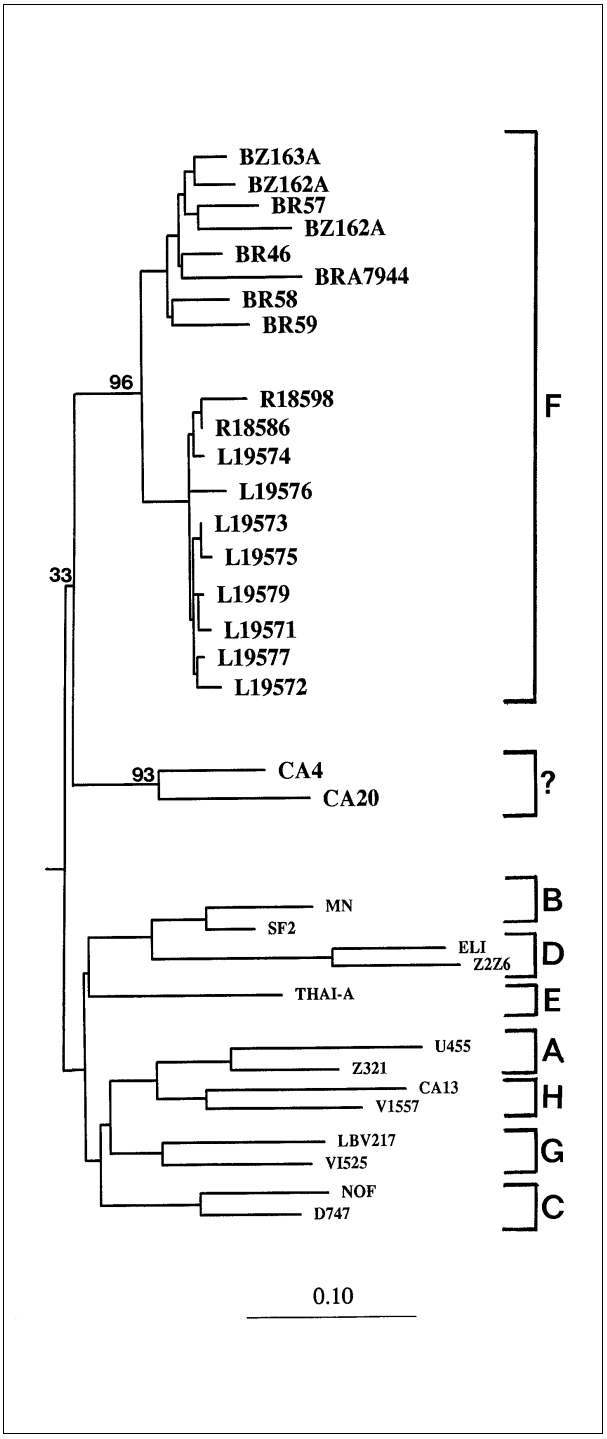

Figure 1. Phylogenetic relationship between Romanian and Brazilian subtype F nucleotide sequences. The tree was constructed by using the neighbor joining method included in the Phylip 3.5c package (7). Three hundred and two aligned nucleotides from the envelope C2-V3 region were used for analysis. The vertical distance between the branches is noninformative and for clarity only. Numbers at the branch nodes indicate bootstrap values. The nucleotide sequence distance among stains can be deduced by using the bar scale included in the figure.

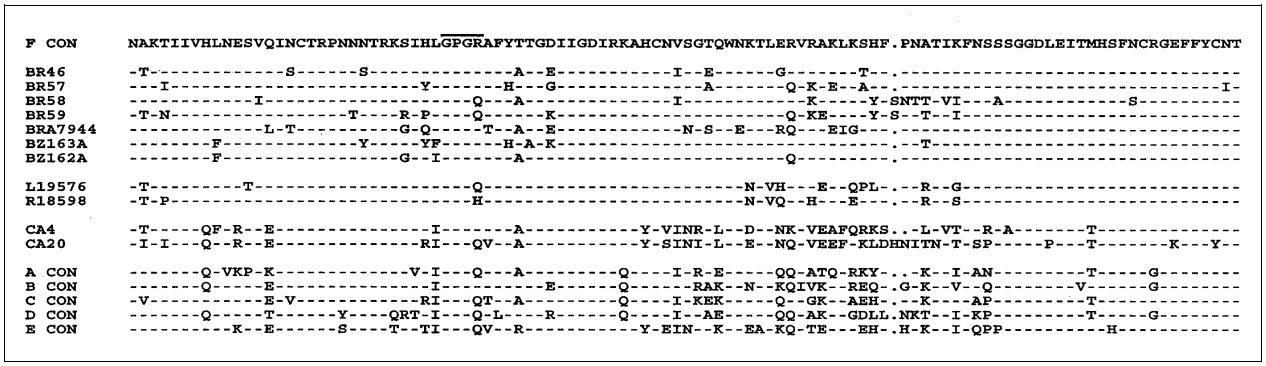

Figure 2. Alignment of deduced amino acid sequences for envelope C2-V3 region of Brazilian and two representative Romanian HIV-1 F subtype strains and their comparison with Cameroonian sequences and the consensus sequences for some of the other subtypes. F CON represents consensus amino acid sequence (single letter code) for the Romanian and Brazilian F subtype HIV-1 viruses presented in this figure. Consensus sequences for the other subtypes are from Ref. 1. Amino acids identical to the F CON are shown as a dash, and the dots represent gaps introduced to align sequences. The top bar shows the peptide motif at the tip of the V3 protein loop.

viruses and indicate that the two regional epidemics arose independently.

