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Emerging Infectious Diseases

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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.

Instructions to Authors

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

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

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

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

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The Ascension of Wildlife Rabies: A Cause for Public Health Concern or Intervention?

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The epidemiology of rabies in the United States has changed substantially during the last half century, as the source of the disease has changed from domesticated animals to wildlife, principally raccoons, skunks, foxes, and bats. Moreover, the changes observed among affected wildlife populations have not occurred without human influence. Rather, human attraction to the recreational and economic resources provided by wildlife has contributed to the reemergence of rabies as a major zoonosis. Although human deaths caused by rabies have declined recently to an average of one or two per year, the estimated costs associated with the decrease in deaths amount to hundreds of millions of dollars annually. In future efforts to control rabies harbored by free-ranging animal reservoirs, public health professionals will have to apply imaginative, safe, and cost-effective solutions to this age-old malady in addition to using traditional measures.

Rabies virus is the type species (serotype 1) of the *Lyssavirus* genus, a group of morphologically similar, antigenically and genetically related, negative-stranded RNA viruses, with a near global distribution (1). The lyssaviruses (Table 1) are well adapted to particular mammalian species (2) and rarely initiate panzootics. The public health threat of rabies as a preeminent zoonosis relates to the acute, incurable encephalitis that results from transmission of the virus by the bite of an infected animal. An estimated 40,000 to 100,000 human deaths are caused by rabies each year worldwide; in addition, millions of persons, primarily in developing countries of the subtropical and tropical regions (3), undergo costly postexposure treatment (PET). Although the number of human rabies cases has been significantly reduced in the United States, the total number of animal rabies cases approached historical limits in 1993. To appreciate the public health significance that lyssaviruses continue to play as persistent and emerging infectious agents, one must understand certain human activities, such as recent animal translocations (i.e., the natural or purposeful change by humans of the normal home range or geographic distribution of an animal) and animal ecology.

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Historical Perspectives

The history of rabies in the New World reflects the interaction of chance, evolutionary constraint, ecologic opportunism, and human surveillance activities. Rabies may have existed in the United States before European colonization and the introduction of domestic animals incubating the disease. Various pathogens could have migrated during the exchanges of fauna and human populations over the Bering Strait some 50,000 years ago; folklore of a rabies-like malady among native people throughout the Pacific Northwest supports this notion (4). Records at the time of the Spanish conquest in Middle America associate vampire bats w ith human illness (5). If chiropteran rabies viruses were present and well established in the New World at the time of continental interchange, terrestrial virus counterparts also could have been present. Nonetheless, the first indication of terrestrial rabies did not surface until 1703 in what is now California (5). Dog and fox rabies outbreaks, reported commonly in the mid-Atlantic colonies throughout the late 1700s (4), were probably exacerbated by the introduction of dogs and red foxes (*Vulpes vulpes*), imported for British-style fox hunting, throughout New England in the 1800s; fox rabies epizootics ensued and spread to the eastern United States by the 1940s to 1950s (5,6). Skunk rabies reports were also frequent throughout the western states by the 19th century, and they were replete with cowboy tales of "phobey cats" (5).

Table 1. Recognized members of the genus Lyssavirus, family *Rhabdoviridae*

Lyssavirus	Reservoir	History
Rabies	Found worldwide, except for a few island nations, Australia, and Antarctica. Endemic and sometimes epidemic in a wide variety of mammalian species, including wild and domestic canids, mustelids, viverrids, and insectivorous and hematophagous bats; >25,000 human cases/year, almost all in areas of uncontrolled domestic dog rabies.	Descriptions of clinical disease in Greek and Roman documents. In the late 1800s, Pasteur attenuated the virus by serial passage and desiccation to vaccinate humans and animals. Pathognomonic inclusions in nerve cells described by Negri in 1903. An immunofluorescence test for rabies viral antigen developed in the 1950s.
Lagosbat	Unknown, but probably fruit bats. 10 cases identified to date, including 3 in domestic animals, in Nigeria, South Africa, Zimbabwe, Central African Republic, Senegal, and Ethiopia. No known human deaths.	Isolated in 1956 from brain of Nigerian fruit bats (*Eidolon helvum*) at Lagos Island, Nigeria, but not characterized until 1970; 3 cases in domestic animals initially diagnosed as rabies, but weak immunofluorescence led to suspicion of "rabies-related" virus, later confirmed by typing with monoclonal antibodies or nucleotide sequence analysis. Marginal cross-protection with rabies vaccines.
Mokola	Unknown, but probably an insectivore or rodent species. Cases identified in Nigeria, South Africa, Cameroon, Zimbabwe, Central African Republic, and Ethiopia; 17 cases known, including 9 domestic animals and 2 human cases.	First isolated from *Crocidura* sp. shrews trapped in Mokola Forest near Ibadan, Nigeria, in 1968. Characterized in 1970. Like Lagos bat virus, evidence of infection with Mokola was recognized only by poor reaction with anti-rabies reagents. 7 domestic animal cases in Zimbabwe in 1981 and 1982 prompted serologic survey and identification of antibodies to Mokola in rodents, especially bushveld gerbils (*Tatera leucogaster*). No cross-protection with rabies vaccines.

Although individual reports document a high incidence of dog rabies at the beginning of the last century, no national surveillance system existed. Human deaths from rabies in the United States were not commonly reported; the highest official record was of 143 cases, from a survey of death certificates in 1890. During 1938, when rabies in humans and other animals became a nationally reportable disease, the total number of rabies cases reported was 9,412 per year (mostly in domesticated species), with 47 human deaths. These numbers are certainly underestimates, since surveillance was limited, and sensitive diagnostic tests for human and animal rabies were not developed until the mid-1950s.

An epizootiologic transition began in the United States in the 1920s, when rabies prevention efforts were no longer focused exclusively on human vaccination but began to include programs for the control of rabies in dogs. Domestic animal cases gradually declined, largely as a result of local dog rabies control programs that included vaccination, stray animal removal, and leash and muzzle ordinances. However, as such cases decreased, surveillance systems designed to track the source of infection for residual domestic animal foci detected increased cases in wild species. By 1960, rabies was diagnosed more frequently among wildlife than among domesticated animals. In 1971, rabies was reported for the first time from all 48 contiguous states and Alaska. Skunks (primarily the striped skunk, *Mephitis mephitis*) formed the major animal reservoir from 1961 to 1989, until they were unexpectedly supplanted by the raccoon (*Procyon lotor*) during the rabies outbreak in the mid-Atlantic and northeastern states (7). This epizootic is believed to have started during the late 1970s by the

Table 1. (continued)

Lyssavirus	Reservoir	History
Duvenhage	Unknown, but probably insectivorous bats. Cases identified in South Africa, Zimbabwe, and Senegal; 4 cases known, including 1 human death. No cases in domestic animals.	First identified in 1970 in rabies-like encephalitis in man bitten by an insectivorous bat near Pretoria, South Africa. Virus named after the victim. Although Negri bodies detected in histologic examination of brain tissue, negative immunofluorescence tests led to suspicion of rabies-related virus, subsequently confirmed by antigenic and genetic typing. Marginal cross-protection with rabies vaccines.
European bat Lyssavirus 1 (EBLV1)	European insectivorous bats (probably *Eptesicus serotinus*); >400 cases in bats. 1 confirmed human case in 1985 and a suspect case in 1977. No known domestic animal cases.	Although cases in European bats were reported as early as 1954, identification of the virus was not attempted until 1985, when the first of 100 infected bats was reported in Denmark and Germany. Almost all cases are in the common European house bat, *E serotinus* . Marginal cross-protection with rabies vaccines.
European bat Lyssavirus 2 (EBLV2)	European insectivorous bats (probably *Myotis dasycneme*); 5 cases identified, including 1 human death. No known domestic animal cases.	First identified in isolate from Swiss bat biologist who died of rabies in Finland. Marginal cross-protection with rabies vaccines.

translocation of infected animals from a southeastern focus of the disease.

The epidemiology of human rabies has also changed considerably over the last 50 years (8,9). From 1946 to 1965, 70% to 80% of human rabies cases occurred after a known exposure (most often a dog bite), and 50% of the cases before 1975 occurred after treatment with suboptimal vaccines. Over the last decade, 80% of rabies-related human deaths were among persons who had no definitive history of an animal bite (Table 2), and none resulted from postexposure prophylaxis failures. Almost all the recent human cases occurred after an animal exposure that was unrecognized by the patient as carrying a risk for rabies infection. The apparent source of human rabies has also changed: 14 of the 18 cases acquired in the United States since 1980 involved rabies variants associated with insectivorous bats (10).

The latest report, in March 1995, typifies recent trends. A bat, subsequently found to be rabid, was found in the bedroom of a 4-year-old girl in Washington State. The child denied any contact with the bat, and no postexposure treatment was initiated. A bat-associated rabies virus variant was later identified in biopsy specimens from the

Table 2. Human rabies cases in the United States by exposure category, 1946-1995*

	Exposure source				Case
Years	Domestic	Wildlife	Other	Unknown (%)	total
1946-1955	86	8	0	26 (22)	120
1956-1965	21	7	0	10 (26)	38
1966-1975	6	7	1	2 (13)	16
1976-1985	6	1	2	11 (55)	20
1986-1995*	2	2	0	14 (78)	18

* Through Oct. 1995.

child and from the bat's carcass (11). Despite the current prominence of raccoons as the largest wildlife reservoir in the United States (12), no documented human rabies cases have been associated with this ubiquitous carnivore.

The Cost of Prevention

Rabies prevention and control strategies in the United States have succeeded in lowering the number of human rabies deaths to an average of one to two per year. However, the reason for this low mortality level is a prevention program estimated to cost $230 million to $1 billion per year (13-15). This cost is shared by the private sector (primarily the vaccination of companion animals) and by the public (through animal control programs, maintenance of rabies laboratories, and subsidizing of rabies PET).

Accurate estimates of these expenditures are not available. The number of PETs given annually in the United States is unknown, although the total must be substantially greater than the minimum of 20,000 estimated in 1980 to 1981 (16) when vaccine distribution was more tightly regulated. As rabies becomes epizootic or enzootic in a region, the number of PETs increases (17). Although the cost varies, a course of rabies immunoglobulin and five doses of vaccine given over a 4-week period typically exceeds $1,000. Potential exposure to a single rabid kitten in New Hampshire recently led to the treatment of more than 650 persons at an estimated cost of $1.5 million (18). Surveillance-related costs also rise as rabies becomes entrenched in wildlife. During 1993, the New York State rabies diagnostic laboratory received approximately 12,000 suspected animal submissions. This compares with approximately 3,000 submissions in 1989, before raccoon rabies became epizootic. In New Jersey, rabies prevention expenditures in two counties increased from $768,488 in 1988, before the raccoon epizootic, to $1,952,014 in 1990, the first full year of the epizootic (15); vaccination of pet animals accounted for 82% of this total. Vaccinated domestic animals are normally administered a booster vaccine dose after a known or suspected rabid animal exposure (19). This further increases costs, as wildlife rabies epizootics escalate. The cost per human life saved from rabies ranges from approximately $10,000 to $100 million, depending on the nature of the exposure and the probability of rabies in a region (20).

What's more, most economic analyses do not take into account the psychological trauma caused by human exposure to rabies, the subsequent euthanasia of pets, or the loss of wildlife resources during rabies outbreaks. Rabies in wildlife has now reached historically high levels in the United States (12), and the costs of preventing human rabies are mounting.

Human Influences and the Role of Translocation

The colonization of the New World had a profound effect upon native fauna and consequent rabies epizootiology. Large-bodied carnivores, such as bears, cougars, wolves, and wolverines, were perceived as dangerous and killed outright. A few Carnivora have persisted and flourished. For example, the coyote (*Canis latrans*), a highly adaptable canid and the subject of many unsuccessful control programs, has been gradually expanding its range northward and eastward. Despite their widespread distribution and abundance (even in suburban neighborhoods), rabid coyotes have been reported rarely and sporadically, except for a brief period from 1915 to 1917, when an extensive outbreak occurred in portions of Utah, Nevada, California, and Oregon.

While dog rabies has been largely controlled, a region of southern Texas that borders Mexico has persisted as a focus of both dog and coyote rabies. The number of cases of coyote rabies has gradually risen in this area since the late 1980s, accounting for 46 of the 50 cases of coyote rabies reported in the United States during 1991, 70 of 75 cases in 1992, and 71 of 74 cases in 1993 (12). The outbreak of coyote rabies has spread to the vicinity of San Antonio. One of the dangers of this outbreak is the continued spillover into the domestic dog population (21); at least 25 rabid dogs were reported from the area in 1991, 41 in 1992, and 54 in 1993 (12). Human rabies closely parallels the disease in domestic animals; at least two human deaths (in 1991 and 1994), probably due to coyote-dog interactions, have been associated with this canid outbreak in Texas (10,22).