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Electronic Communication Facilitates Investigation of a Highly Dispersed Foodborne Outbreak: *Salmonella* on the Superhighway

Widely dispersed foodborne disease outbreaks are an emerging public health problem (1-3). Increasing population mobility and the wide distribution of centrally produced foods mean that when an outbreak of foodborne disease occurs, the affected persons may be distributed across the country or even the world. Widely dispersed outbreaks challenge limited public health resources; they can be difficult to detect, labor-intensive, and time- consuming to investigate. We report the rapid, efficient investigation of a widely dispersed interstate outbreak through electronic communication between the possible patients and public health workers.

On August 4, 1994, a resident of a western state contacted the Centers for Disease Control and Prevention (CDC) regarding a possible outbreak of foodborne illness. On July 22, the day after returning from a conference in Baltimore that included attendees from all 50 states, he became ill with diarrhea, and *Salmonella* was isolated from his stool. He contacted four other conference attendees who had taken the same flight. One of these also had culture-confirmed *Salmonella* infection; a second, who was taking antibiotics for other reasons, had a diarrheal illness with a negative stool culture, and two had nonspecific diarrheal illnesses. Because of the possibility of a multistate outbreak involving the airline or the conference and affecting many people, we initiated a survey of conference attendees to determine the rate and correlates of diarrheal illness.

Traditionally, surveys of dispersed populations have been conducted either by telephone, requiring many person-hours of interviewing, or by mail, leading to many days' delay while questionnaires are distributed and returned. However, in this case, the organization that sponsored the conference had an internal electronic mail (e-mail) system. Each section of the organization, although not each person, had a computer that could receive e-mail messages. On August 5, 1994, the organization's central office e-mailed a questionnaire, developed in consultation with CDC, to all computers in the organization with instructions to organization staff to print and distribute the questionnaire to conference attendees. The questionnaire contained items regarding diarrheal illness, meal and flight exposures, and demographic information. Because the organization's e-mail system was only internal, responses could not be made to CDC by e-mail. Therefore, attendees were instructed to send their completed questionnaires to CDC by fax. By August 12 (7 days later), sufficient responses had been received to evaluate the flight and the conference as sources of an outbreak of salmonellosis (Figure 1).

Of 390 persons registered at the conference, 86 (22%) returned questionnaires by August 12. A questionnaire was returned by the index patient who made the initial call to CDC but not by the four other persons he contacted who were passengers on the same flight. Six (7%) of the 86 respondents reported having diarrhea (three or more loose stools in a 24-hour period) during the period beginning 12 hours after the conference started and ending 5 days after the conference ended (July 20 to 26). Among questionnaire respondents, only the index patient was diagnosed with salmonellosis. Three respondents had taken the initially suspect flight. Illness was not associated with taking the same flight as the index patient ($p = 0.20$, Fisher's Exact Test, 2-tailed).

To further investigate the reports of diarrhea, we interviewed the six persons who reported diarrheal illness by questionnaire, as well as the four persons initially contacted by the index patient who had not completed questionnaires. This group included the two persons with known *Salmonella* infection, of whom one had completed a questionnaire and one had not. Seven of the other eight persons had mild, nonspecific symptoms of less than 2 days' duration; the onset dates of their illnesses spanned a 5-day period, and none sought medical attention. Because few conference attendees or flight passengers became ill with symptoms suggestive of salmonellosis during a likely period, we thought that an airplane- or conference-associated outbreak was improbable.

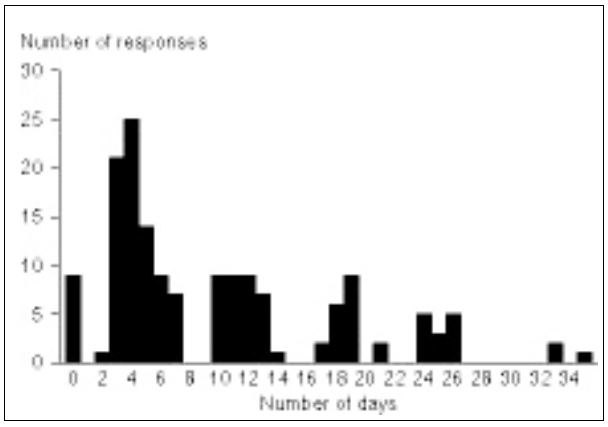

Figure 1. Number of days from questionnaire distribution by e-mail to questionnaire return by fax (n=156). Day 0 is Friday, August 5, 1994. Range, 0 to 35 days; median = 6 days.

By September 9, questionnaires were returned by 156 (40%) of the conference attendees. No additional cases of diarrhea were reported, confirming our initial conclusion that the *Salmonella* infections were not associated with the flight or the conference.

The *Salmonella* isolates were identified at CDC as *Salmonella* serotype Norwich, of the *Salmonella* serogroup C1. *S.* Norwich is rare; in 1993 and 1994, respectively, 63 and 102 isolates of this serotype were reported to the Public Health Laboratory Information System (PHLIS), a nationwide electronic laboratory-based surveillance system that collects and summarizes data on isolates from state public health laboratories (4). Because infection with *S.* Norwich is so uncommon, it still seemed likely that the two infections could have a common source, such as a restaurant.

Subsequent investigation focused on meals that the two persons with salmonellosis shared outside the conference and ultimately revealed the source, a restaurant in Baltimore. In late July 1994, the Maryland Department of Health and Mental Hygiene received reports that *Salmonella*, serogroup C1, had been isolated from five other persons who visited Baltimore around the time of the conference. Two persons from one family had driven to Baltimore on July 17, eaten only at one restaurant, then returned to their home state of Pennsylvania. Three persons in a second family, from a different part of Pennsylvania, ate at the same Baltimore restaurant on July 21 during a vacation trip. The *Salmonella* isolates from members of both families were initially misidentified as other serogroup C1 serotypes. They were retested because of this outbreak and were confirmed as *S.* Norwich. The two conference attendees with *S.* Norwich infection also ate at the implicated restaurant on July 21. No single menu item had been eaten by all ill persons. In response to a complaint by the first family, the restaurant had been inspected by the local health department; multiple violations of food safety regulations were found. *S.* Norwich was isolated from a stool specimen from an employee who reported a diarrheal illness beginning on July 22 and who ate the restaurant's food. In the month following the inspection of Restaurant A and subsequent corrective action, no further cases of *S.* Norwich were reported to PHLIS from Maryland or Pennsylvania.