The translocation of infected coyotes from the south Texas focus is believed to be responsible for the transmission of this rabies variant to dogs in at least two other states: a single hunting dog in Alabama during 1993 (12) and at least seven cases of apparent dog-to-dog rabies transmission in Florida in 1994 (21). Expanded surveillance similar to that done in 1977 with the raccoon

rabies focus in the mid-Atlantic region (7) is warranted for this canid virus. In this effort, state health departments should monitor unusual occurrences (such as the increased su bmission of canid specimens to the diagnostic facility), tracking of their time and location, and establishment of suitable public health interventions. These would include restricting further animal movements and enforcing mandatory companion animal rabies vaccination. Assessing control efforts is an important component of any intervention. In addition to the problems posed by the emergence of the coyote as a reservoir for rabies, the potential translocation of other species should be recognized.

Since the transmission of rabies by a bat was first reported in 1953, rabid insectivorous bats have caused an average of 700 to 800 cases annually, and have been found throughout the United States, excluding Alaska and Hawaii (12). The discovery of these cases, coincident with the marked reduction of canine rabies cases, has afforded a certain epidemiologic luxury to enhance surveillance among wildlife. Similar to the Carnivora, the chiropteran families most important in rabies perpetuation (e.g., *Vespertilionidae, Molossidae*) have several species that are highly adaptable, abundant, and widespread. Rabies virus variants maintained by insectivorous bats appear to be exchanged largely independently from those in terrestrial mammalian reservoirs (23), despite documented spillovers. A similar epidemiologic situation exists among European bats, but with *Lyssavirus* genotypes (24) that can be readily differentiated from New World rabies isolates. The role of bats in Africa (25,26) in *Lyssavirus* maintenance is less clear (Table 1). Infections with non-rabies lyssaviruses have resulted in rabies vaccine failures (27). Such infections raise the specter of potentially serious public health consequences if introduced and subsequently established in susceptible bat populations. How probable is this scenario?

The distances between Africa, Eurasia, Pacific Oceania, and the New World mitigate against the dispersal, migration, and introduction of healthy bats without human intervention (28). However, several recent events illustrate the opportunity for the transoceanic transfer of rabies-infected bats. In March 1986, researchers from Canada inadvertently shipped a big brown bat (*Eptesicus fuscus*) that was incubating rabies virus to colleagues in Tubingen, Germany. When the bat became ill and was euthanized, a diagnosis of rabies was made (29). A similar event occurred when Boston researchers collected a dozen wild big brown bats from Massachusetts during July 1994 and exported them to researchers in Denmark. By December 1994, six of the imported bats had died and were confirmed as positive for rabies virus by the Danish Veterinary Services, State Institute for Virus Research (L. Miller, pers. comm.).

Commercial enterprises also serve as vehicles for the accidental translocation of animals infected with rabies virus. The first confirmed nonindigenous case of rabies in Hawaii resulted from the accidental introduction of a big brown bat (30). In March 1991, a bat was captured within a transport container unloaded from a ship in Honolulu harbor. The container held automobiles from Michigan loaded into the container ship in California. The local department of health laboratory diagnosed rabies; this was later confirmed, and the virus was characterized antigenically at the Centers for Disease Control and Prevention. The strain was a variant common to *E. fuscus* in the midwestern and western United States. None of the three instances cited above appear to have resulted in secondary cases or establishment of the virus in foreign animal populations.

No unintended importations of non-rabies lyssaviruses to the United States have been documented. The likelihood of accidental introduction, escape, survival, and perpetuation of infected exotic bat species into the United States is remote. However, other more recent deliberate translocation activities may significantly enhance the probability of such introductions.

During 1994, a number of improperly issued federal permits allowed as many as several thousand wild bats to be imported to the United States for sale in the commercial pet trade. These animals were primarily Egyptian tomb bats (*Rousettus aegyptiacus*), although several other bat species were imported as well. Sales of imported bats (and their offspring) to private collectors or as pets in the United States are prohibited, according to the Foreign Quarantine Regulations (42 CFR 71.54). Animals that may be vectors of diseases of public health concern are eligible for entrance only for restricted uses at accredited zoos or research institutions, where contact with the general public is limited. Imported bats that

will be legally displayed normally undergo an extended quarantine period.

Although no reports of lyssaviruses isolated from Egyptian fruit bats exist, active surveillance for such viruses has not been conducted. These bats are relatively common and widespread throughout the area that extends from Turkey and Cyprus to Pakistan, the Arabian peninsula, Egypt, and most of sub-Saharan Africa (31). Because they may roost by the thousands in a wide variety of habitats, there is ample opportunity for interaction with other Chiroptera, such as the widely distributed straw-colored (*Eidolon helvum*) or epauletted (*Epomophorus wahlbergi*) fruit bats; both of these species have been implicated in *Lyssavirus* epizootiology in Africa (25,26). The adaptability of Egyptian fruit bats should be a cause for concern because of the potential for survival and interaction among indigenous bat fauna, particularly in the southern United States. Additionally, beyond the obvious public health risks and foreign animal disease introduction, imported bat species should not be released into the wild because they may cause serious harm to local agriculture and may displace native species.

Bats serve many critical ecologic functions worldwide and generally avoid contact with humans. However, they may be infected with many pathogens without demonstrating obvious clinical signs of infection. When bats are placed in a private household or pet shop, the hazard of disease transmission to humans is greatly increased. Persons currently possessing imported bats should be advised not to display them in settings where human contact can occur.

Intervention

Widespread, sustained population reduction of mammalian reservoirs to eliminate rabies is not justified (32) for ecologic, economic, and ethical reasons. Given the multispecies complexity and considerable geographic areas affected by wildlife rabies, and the opportunities for translocation, what alternative preventive strategies exist? Recent progress in implementing terrestrial wildlife rabies control programs elsewhere in the world has public health relevance for the United States. Oral rabies vaccination of the red fox with vaccine-laden baits is an integral aspect of rabies control throughout southeastern Canada and Europe, where more than 75 million doses of vaccine have been distributed over 5 million km^2 during the past two decades (33). Consequently, rabies incidence among wild and domestic animals has fallen, as have PETS for human rabies.

The raccoon rabies epizootic in the eastern United States provided renewed impetus for reconsidering oral vaccination technology, first conceived at the Center for Disease Control in the 1960s (34). The shift of the vaccination and baiting methods from a fox model to the raccoon involved extensive field and laboratory research during the 1980s. The existing attenuated rabies vaccines for foxes were shown to be less effective for raccoons and other carnivores (35,36). Additionally, studies of new candidate vaccines raised safety issues regarding vaccine-induced disease in wildlife (36).

In 1983, a vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine was developed (37) that has proven to be an effective oral immunogen in raccoons and various other important reservoir species (38); vaccine advantages include improved thermostability and an inability to cause rabies. (Only the gene for the surface glycoprotein of a vaccine strain of rabies virus was included in the recombinant virus.) When vaccine-laden baits are offered under natural conditions, contact with them by nontarget wildlife species cannot be totally excluded. However, studies of V-RG virus have shown no vaccine-associated morbidity, mortality, or gross pathologic lesions in more than 40 warm-blooded vertebrate species examined. Moreover, with rare exceptions, there has been no contact-transfer of vaccine between vaccinated and control animals housed together (38); viral recovery has been limited to a few anatomical sites over a 48-h interval (39).

While laboratory evaluations of target and nontarget species proceeded during 1987 to 1989, small-scale trials of V-RG were conducted in Belgium and France, with promising results (40). The first North American V-RG vaccine field trial began on August 20, 1990, on Parramore Island off the eastern shore of Virginia (41,42). This limited field trial demonstrated vaccine safety. Efficacy was also suggested: more than 80% of field-vaccinated raccoons survived a severe laboratory rabies challenge (7 months after V-RG release) to which more than 90% of control raccoons succumbed (43).

A 1991 Pennsylvania study site closely approximated the ecologic communities of the eastern United States targeted for use of V-RG

vaccine, while still maintaining relative biosecurity through its geographic barriers. The study at this site evaluated the rate of vaccine-laden bait contact and potential vaccine-related adverse effects among nonraccoon species, including rodents, carnivores, insectivores, and opossum. Raccoons and other furbearers demonstrated no adverse effects associated with vaccine contact. Examination of more than 750 nontarget individuals, representing 35 species, failed to demonstrate gross lesions suggestive of V-RG contact.

After these safety trials, the first efficacy field experiments began in New Jersey during 1992 (44). Between spring 1992 and autumn 1994, more than 100,000 vaccine-laden fishmeal polymer baits were distributed by hand and helicopter over an area of 56,000 hectares. This trial attempted to create a population of immunized raccoons across the northern Cape May Peninsula to prevent the spread of epizootic raccoon rabies from affected portions of the state. Surveillance demonstrated a significant decrease in the rate of spread and overall rabies incidence in the target and other monitored areas (44), suggesting the potential effectiveness of this strategy.

In the United States, oral vaccination of raccoons is now under way in Massachusetts (45), New York (46), and Florida, and an experimental extension of the program to coyotes is under way in south Texas. However, the future of such vaccination for wildlife in the United States may be seriously questioned. For oral vaccination to become an adjunct to traditional methods, the following major questions need to be answered: 1) What is the relationship between animal population density and the minimum density of vaccine/baits needed? 2) What level of herd immunity is necessary to eliminate rabies under various environmental circumstances? 3) What bait distribution techniques are optimal? 4) How can these methods be generalized from foxes and raccoons to other species, such as skunks, mongooses and dogs? 5) What long-term funding sources are available? 6) What are the various costs of rabies control and prevention methods? Given the problems inherent in wildlife control, the greater issue of extending these methods to the control of dog rabies in the developing world will be a challenge well into the next century.

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Diagnosis of Tuberculosis in Children: Increased Need for Better Methods

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In the last decade tuberculosis (TB) has reemerged as a major worldwide public health hazard with increasing incidence among adults and children. Although cases among children represent a small percentage of all TB cases, infected children are a reservoir from which many adult cases will arise. TB diagnosis in children usually follows discovery of a case in an adult, and relies on tuberculin skin testing, chest radiograph, and clinical signs and symptoms. However, clinical symptoms are nonspecific, skin testing and chest radiographs can be difficult to interpret, and routine laboratory tests are not helpful. Although more rapid and sensitive laboratory testing, which takes into account recent advances in molecular biology, immunology, and chromatography, is being developed, the results for children have been disappointing. Better techniques would especially benefit children and infants in whom early diagnosis is imperative for preventing progressive TB.

Despite the availability of effective preventive measures and chemotherapy, the prevalence of tuberculosis (TB) is increasing in the developing world and in much of the industrialized world as well (1-4). According to World Health Organization (WHO) estimates, in 1990 there were 8 million new cases of TB and 3 million deaths due to the disease worldwide; 1.3 million new cases and 450,000 deaths were among children under 15 years of age (5). WHO projects that 90 million new cases and 30 million deaths–including 4.5 million deaths among children–will occur in the 1990s (6,7). In developing countries, the risk for TB infection and disease is relatively uniform in the population; annual rates of infection often exceed 2% (5,6). In industrialized countries, risk is more uneven and depends on the individual's past or present activities and exposure to persons at high risk for the disease (Table 1). From 1987 to 1991, the number of TB cases among children under 5 years of age in the United States increased by 49% from 674 cases to 1006 (8). Although cases among children represent a small percentage of all TB cases, infected children are a reservoir from which many adult cases will arise. The risk for infection by *Mycobacterium tuberculosis* among children depends primarily on the level of risk of developing infectious TB for the adults in their immediate environment, especially their household. Because most current diagnostic tests for TB infection and disease have low specificity and therefore low positive predictive values, epidemiologic investigation continues to be important in establishing the diagnosis of TB in children. In industrialized countries, clinicians and public health professionals in TB services must always ask: Has the child been exposed to an adult with infectious pulmonary TB?