E-mail can expedite questionnaire distribution, especially when the population of interest is on one network. The computer system used to send the e-mail message in this outbreak was not linked to individual conference attendees; therefore, we could not evaluate the rates at which individual attendees obtained and responded to the message. If we had been able to reach attendees directly, our response rate may have been higher, and we would have been able to send additional messages to nonresponders. In the future, when outbreaks occur among persons accessible by e-mail, it may be possible to evaluate strategies to improve response rate and to compare the effectiveness of the delivery of questionnaires by e-mail and by more traditional means.

This outbreak illustrates the usefulness of rapid electronic communication in a public health setting. Isolation of a rare *Salmonella* serotype and national electronic reporting to PHLIS assisted in the detection and investigation of a widely dispersed multistate outbreak of salmonellosis. Without the national *Salmonella* serotyping system, the outbreak would not have been recognized. Questionnaires were distributed rapidly by e-mail; the utility of this method is likely to increase as more people become accessible by e-mail. Fax provided a means for respondents to return questionnaires quickly. Continued on-line analysis of surveillance data with PHLIS confirmed that the outbreak was controlled. Rapid communication between public health workers in Maryland and Pennsylvania and at CDC was also essential. The usefulness of electronic communication is not limited to outbreak investigation. New technologies will undoubtedly continue to be useful in addressing emerging public health problems.

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Reemergence of Ebola Virus in Africa

Members of the family *Filoviridae* , which currently consists of Ebola and Marburg viruses, cause severe and often fatal hemorrhagic fevers in humans and nonhuman primates. The recent isolation and identification of a new Ebola virus from a single nonfatal human case in Côte d'Ivoire (1) and the more recent outbreak of Ebola hemorrhagic fever in and around Kikwit, Zaire (2, 3), have raised concerns about the public health threat of these human pathogens. Filoviruses are classified as biosafety level 4 agents because of the extreme pathogenicity of certain strains and the lack of a protective vaccine or effective antiviral drug. Moreover, filoviruses are among the most mysterious groups of viruses known because their natural history and reservoirs remain undefined and their pathogenesis is poorly understood.

Ebola virus infections were first recognized in 1976, when simultaneous but separate outbreaks of human disease caused by two distinct virus subtypes erupted in northern Zaire and southern Sudan (4) and resulted in hundreds of deaths. The Zaire subtype of Ebola virus had a higher case-fatality, nearly 90%, while the Sudan subtype had a case-fatality rate of approximately 50%. Before 1995, the last identified outbreak of Ebola disease in Africa occurred in 1979, when the Sudan subtype of Ebola virus infected 34 persons (5). In late 1989, in Reston, Virginia, a novel Ebola virus infected a colony of cynomolgus macaques that had been imported from the Philippines (6). The new virus, named Reston virus, was shown by researchers at the Centers for disease Control and Prevention (CDC) to be antigenically and genetically distinct from the African Ebola viruses, yet despite its high pathogenicity for nonhuman primates, it did not appear to cause disease in humans. Several persons who handled the infected animals developed antibody to Ebola virus but showed no signs of disease; one of these persons was infected while performing an autopsy on an animal that had died of a Reston virus infection. In 1992, a repeat of the 1989 Reston episode occurred in Siena, Italy when macaques were received from the same Philippine exporter; no evidence of a human infection was found (7). The new Ebola virus recently isolated from a patient in the Côte d'Ivoire has been shown to be genetically distinct from previous Ebola isolates (A. Sanchez, unpublished data) and is the first evidence of Ebola virus in West Africa.

Investigations of these outbreaks, as well as of those caused by Marburg viruses, have yet to produce any substantial evidence for the natural reservoir(s) of filoviruses. Filoviruses do not persist in experimentally infected nonhuman primates; therefore, nonhuman primates are likely not the natural reservoir. Like humans, these species probably are infected when direct or indirect contact is made with the natural host.

The recent news of a large Ebola outbreak in Kikwit, Zaire, alarmed a worldwide audience already sensitized by an array of books, magazine articles, television programs, and movies dealing with the danger of Ebola virus disease. The public concern is underscored by the potential for the spread of these viruses to far regions of the world as a result of international commerce and jet travel. The Kikwit outbreak was similar to the original 1976 episode in Zaire, which was centered around the small village of Yambuku some 1000 km to the north (8). As in the 1976 outbreak, secondary transmission of the virus in Kikwit occurred through close personal contact with infectious blood and other body fluids and was facilitated by the lack of modern medical facilities and medical supplies that could protect those giving care to the initially affected patients. The chief difference between the Yambuku episode and this year's outbreak is that Kikwit is a large and densely populated center close to larger cities, such as Kinshasa and Brazzaville, and the potential for communitywide transmission and spread to neighboring areas is greater. Retrospective case surveillance suggests that the index case may have been a charcoal maker that worked in the forest outside Kikwit. Human to human transmission occurred without being recognized until the end of April 1995. Ebola hemorrhagic fever was suspected when nosocomial infections in the surgical teams and the nursing staff followed repeated laparotomies on an infected laboratory technician in Kikwit General Hospital. Specimens were send to CDC through the Tropical Institute of Antwerpen (Belgium). Teams of experts from CDC, the World Health Organization, Belgium, France, South Africa, and Sweden traveled to the region to assist in implementing safe patient care, management, and containment of the Ebola virus outbreak. As of July 1, 1995, 233 deaths had been reported among the 293 cases.