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Natural History of TB in Children

The natural history of TB in children follows a continuum; however, it is useful to consider three basic stages: exposure, infection, and disease (1). Exposure implies that the child has had recent and substantial contact with an adult or adolescent who has suspected or confirmed contagious pulmonary TB (a source case). Exposed children are usually identified during followup investigations for persons with suspected pulmonary TB by public health workers (9); the child's tuberculin skin test (TST) is nonreactive, the results of the chest radiograph are normal, and the child is free of physical signs or symptoms of TB. Some exposed children are infected with *M. tuberculosis*. The clinician cannot know immediately which exposed children are infected because the development of delayed-type hypersensitivity to tuberculin may take up to 3 months. Unfortunately, in children under 5 years of age, severe TB—especially meningeal and disseminated

Table 1. Persons at high risk for *Mycobacterium tuberculosis* infection in industrialized countries

Persons likely to be exposed to or become infected with *M. tuberculosis*

- Close contacts of a person with infectious tuberculosis (TB)
- Foreign-born persons from high-incidence areas (e.g., Asia, Africa, Latin America)
- The elderly
- Residents of long-term care facilities (e.g., correctional facilities and nursing homes)
- Persons who inject drugs
- Other groups identified locally as having increased prevalence of TB (e.g., migrant farm workers or homeless persons)
- Persons who may have occupational exposure to TB

Persons at high risk of developing TB disease once infected

- Persons recently infected with *M. tuberculosis* (within the past 2 years)
- HIV-infected persons
- Persons with immunosuppressing conditions or medication use
- Persons with a history of inadequately treated TB
- Infants

disease—can occur in fewer than 3 months, before the TST becomes reactive (10). Young children in the exposure stage should receive chemotherapy, usually isoniazid, until infection can been excluded.

TB infection is first signaled by a reactive Mantoux TST. In this stage, there are no signs or symptoms, and the results of the chest radiograph are either normal or show only fibrotic lesions or calcifications in the lung parenchyma or regional lymph nodes. In developing countries, TB infection is rarely discovered and almost never treated. In most industrialized countries, children with a positive TST receive isoniazid for 6 to 12 months.

TB disease occurs when signs and symptoms or radiographic manifestations caused by *M. tuberculosis* appear. Radiographic abnormalities and clinical manifestations in infected children probably are influenced by the host inflammatory reaction more than by the number of organisms. Studies show that in 40% to 50% of infants with untreated TB infection disease develops within 1 to 2 years (11). The risk decreases to 15% among older children. In 25% to 35% of children TB is extrapulmonary and more difficult to confirm bacteriologically.

In adults, the distinction between TB infection and disease is usually clear because most disease is caused by reactivation of dormant organisms years after infection. Disease in adults is usually accompanied by symptoms, and patients frequently are infectious. In children, who most often have primary disease, the interval between infection and disease can be several months to several years, and radiographic abnormalities often are not accompanied by symptoms; moreover, these children are rarely infectious. The major reason for separating infection from disease in children is that the perception affects the approach to treatment: infection is generally treated with a single anti-TB drug, whereas active disease is treated with two or more drugs. The rationale for the difference in treatment is that the likelihood of emergence of resistance to a drug increases as the bacillary population increases (3). This distinction is somewhat artificial in children since infection and primary disease are parts of a continuum. Because anti-TB medications are well tolerated by children and are relatively inexpensive in industrialized countries, the usual paradigm of infection and disease encourages overtreatment rather than undertreatment. Asymptomatic lymphadenopathy and mild lung parenchymal changes are labeled and treated as disease.

When evaluating new diagnostic tests the basic differences between the pathophysiology of TB in adults and children should be considered. Among children with recent TB infection, active multiplication of mycobacteria occurs with or without the presence of radiographic abnormalities or clinical symptoms. For example, gastric aspirate cultures yield *M. tuberculosis* from a small proportion of recently infected children with normal chest radiographs. One can anticipate that most diagnostic tests designed to detect *M. tuberculosis* in adults with TB disease will be positive in some proportion of children who have what is usually called TB infection. It will take careful consideration and investigation to determine if and how the results of these new tests should influence the definitions and treatment of TB infection and disease in children.

Established Diagnostic Methods

Tuberculin Skin Test (TST)

The Mantoux TST, which uses five tuberculin units of purified protein derivative, is the standard method for detecting infection by *M. tuberculosis*. The reaction is measured as millimeters of induration after 48 to 72 hours. Since TST is the only way to determine asymptomatic infection by *M. tuberculosis*, the false-negative rate cannot be calculated. A negative TST does not rule out TB disease in a child. Approximately 10% of otherwise normal children with culture-proven TB do not react to tuberculin initially (12,13). Most of these children have reactive skin tests during treatment, which suggests that TB disease contributed to the immunosuppression. In most cases, the anergy occurs to all antigens, but in some cases, reactions to tuberculin are negative but reactions to other antigens remain positive (13). The rate of false-negative TST is higher in those who are tested soon after becoming infected with severe TB; in children with debilitating or immunosuppressive illnesses, malnutrition, or viral and certain bacterial infections; in infants; and if poor technique is used (1,14). The rate of false-negative TST in children with TB who are infected with human immunodeficiency virus (HIV) is unknown, but it is certainly higher than 10%.

False-positive reactions to TST are often attributed to asymptomatic infection by environmental nontuberculous mycobacteria (NTM). Vaccination with *M. bovis* can cause transient reactivity to a subsequent TST, but the association is weaker than commonly recognized. Most–80% to 90% in several studies–children who received BCG as infants have a nonreactive TST at 5 years of age (15-17). Among older children or adolescents who receive BCG, most develop a reactive skin test initially; however, by 10 to 15 years postvaccination, 80% to 90% have lost tuberculin reactivity (18,19). Skin test reactivity can be boosted, probably by antigenic stimulation, by serial testing in many children and adults who received BCG (20). Various factors determine the TST reaction size after receipt of BCG (1). Many recipients of BCG have a reactive TST because they are infected with *M. tuberculosis* and are at risk for disease, especially if they have had recent contact with an infectious TB patient (18). In general, TST reaction should be interpreted in the same manner for persons who have received BCG (20) and for unvaccinated persons.

The relatively low sensitivity and specificity of TST make the test very useful for persons at high risk for TB infection or disease but undesirable for use in persons at low risk (21,22). The predictive values of TST can be improved by varying the size of induration considered positive according to epidemiologic risk factors for infection (Table 2). However, most of even 15-mm reactions in children at low risk are false-positive results, and testing of persons at low risk should be discouraged. Although the scheme in Table 2 is scientifically and mathematically valid, it assumes that the clinician and family are willing and able to develop an accurate history for TB risk factors for children and adults in their environments.