Rapid diagnosis and characterization of Ebola virus was performed at CDC in Atlanta on blood specimens from 14 patients received on May 9. Nine hours after the specimens had been delivered to CDC, Ebola virus antigen and/or antibody to this virus was confirmed in specimens from 13 of the patients. Four hours later, reverse transcriptase-polymerase chain reaction (RT-PCR) assays targeting conserved regions of filovirus polymerase or Ebola virus glycoprotein genes each detected Ebola virus RNA in 12 of the patients. Subsequent analysis of the genetic profile of the virus was especially important to understanding the epidemiology of the Kikwit outbreak. Within 48 hours of receiving the specimens, sequence analysis on the PCR DNA (528 bp) amplified from the glycoprotein gene derived from four different patients showed that the Ebola virus

was a Zaire subtype that differed from the original 1976 strain in four bases (<1%). No differences were seen when the polymerase gene PCR products (~350 bp) from those four patients were sequenced, which indicated that they had been infected with the same virus. Three days later, sequence data from expanded analysis of the entire glycoprotein gene were compared with those of the original 1976 Yambuku isolate (9) and showed that the overall difference between these Ebola viruses was less than 1.6%. Such little change in viruses that caused outbreaks of disease at extreme ends of Zaire separated by a span of nearly 19 years, may indicate that the genomes of Ebola viruses (and filoviruses in general) are unusually stable and have evolved to occupy special niches in the wild.

The capability to rapidly diagnose and characterize filovirus infections is critical to the ability of public health professionals to identify and limit the spread of future outbreaks of filovirus disease. A continued commitment to research and modern disease-surveillance programs is necessary to minimize or preclude filovirus outbreaks similar to that in Kikwit. The possibility of outbreaks is increasingly likely given the continued human incursions into the African forests and the vulnerability of large impoverished populations to rapid transmission of disease as a result of inadequate public health services. With the current outbreak under control, CDC and collaborators have begun their efforts to identify the natural reservoir by sending teams of scientists to collect specimens from the area where the putative index patient worked. Attempts to identify the reservoir after outbreaks in 1976 and 1979 were handicapped by the lack of satisfactory diagnostic tools that are critical to detecting small quantities of the virus. However, now that sensitive enzyme immunoassays and PCR assays have been developed for filoviruses, the chances are much better that, if appropriate materials can be collected in the field, the virus can be detected.

In conclusion, we want to alert physicians and public health agencies who encounter persons that have clinical signs and symptoms of hemorrhagic fever disease to the reemergence of Ebola virus. Recommendations for the management of viral hemorrhagic fevers attributable to filoviruses in the United States were recently published in CDC's *Morbidity and Mortality Weekly Report* (1995;44:475-79).

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Prospects for the Control of Bolivian Hemorrhagic Fever

Bolivian hemorrhagic fever (BHF) was first identified in 1959 as a sporadic hemorrhagic illness in rural areas of Beni department, Bolivia. Clusters of BHF patients were noted the same year, and by 1962 BHF was recognized as a new epidemic infectious disease. In 1963, Machupo virus (a member of the family *Arenaviridae*) was first isolated from patients with acute hemorrhagic fever in San Joaquin, Bolivia (1). Ecologic investigations established the rodent *Calomys callosus*, which is indigenous to the disease-endemic region of northern Bolivia, as the reservoir for Machupo virus (2,3).

Machupo virus infection in *C. callosus* results in asymptomatic infection with shedding of virus in saliva, urine, and feces; 50% of experimentally infected *C. callosus* are chronically viremic and shed virus in their bodily excretions or secretions (2). Although the infectious dose of Machupo virus in humans is unknown, exposed persons may become infected by inhaling virus shed in aerosolized secretions or excretions of infected rodents, by eating food contaminated with rodent excreta, or by direct contact of excreta with abraded skin or oropharyngeal mucous membranes (4). Reports of person-to-person

transmission are uncommon; however, hospital contact with a patient resulted in person-to-person spread of Machupo virus to nursing and pathology laboratory staff (5). In 1994, the fatal secondary infection of six family members in Magdalena from a single naturally acquired infection further suggested the potential for person-to-person transmission (Ksiazek et al., manuscript in preparation).

The pathogenesis of BHF, which resembles that of other South American hemorrhagic fevers due to Arenavirus infection (e.g., Argentine hemorrhagic fever), has been described in clinical and pathologic investigations of naturally infected patients (6,7). Experimental infection of rhesus monkeys with Machupo virus demonstrated an incubation period of 7 to 14 days, which is consistent with clinical observations in human infection (8). Early clinical manifestations in humans are characterized by nonspecific signs and symptoms including fever, headache, fatigue, myalgia, and arthralgia. Later in the course of disease (usually within 7 days of onset), patients may develop hemorrhagic signs, including bleeding from the oral and nasal mucosa and from the bronchopulmonary, gastrointestinal, and genitourinary tracts.

During the BHF epidemics in the 1960s, rodent control was recognized as the primary method for the prevention of Machupo virus transmission (9). Since *C. callosus* was frequently found in domestic and peridomestic environments, rodent control measures (e.g., trapping, poisoning) resulted in an immediate reduction in the number of *C. callosus* and control of BHF outbreaks; an epidemic in 1964 ended after 2 weeks of continuous trapping for *C. callosus* in homes of the affected community (10). Rodent control programs became a new priority for health officials in Bolivia, and active interventional programs were carried out for many years by survivors of past BHF epidemics known to be immune to Machupo virus (11).