Clinical Signs and Symptoms

Two scenarios lead the clinician to suspect that a child has TB disease. The first occurs when TB is considered during the differential diagnosis for an ill child. This is a common scenario in the developing world but is less common in developed countries. Infants are more likely to be symptomatic than older children with pulmonary TB. The most common symptoms are cough, fever, wheezing, and failure to gain weight (13). Clinical

Table 2. Cut-off size of reactive area for a positive Mantoux tuberculin reaction

≥5 mm	≥10 mm	≥ 15 mm
Persons who had contact with infectious persons	Foreign-born persons from high-prevalence countries	No risk factors
Persons with an abnormal chest radiograph	Residents of prisons, nursing homes, institutions	
HIV-infected and other immunosuppressed persons	Persons who inject drugs	
	Persons with other medical risk factors	
	Health-care workers	
	Locally identified populations at high risk	
	Children in contact with adults at high risk	
	Infants	

signs are surprisingly meager, but rales and wheezes over the affected lung field are most common. Signs and symptoms of extrapulmonary TB are referable to the involved organ. The sensitivity and specificity of signs and symptoms are extremely low and can lead to both overdiagnosis and underdiagnosis when radiographs and other tests are not available.

The second scenario occurs when evaluating a child who has had significant contact with an adult with suspected or confirmed TB. Usually the TST is applied first and is reactive. A subsequent chest radiograph or physical examination leads to discovery of early disease. The child is usually relatively asymptomatic. In the United States, about 50% of childhood TB cases are discovered in this manner (13).

Radiologic Studies

Evidence of pulmonary TB in chest radiographs varies (23,24), but usually radiographs show enlargement of hilar, mediastinal, or subcarinal lymph nodes and lung parenchymal changes (Figure 1). Most of the radiographic abnormalities are caused by a combination of lung disease and the mechanical changes induced by partial or complete airway obstruction resulting from enlarging intrathoracic nodes. The most common findings are segmental hyperinflation then atelectasis, alveolar consolidation, interstitial densities, pleural effusion, and, rarely, a focal mass. Cavitation is rare in young children but is more common in adolescents, who may develop reactivation disease similar to that seen in adults.

The development of radiographic techniques, such as computed tomography (CT) scanning, illustrates some of the issues that arise when newer and more sensitive diagnostic tests become available (24). A CT scan may show enlarged or prominent mediastinal or hilar lymph nodes in some children with recent TB infection and a normal chest radiograph (25). In the absence of a CT scan, the child's disease stage would be called TB infection, and single drug therapy would be used. Many studies, involving thousands of children have shown this treatment to be successful. However, when the CT scan shows mild adenopathy, the clinician may consider this finding indicative of TB disease and treat with several drugs, although this probably is not necessary in the absence of drug resistance. These findings reinforce the idea that pediatric TB is a continuum, and the distinction between infectionand disease is somewhat artificial. There is no current role for the CT scan in the evaluation of the asymptomatic TB-infected child with a normal chest radiograph. This scan can be helpful in selected cases to demonstrate endobronchial disease, pericardial invasion, early cavitation, and bronchiectasis resulting from pulmonary TB when the chest radiograph is abnormal but the pathologic process is not clear.

Figure 1. Chest radiograph of a girl with pulmonary tuberculosis. Note the significant hilar adenopathy in association with atelectasis, the so-called collapse-consolidation lesion.

Mycobacterial Detection and Isolation

Despite recent advances, early mycobacteriologic diagnosis of TB still relies primarily on examination of acid-fast-stained smears from clinical specimens. It is the easiest, least expensive, and most rapid procedure for obtaining preliminary information. However, children under 12 years of age with pulmonary TB rarely produce sputum and are usually unable to expectorate voluntarily. When sputum samples cannot be obtained, gastric aspirate samples are used for detection and isolation of *M. tuberculosis*. Even though an acid-fast bacilli (AFB) stain of sputum is positive in up to 75% of adults with pulmonary TB, fewer than 20% of children with TB have a positive AFB smear of sputum or gastric aspirate (26,27). The newer fluorochrome stains, such as auramine and rhodamine, are superior to classic carbolfuchsin stains (28). The rates of positive AFB stain from body fluids and tissues in children with extrapulmonary TB also are low, and false-positive results caused by NTM disease are common, especially in cervical lymph nodes.

For most children with pulmonary TB, culture confirmation is not needed. Diagnosis is made on the basis of a positive TST, clinical and radiographic findings suggestive of TB, and history of contact with an adult source case. The drug-susceptibility test results from the source case isolate can be used to design the optimal treatment for the child. However, cultures should be obtained from the child if the source patient is unknown or has a drug-resistant organism and if the child is immunocompromised or has extrapulmonary TB.

The best specimen for culture from children with suspected pulmonary TB is the early morning gastric aspirate obtained in the hospital by using a nasogastric tube before the child arises and peristalsis empties the stomach of the respiratory secretions swallowed overnight (29,30). Three consecutive morning gastric aspirates yield *M. tuberculosis* in only 30% to 50% of cases, although the yield from infants is as high as 70% (14). The culture yield from other body fluids or tissues from children with extrapulmonary TB is usually less than 50% (13). Gastric aspiration is inconvenient, expensive, and uncomfortable. The culture yield from random, outpatient gastric aspirates has not been determined recently. Therefore, this procedure cannot be recommended but should be studied.

Bronchoscopy

The role of bronchoscopy in evaluating children for TB is controversial. The culture yield is lower from bronchoscopy specimens than from properly obtained gastric aspirates (29,31). Most children do not need flexible fiberoptic bronchoscopy, but the procedure may be useful in diagnosing endobronchial TB and excluding other causes of pulmonary abnormality, particularly in immunocompromised children, such as those with HIV infection in whom other opportunistic infections may coexist with or mimic TB. In a recent study of 36 children with pulmonary TB, bronchoscopy showed endobronchial involvement in 42%; most (63%) of these children had no clinical or radiographic evidence of endobronchial TB (31). This technique may be used to determine if a child might benefit from corticosteroid therapy, but guidelines for making this decision have not been established.

Clinical Scoring System

TB is an enormous problem in developing countries, where about 95% of cases occur (6). Cost, technical difficulties, and lack of resources make TB diagnosis in children very difficult in these countries. Various clinical scoring systems have been proposed on the basis of available information and tests (32,33) (Table 3). Although helpful, many of these systems have low sensitivity and specificity. However, even in industrialized countries, the triad of a positive tuberculin skin test, an abnormal radiograph, and a history of exposure to an adult with TB remains the most effective method for diagnosing TB in children.