From 1973 to 1992, no cases of BHF were reported, possibly because of effective control of rodent reservoir populations (12). Since the late 1960s, no epidemics of BHF have occurred that involve rural communities, but recent sporadic cases have been identified in the disease-endemic region (13). Although patients with BHF have been treated at hospitals outside the disease-endemic region, these patients had a history of exposure to Machupo virus in the disease-endemic region or secondary contact with BHF patients who became infected in the endemic region. Additionally, no documented cases of BHF have been exported to other countries.

Concurrently with the lack of identification of BHF patients during the 1970s and 1980s, the emphasis on conducting rodent control programs in the BHF-endemic areas also diminished. Moreover, in recent years, Bolivian health officials have been faced with numerous other public health problems, including diarrheal disease, tuberculosis, Chagas' disease, sexually transmitted diseases, and acquired immunodeficiency syndrome. Thus, local health authorities are confronted with the challenge of allocating limited health resources for the control of BHF as the demand for work with other important diseases increases.

Agricultural activities dominate the economy of northern Bolivia where many workers are employed in farming and animal husbandry (14). Farm workers may reside for prolonged periods in rural areas also inhabited by *C. callosus,* and farm houses constructed with partially open walls may allow rodents access to living areas. Thus, human exposure to infected rodents may occur in and around farm workers' shelters or during work in the fields and grasslands of the BHF-endemic region. Given the projected economic growth in Bolivia, it is likely that agricultural workers' risk for exposure to *C. callosus* will continue and even increase as development modifies the natural habitat of the rodent reservoir leading to increased contact with humans (e.g., focused rodent habitats with increased densities) (15).

Future efforts to control BHF may benefit from recent experience in neighboring Argentina where ongoing work has led to the control of Argentine hemorrhagic fever (AHF), caused by Junin virus, an arenavirus genetically related to Machupo virus. Extensive study of AHF by Maiztegui, Enria, and colleagues has provided new insights into the epidemiology, pathogenesis, treatment, and control of this disease (16,17) and has led to an effective Candid #1 vaccine against Junin virus as well as phase 2 clinical trials that suggest ribavirin may be effective in patients with AHF (18,19). The use of an effective vaccine against AHF and evidence for its cross-protection against Machupo virus suggest that vaccination may play a role in the prevention of BHF for persons at highest risk, such as workers who trap rodents for control programs (20). Intravenous ribavirin has shown promise for the treatment of clinically diagnosed BHF cases subsequently confirmed in the laboratory (Kilgore, manuscript in preparation). Intravenous ribavirin also appeared effective in the treatment of a laboratory-acquired infection with Sabiá virus, a related Arenavirus first isolated in Brazil (21). Ribavirin could be administered to patients whose symptoms meet a clinical case definition with subsequent laboratory confirmation of Machupo virus infection. Local laboratory handling of specimens or testing by effective rapid enzyme-linked immunosorbent assays for antigen and IgM antibodies is ideally performed under biosafety level 4 containment, but use of biological safety cabinets and addition to samples of inexpensive reagents such as Triton X-100, which reduce

viral titers, allow the development of capability for real time testing.

The family cluster of BHF patients and later sporadic cases in September and October 1994 highlighted the diagnostic challenge of BHF for clinicians. Even local physicians may rarely evaluate BHF patients, and other diseases (e.g., malaria, dengue fever, and yellow fever) that coexist in the BHF-endemic region may resemble BHF in the early phases of illness. Moreover, no readily available diagnostic tests exist locally to differentiate BHF from other diseases (22). Bolivian health care providers and public health officials recognized the need for education of health care providers and subsequently established a training program aimed at increasing clinicians' recognition of BHF particularly in the disease-endemic region.

The cluster of patients in 1994 also focused public attention on BHF because the illnesses had higher case-fatality rate than other diseases in the region where BHF is endemic. The underrecognition of these illnesses as dangerous and potentially fatal in disease-endemic communities suggests the need for increased public health education to reduce virus exposure and transmission. Proven control measures must be reinforced even in towns affected by large epidemics 30 years ago where younger residents have no recollection of the heavy toll exacted by BHF. Prevention of communitywide epidemics through rodent control programs may be combined with the application of barrier precautions (e.g., gloves, masks) in hospitals or clinics to minimize secondary person-to-person transmission of Machupo virus. After the familial cluster of BHF in 1994, results of rodent trapping confirmed the absence of reinfestation in towns and indicated that the density of rodent reservoirs was not unusually high in areas of probable exposure for the index patient. The absence of communitywide epidemics of BHF suggests that focused rodent control in towns of the disease-endemic region prevented large urban outbreaks. Prevention of sporadic illness in farm workers through widespread elimination of reservoirs may not be feasible, but other measures, such as the administration of Candid #1 AHF vaccine to workers at high risk, may offer a more realistic alternative. Finally, agricultural workers in the disease-endemic region should be taught methods to reduce exposure to rodent reservoirs, especially around rural shelters as a means of reducing their risk of exposure to Machupo virus in the environment.

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Conference on "Emerging Infectious Diseases: Meeting the Challenge"

Emerging infectious diseases, the leading cause of death worldwide, continue to pose difficult challenges to clinicians, public health professionals, and biomedical researchers in academic settings and industry. Addressing these challenges requires a cohesive effort to develop prevention strategies and to communicate them effectively to the health care community, the public, and policy makers.

On June 5-6, 1995, the New York Academy of Medicine and the New York State Department of Health convened to examine the problem of emerging infections. The speakers addressed four themes: 1) emerging infectious diseases: why and why now? 2) transmission of emerging infectious diseases: old modes, new agents; 3) surveillance and sentinel systems for infectious diseases; and 4) emerging infectious diseases: what is to be done?

The first three themes were addressed through presentations by 20 experts. The fourth was divided into six segments focusing on diagnosis, the role of the microbiology laboratory in surveillance, other surveillance issues, approaches to epidemic investigations, risk perception, and global issues.

Speakers consistently alluded to recent complacency about infectious diseases in the United States and stressed the need for the clinical, public health, and research communities to work with the biomedical industry in confronting emerging infectious disease challenges in this era of transition to managed health care. In his opening address Joshua Lederberg from Rockefeller University reminded participants that the struggle between humans and microbes could be characterized as a battle of "wits versus genes." Margaret Hamburg, Commissioner of the New York City Department of Health, emphasized that plague in India and Ebola virus infection in Zaire were reminders that the world is a global village, that considering domestic and international diseases as separate entities is an outmoded concept, and that many conditions that contribute to disease emergence or reemergence in the developing world are also present in the United States, adding to our domestic vulnerability to emerging infections.

Other speakers focused on the evolution of virulence, the molecular basis of pathogenesis, observations on factors contributing to the plague epidemic in India in 1994 and the Ebola outbreak in Zaire in 1976; foodborne and waterborne diseases; airborne diseases; zoonoses; sexually transmitted and bloodborne diseases and the increasing problem of antimicrobial resistance in both hospital and community settings. Concerns were expressed about the possibility of a "post-antimicrobial era" in which available drugs are no longer effective against common bacterial infections. Other speakers focused on innovative approaches to surveillance at the local, state, national, and international levels. James LeDuc from the World Health Organization (WHO) provided an update on the emerging infections resolution passed by the World Health Assembly in May 1995 and other WHO activities related to detecting and responding to emerging and reemerging diseases.

Among the themes recurring throughout the conference were the challenges that microbes will continue to pose; the critical role of the modern microbiology laboratory in detecting and responding to emerging and reemerging infections; and the limitations of existing capacity at the local, state, national, and international level to respond to these challenges. Human resource, equipment, diagnostic reagent, and facility needs were addressed, and resource needs were emphasized. Training needs of medical students, clinicians, epidemiologists, microbiologists, entomologists, mammalogists, behavioral scientists, and other researchers were also stressed. Additional emphasis was placed on the critical importance of communicating alerts about clusters of illness, data on disease trends, and guidelines for disease prevention; the need for educating professionals, the public, and policy makers about the critical importance of these issues; the need for strengthening existing partnerships and developing new ones, particularly with health maintenance organizations, the pharmaceutical industry, and nongovernmental organizations (including medical missionary organizations); and the need to carefully identify priorities.

Conference participants resounded the message of the 1992 Institute of Medicine Report, *Emerging Infections: Microbial Threats to Health in the United States,* "Pathogenic microbes can be resilient, dangerous foes. Although it is impossible to predict their individual emergence in time and place, we can be confident that new microbial diseases will emerge." Particular future concerns included a possible influenza pandemic, the emergence of vancomycin resistance in *Staphylococcus aureus* , the occurrence of large dengue hemorrhagic fever epidemics in the Western Hemisphere, and the likelihood that additional chronic diseases will be found to have infectious etiologies. Concerns were also expressed about the possibility of a terrorist incident involving an infectious agent and the potential difficulties in detecting and responding to such an episode.

The New York Academy of Medicine plans to use the discussions during the conference in formulating an agenda for further action.

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Japanese Encephalitis Acquired in Australia

Japanese encephalitis (JE), a mosquito-borne flaviviral disease of humans and animals, is a major public health problem in Asia, where an estimated 50,000 cases occur each year. There has been concern that the range of epidemic JE may be expanding.

On April 5, 1995, an outbreak of three cases of JE was recognized in Australia. Two of the cases were fatal; all were among residents of an island in Australia's Torres Strait, which lies between mainland Queensland and Papua New Guinea. JE was confirmed in two of the patients by polymerase chain reaction (Jeffrey Hanna, Queensland Health, pers. comm.). No other cases were reported. This is the first recognized episode of JE acquired in Australia.

Control activities on the Australian island began on April 7. The community was informed about the importance of personal mosquito protection measures. In addition, larvicides were applied, and areas were fogged to kill adult mosquitoes.

The patients were all male, aged 6 to 44 years. All were hospitalized with symptoms that included fever (up to 40 °C), stiff or painful neck, headache, and abdominal pain. Two patients were unconscious at the time of admission.

Acute-phase sera showed elevated JE virus immunoglobulin M (IgM) titers. Two of the patients also had detectable levels of Kunjin and Murray Valley encephalitis virus IgM, but the JE IgM titers were significantly higher in each case.

Flaviviruses have also been isolated from the sera of each of two asymptomatic island residents. Preliminary tests suggest that these are both JE virus. Blood taken from 10 horses and 12 domestic pigs living near humans on the island was also tested. All 12 pigs and 9 of the horses had high JE titers by hemagglutination inhibition assay. Neutralizing antibody to JE virus was detectable in all the pigs and in four of the horses tested to date.

Details of the index case are as follows: The patient, a 16-year-old male, was admitted to Thursday Island Hospital on March 22, 1995. He was unconscious and was responsive only to painful stimuli. His neck was stiff, and he showed a preference for moving his right side. His illness had begun 3 days before. The day before admission he complained of abdominal pain. This patient had been mildly mentally retarded since birth and occasionally had generalized seizures but was generally healthy. He was transferred to Cairns Base Hospital, where a cerebral CT scan showed a nonenhancing hypodense lesion in his posterior right basal ganglia.

He had a leukocytosis of 17.3 $\times 10^9$L, neutrophils, 15.2 $\times 10^9$. His cerebrospinal fluid contained 150 leukocytes/ µl with a differential count of 50% polymorphs and 50% mononuclear cells.

He had a generalized seizure and 2 days after admission, required mechanical ventilation. He never regained consciousness and died on day 17 of hospitalization (April 8).

Adapted from Hanna J, Ritchie S, Loewenthal M, et al. Probable Japanese encephalitis acquired in the Torres Strait. Communicable Diseases Intelligence 1995;19:206-7.