New Diagnostic Techniques

Polymerase Chain Reaction (PCR)

Diagnostic PCR is a technique of DNA amplification that uses specific DNA sequences as markers for microorganisms (34). In theory, this technique can detect a single organism in a specimen such as sputum, gastric aspirate, pleural fluid, cerebrospinal fluid, or blood. Recent publications show that various PCR techniques, most using the mycobacterial insertion element IS6110 as the DNA marker for *M. tuberculosis* -complex organisms, have a sensitivity and specificity greater than 90% for detecting pulmonary TB in adults (35,36). However, these tests are not performed correctly in all clinical laboratories (36)

Table 3. A set of criteria for the diagnosis of pulmonary tuberculosis (TB) in children when culture is not available

A. Positive acid-fast stain of sputum or gastric aspirate

or

B. Two or more of the following:

- History of contact with a tuberculous adult
- Cough lasting longer than 2 weeks
- A reactive tuberculin skin test
  - ≥ 10 mm in children without prior BCG vaccination
  - ≥ 15 mm in children with prior BCG vaccination
- Radiographic findings compatible with TB
- Response to anti-TB therapy (increased body weight by 10% after 2 months, decrease in symptoms)

Source: ref. 33.

and may offer little advantage over high-quality microscopic examination of sputum (34). The cost involved and the need for sophisticated equipment and scrupulous technique to avoid cross-contamination of specimens preclude the use of PCR techniques in many developing countries. PCR may have a special role in the diagnosis of extrapulmonary TB and pulmonary TB in children since sputum smears are usually unrevealing in these cases.

Use of PCR for detecting *M. tuberculosis* in children has not been evaluated extensively. Pierre et al. (37) used an IS6110-based PCR to detect *M. tuberculosis* in gastric aspirate samples from 22 children with pulmonary TB. They found that 15 (25%) of 59 samples were positive; however, testing multiple samples or testing samples at least twice improved the sensitivity. When three samples from the same patient were tested two times each, two or more positive results were obtained from 9 of 15 children with TB, but from 0 of 17 controls. However, 2 of 65 single samples from controls were positive by PCR. Using an IS6110-based PCR assay, Starke et al. (38) tested gastric aspirates from 35 hospitalized children with pulmonary TB and 30 controls to detect *M. tuberculosis* . When compared with the clinical diagnosis, PCR had a sensitivity of 40% and specificity of 80%. Six controls had false-positive PCR results; one had a recent TB infection, two had NTM disease, and three had conditions unrelated to mycobacterial infection. Delacourt et al. (39) studied 199 specimens from 68 children with suspected TB. An IS6110-based PCR identified *M. tuberculosis* in clinical samples from 83% of children with disease compared to the low yield from positive AFB smears (21%) and positive cultures (42%) (39). PCR identified 70% of children with clinical pulmonary TB but no other microbiologic proof of the infection. However, 39% of children with infection but no radiographic or clinical disease also had positive PCR results. These results again demonstrate the arbitrariness of the distinction between TB infection and disease in children.

It appears that PCR may have a useful but limited place in evaluating children for TB. A negative PCR result never eliminates TB as a diagnostic possibility, and a positive result does not confirm it. PCR's major use will be in evaluating children with significant pulmonary disease, when the diagnosis is not easily established by clinical or epidemiologic grounds. PCR may be particularly helpful in evaluating immunocompromised children with pulmonary disease, although published reports of PCR performance in such children are lacking. PCR may also aid in establishing the diagnosis of extrapulmonary TB, though only rare case reports have been published. However, performing PCR on gastric aspirates is not a useful test to distinguish between TB infection and disease and should not be used for children with normal chest radiographs.

Serology and Antigen Detection

Despite dozens of studies published over the past several decades, serology has found little place in the routine diagnosis of TB in adults or children. Several recent studies have used the enzyme-linked immunosorbent assay (ELISA) to detect antibodies to various purified or complex antigens of *M. tuberculosis* in children. Rosen (40) used mycobacterial sonicates in an ELISA on samples from 31 children with clinical TB and found a sensitivity of 26% and a specificity of 40%. This ELISA was influenced by recent BCG vaccination in children under 5 years of age. Barrera et al. (41) used an ELISA that detects antibodies to purified protein derivative and found a sensitivity of 51% for culture-positive pulmonary TB cases in children, but the sensitivity was only 28% for the clinical cases. Hussey et al. (42) used an autoclaved suspension of *M. tuberculosis* to detect antibodies in serum from 132 children with clinical pulmonary TB; the test was 62% sensitive and 98% specific. Higher sensitivity was obtained among patients with positive culture results (69%, n=35), miliary TB (100%, n=6), tuberculous meningitis (80%, n = 15), and pleural effusion (78%, n=16). No correlation was observed with the tuberculin skin-test result, BCG vaccination, or nutritional status whereas duration of therapy, increasing age and chronicity of infection were positively correlated. Delacourt et al. (43) used an ELISA to detect IgG and IgM antibodies directed against mycobacterial antigen A60 in children with TB. At a chosen specificity of 98%, IgG was detected in 68% of children with clinical disease when results were highly controlled for age and prior BCG vaccination. IgM detection had only a 19% sensitivity. However, using the same anti-A60 ELISA at a defined specificity of 95%, Turneer et al. (44) found the IgG sensitivity to be 26% for past TB, 6% for asymptomatic primary

TB, 14% for symptomatic TB, and 9% for NTM adenitis. No available serodiagnostic test for TB has adequate sensitivity, specificity, or reproducibility under various clinical conditions to be useful for diagnosing TB in children.

Mycobacterial antigen detection has been evaluated in clinical samples from adults, but rarely from children (45,46). Two recent assays detecting *M. tuberculosis* -specific antigens yielded high sensitivity and specificity in various clinical specimens from adults with TB (47,48). Measurement of tuberculostearic acid, a mycobacterial mycolic acid, has been used to detect *M. tuberculosis* in clinical specimens (49). Brooks et al. (50) demonstrated a sensitivity of 95% and specificity of 91% when chromatographic profile of carboxylic acids and detection of tuberculostearic acid were combined and compared with culture results and clinical findings in adults with pulmonary TB; however, these techniques require technically advanced equipment and expertise, which are not available where TB in children is most common. Their sensitivity and specificity in children are unknown.