USPHS and IDSA Collaborate on Guidelines to Prevent Opportunistic Infections in HIV-Infected Persons

U.S. Public Health Service (USPHS)/Infectious Diseases Society of America (IDSA) Guidelines for Preventing Opportunistic Infections in HIV-Infected Persons will be published in an August 1995 supplement of *Clinical Infectious Diseases* . The guidelines, which are intended for health care providers, are the result of collaboration between the Centers for Disease Control and Prevention (CDC), the National Institutes of Health, IDSA, numerous federal and nonfederal organizations, community groups, and HIV-infected persons. The guidelines are endorsed by the American Academy of Pediatrics, the Infectious Diseases Society of Obstetrics and Gynecology, and the Society of Healthcare Epidemiologists of America. Jonathan E. Kaplan, M.D. (CDC), Henry Masur, M.D. (NIH), and King Holmes, M.D., Ph.D. (University of Washington), chaired the USPHS/IDSA Prevention of Opportunistic Infections Working Group and are guest editors of the *Clinical Infectious Diseases* supplement.

CDC initiated work on the guidelines in early 1994; meetings were held in Atlanta in June and September to discuss and refine the recommendations.

The USPHS/IDSA guidelines address 17 opportunistic infections from three angles: 1) preventing exposure to opportunistic pathogens (e.g., sexual, occupational, and environmental exposure as well as exposure through pets, food, water, and international travel); 2) preventing opportunistic disease by chemoprophylaxis and vaccination; and 3) preventing disease recurrence. In this document, new recommendations were made and earlier recommendations were updated. For example, new guidelines recommend that in nonemergency situations, cytomegalovirus (CMV)-seronegative HIV-infected persons who require blood transfusions receive only

CMV-antibody-negative or leukocyte-reduced cellular blood products. The guidelines also recommend that *Toxoplasma* -seropositive HIV-infected persons who have a CD4+ lymphocyte count <100 cells/ μL received chemoprophylaxis against toxoplasmosis (such chemoprophylaxis is generally accomplished with anti- *Pneumocystis carinii* medication). Earlier recommendations for chemoprophylaxis against *Pneumocystis carinii* pneumonia and *Mycobacterium avium* complex disease have also been updated.

In addition to disease-specific recommendations, the guidelines include an overview article designed to prioritize the recommendations for health care providers. This article provides an approach to the initial and follow-up evaluations of the HIV-infected patient and also contains sections on HIV-infected pregnant women and HIV-exposed/infected children. The guidelines are followed by 15 background articles, which provide the information on which the recommendations were based and include research priorities generated by the development of the prevention recommendations.

The guidelines conclude with quality standards and implementation steps on the most standard-of-care recommendations, such as chemoprophylaxis against *Pneumocystis carinii* pneumonia. This final section provides a mechanism by which health care facilities can assess their degree of compliance with the recommendations, so that they can detect and correct compliance-related problems.

An abbreviated version of the USPHS/IDSA Guidelines will be published in CDC's *Morbidity and Mortality Weekly Report* in July.

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Recommendations for a Regional Strategy for the Prevention and Control of Emerging Infectious Diseases in the Americas

On June 14-15, 1995, a conference on "Combating Emerging Infectious diseases: Challenges for the Americas" was held at the Pan American Health Organization (PAHO) Headquarters in Washington, D.C. The meeting was designed to shape a regional strategy for preventing and controlling emerging infectious diseases that could pose serious threats to the peoples of the Americas.

Participants, convened by PAHO, included top officials and infectious disease experts from that organization as well as the World Health Organization, the U.S. Centers for Disease Control and Prevention, the Canadian Laboratory Center for Disease Control, the U.S. Department of Defense, and several Latin American and Caribbean countries.

This international group of experts noted that an increasing number of new, emerging, and reemerging infectious diseases have been identified in both developed and developing nations and that these diseases threaten to increase in the near future. They include human immunodeficiency virus/acquired immunodeficiency syndrome, which emerged in the l980s and now affects some 16 million people worldwide; and cholera, which returned to the Western Hemisphere for the first time this century in 1991 and has caused more than 1 million cases and 9,000 deaths in the Americas. PAHO estimates that it will take more than a decade and over $200 billion to control the current pandemic of this disease.

The experts concluded that both early warnings of, and rapid responses to, infectious disease threats are needed. The group made several major recommendations to PAHO and its member states to improve surveillance, research, and communications in developing countries. They also issued more detailed recommendations in the areas of antimicrobial resistance, outbreak control, and information and communication. In addition, a plan of action is forthcoming.

The group made the following recommendations for PAHO and its member countries:

General Recommendations

- Develop and frequently update prioritized disease-specific guidelines for the prevention and control of diseases that are emerging or reemerging, both at the public health and individual levels. This should include biologic and behavioral change measures and will require groups of experts for each disease as well as communications experts. Diseases of interest include yellow fever, dengue, antimicrobial-resistant organisms (malaria, tuberculosis, and enteric diseases), measles, polio, cholera and other foodborne and waterborne diseases, viral hemorrhagic fevers, plague, rabies and other zoonoses, and trypanosomiasis and other vector-borne diseases.
- Identify points of contact in the field to receive and transmit information in countries. These contacts should include organizations and individuals outside the government.

- Develop plans to distribute accurate and timely information to the general public.
- Develop plans to improve and make more efficient two-way communication on reporting, control, and modification measures. This may require contracting information management specialists to identify and implement the most efficient means.
- Make efficient use of the press, including radio, television and newspapers, fliers, and other methods to educate the public and the medical community, with an eye toward social mobilization of communities to fight emerging diseases. This will require expertise in communications and support to the countries in developing information dissemination plans. Countries should define populations at greatest risk and focus the information and control measures in these populations.
- Define different approaches for educating the public and the medical community.
- Focus efforts on intersectorial action, including education of policy makers outside the health community.

Antimicrobial Resistance

The expert group recommended that both PAHO and its member countries, where applicable, do the following:

- Seek ways to reduce availability of over-the-counter antimicrobial agents, including those used in veterinary medicine; this will require efforts beyond the health care community and involve education and dissemination of information to all sectors.
- Intensify assistance to the countries in developing rational drug policies.
- Monitor sensitivity to antibiotics in each country to allow for optimum antibiotic use for individual cases and to eliminate antibiotics with little therapeutic value. Employ mechanisms such as WHONET and PHLIS to centralize, analyze, and distribute antimicrobial sensitivity data.
- Develop and distribute specific recommendations to extend the useful life of antimicrobial drugs.
- Frequently revise the list of essential antimicrobials based on sensitivity data.
- Initiate educational campaigns on the cost-effectiveness of rational drug use in hospitals.
- Initiate collaboration with the pharmaceutical industry on rational drug use, standardized labels and warnings, and ethical marketing strategies.

Outbreak Control

The expert group endorsed the leadership role of PAHO in developing and disseminating guidelines for outbreak evaluation and control and recommended that PAHO

- Make timely recommendations to coordinate response to outbreaks or threats, including issues related to travel advice, quarantine, and commerce.
- Develop policies and standard operating plans for response to outbreaks at the regional and country levels. Assist countries in developing national outbreak response plans and assist in training teams.
- Identify and list individuals and groups with disease-specific expertise, laboratories with disease-specific diagnostic capabilities, and products, including diagnostic reagents, drugs, and vaccines (both licensed and investigational products). Frequently update these lists.
- Establish a standard system for rapid procurement of vaccines, reagents, insecticides and antimicrobial drugs for prompt response to outbreaks.
- Establish information management and dissemination procedures for use during outbreaks, including accurate and frequent release of information to the press and public.
- Conduct formal evaluations of responses to each outbreak and use the lessons learned to improve responses to subsequent outbreaks.

Information and Communication

The experts recommended communicating with high-level government officials and emphasizing to them the importance of a basic public health infrastructure—including improvements in water, sanitation, and social and economic conditions—in preventing diseases. The group suggested disseminating more information about public health implications of development (such as deforestation, dam construction, urbanization, and other measures) and seeking effective interaction with other sectors.

Other Recommendations

PAHO should

- Create interagency task forces for emerging diseases at regional and country levels.
- Inform regional governments, other organizations, and the public about the emerging disease initiative and strive for the highest level of political support.

- Solicit and allocate specific resources to deal with the emerging diseases initiative, both at the regional and country levels. A portion of these funds should be immediately available when outbreaks are recognized.

For more information on these recommendations, the conference, or its plan of action, contact PAHO.

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Emerging Infectious Diseases Laboratory Fellowship Program

A partnership has been established between the Association of State and Territorial Public Health Laboratory Directors and the Centers for Disease Control and Prevention (CDC) to develop and initiate an emerging infectious diseases laboratory fellowship program in January 1996. A goal of this fellowship program is to strengthen local, state, and federal public health infrastructures to support surveillance and implement prevention and control programs. The fellowship program will help recruit and train microbiologists for laboratories nationwide and provide opportunities for doctoral level scientists to conduct high-priority infectious disease research.

The emerging infectious diseases fellowship program will offer a 2-year laboratory research track for doctoral level scientists, with emphasis on applied research or development in infectious diseases and a 1-year advanced laboratory training track for bachelor's and master's level scientists, with emphasis on the practical application of emerging infectious diseases technologies, methods, and practices. Fellow training and research will take place at CDC and state and local public health laboratories.

For applications or additional information, contact

Emerging Infectious Diseases Fellowship Program
Association of State and Territorial Public Health Laboratory Directors
1211 Connecticut Avenue, Suite 608
Washington, D.C. 20036
Phone: 202-822-5227, Fax: 202-887-5098

Tenth Annual ASTPHLD Conference on Human Retrovirus Testing

The Tenth Annual Conference on Human Retrovirus Testing, sponsored by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), was held March 6 to 9, 1995, in Reno, Nevada. The conference, which was attended by more than 300 representatives of public and private sector laboratories as well as test kit manufacturers, emphasized three themes: new human immunodeficiency virus (HIV) variants, international issues, and HIV testing of newborns. The topics discussed included sequence data for type O isolates, the search for new HIV variants, zidovudine (AZT) resistance, decreased maternal-neonatal transmission due to AZT prophylaxis, results of the national anonymous survey of HIV prevalence in the United States, and the ethical concerns of perinatal screening.

An international perspective on HIV testing was brought to the conference by presentations that focused on India and Latin America. Results were given of a project, funded by a 12-month study grant from the World AIDS Foundation, to provide training on HIV testing to laboratories in India. Four Indian facilitators were trained in the United States; they provided translation and other assistance to eight ASTPHLD faculty, who gave workshops in four training centers in India. This training, which focused on enzyme immunoassay, linked trainees with staff from Indian reference centers and established training materials and trainers for future workshops to be conducted by Indian staff.

Laboratory aspects of HIV testing in Latin American and the Caribbean were also discussed by a member of the Pan American Health Organization (PAHO), who described the spectrum of HIV incidence rates and testing algorithms. PAHO is asking countries of the region to assess their algorithms in terms of sensitivity, specificity, and cost. PAHO aims to support national laboratories by providing guidelines and quality assurance. Proficiency testing, which is encouraged, will be provided by the Centers for Disease Control and Prevention.

ASTPHLD's 11th Annual Human Retrovirus Conference is set for March 6-8, 1996, in Orlando, Florida. Requests for additional information are available; FAX request to 202-887-5098.

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