Implications for HIV Infection and Drug Resistance

The resurgence of TB over the last decade has coincided with the HIV pandemic. HIV-infected infants and children are in close contact with their caregivers, who may be infected with HIV and *M. tuberculosis* and are at high risk of developing infectious TB as they become immunocompromised. None of the available diagnostic tests for TB infection or disease in children has been evaluated systematically in children with HIV infection and pulmonary disease or suspected TB. In adults with HIV infection and TB, the sensitivity of diagnostic tests that rely on the host immune response, such as the TST or serology, is much lower than in nonimmunocompromised TB patients. It is likely that the tests' sensitivity also will be lower in HIV-infected children with TB. Tests that directly detect *M. tuberculosis* , such as PCR or antigen detection assays, may be particularly important for HIV-infected children. The culture yield of *M. tuberculosis* from children with HIV infection and TB is unknown but appears to be similar to that from non-HIV-infected children. The most important diagnostic clue for detecting TB in HIV-infected children is a history of contact with an adult who has infectious TB. Since TB may not have yet been diagnosed in this adult, a rapid and aggressive evaluation for TB in adults who care for the child is a critical part of the evaluation of the child.

The current prevalence of drug resistance among *M. tuberculosis* isolates in the United States is 8% to 14% (51, 52). Drug resistance is most common in patients who received treatment, are not responding to therapy, do not adhere to treatment, live in developing countries, are immunocompromised, are prisoners, are homeless, or are children exposed to adults at increased risk for drug resistance. Drug-resistant TB has increased significantly among children (52). Because of low culture yields from children with TB, the clinician must often rely on the antimicrobial susceptibility results for the *M. tuberculosis* isolate obtained from the adult source case who presumably infected the child. This again emphasizes the crucial need to identify and evaluate the source case for every child with TB. The rapid identification of drug-resistant organisms is necessary for control of drug-resistant TB. Various new methods, such as high-performance liquid chromatography or PCR and DNA sequence analysis, may help to identify and test for antimicrobial susceptibility within a few days of diagnosis, but these techniques remain experimental.

Summary

Most recently developed sensitive and specific diagnostic tests have not found a place in the routine evaluation of children with suspected TB. Clinical criteria, particularly skin-test results, radiographic changes, and documented exposure to an infectious adult remain standard diagnostic methods. In industrialized countries, the local public health entity is a crucial partner to the clinician in establishing the diagnosis in the child and determining if drug resistance is present. As new diagnostic tests are developed, they must be evaluated against clinical criteria. The basic differences in pathophysiology of TB in adults and children must be considered before new tests are applied in pediatrics. It will be crucial to study the new techniques in children and not simply extrapolate from results for adults with TB.

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Data Management Issues for Emerging Diseases and New Tools for Managing Surveillance and Laboratory Data

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Data Management Issues for Emerging Diseases

Since 1976, when Legionnaires' disease affected attendees at the American Legion Convention in Philadelphia (1), the scope of public health has expanded. During the 1976 outbreak investigation, public attention was drawn to news accounts of the increasing numbers of cases and deaths as well as to speculations about diseases causes and prevention. After the outbreak, public health officials contended with volumes of information, including clinical data, epidemiologic survey results, and records of specimens collected from patients and the environment. This information was managed on mainframe computers.

In 1980, a cluster of cases of unrecognized illness, primarily affecting young women, created a data management situation similar to that surrounding the Legionnaires' disease outbreak. A major epidemiologic investigation, which included examining a multitude of laboratory specimens and analyzing volumes of data, was undertaken by a large team of federal, state, and local public health officials, as well as numerous academic institutions and private industries. The problems with establishing databases and implementing a data management system for toxic shock syndrome (2) were essentially the same as the data management problems of Legionnaires' disease, except that computer technology had crept forward slightly in public health offices.

During the spring of 1993, a cluster of cases of another unknown illness, eventually attributed to hantavirus (3), occurred in the southwestern United States. The reaction to this unknown disease by public health officials reflected a startling fact: even though the epidemiologic and laboratory methods for curtailing the outbreak were in place, a consistent data manag ement strategy had not been established. Ad hoc databases built by outbreak investigators for a multitude of purposes began to bog down the investigation. Cases were recorded in multiple databases that did not recognize duplicate reports of cases. Updates of data about cases were done in some, but not all, databases. Laboratory data about specimens from patients were not linked to other clinical and epidemiologic data about a patient. No single database was available with well-edited, complete data about all the cases. Parallel, fragmented data management efforts evolved in at least 15 locations, with no coordinated mechanism to integrate them into one system.

Introducing a single system for data management in the midst of the hantavirus outbreak involved more than the data management issues encountered in the earlier outbreaks. Previously, computer technology was viewed as a solution that, although somewhat cumbersome, enabled officials to move from data management by hand to electronic management. However, during the hantavirus outbreak, computer technology became part of the problem; it initially prevented good data management and may have hindered some of the laboratory and epidemiologic efforts to control the outbreak. Data were essentially being locked into various databases and could not be adequately analyzed or merged with data in other databases. In some instances, this peculiar circumstance caused investigators to perform analyses by hand using printouts from electronic databases or entering data again into other systems.

In recent years, legal considerations, such as the Privacy Act enacted in 1974 and the Freedom of Information Act enacted in 1966 (4,5), have also complicated data management. These acts, in their efforts to protect individual privacy and ensure availability of data, have in some cases, constrained public health responses to emergency situations and subsequent surveillance efforts by enforcing strict database design and handling requirements.

Data Management Requirements

In epidemiologic investigations, disease problems are generally characterized by person, place, and time, whether the problem concerns the emergence of a new disease, a change in the resistance pattern of a known pathogen, an emergency response to an outbreak, or a routine

disease surveillance program. The principles of data gathering, management, and analysis are essentially the same for all these purposes. Computer systems developed to manage data associated with these problems should be regarded as tools for the epidemiologic character ization of pathogens, syndromes, cases, and risk factors. Therefore, laboratory data management and reporting systems must be able to handle data about all of these.

The most stringent requirements for data management are imposed by data from laboratory testing of specimens from patients, human and nonhuman sources, and the environment. A system having a relational data model adequate to properly handle the laboratory data requirements will almost certainly be adequate to handle the clinical, exposure, and demographic data requirements.

Two primary data management functions can satisfy the laboratory data demands with multiple requirements in each function. The first function, internal laboratory data management, consists of entering test results and tracking specimens. The second, surveillance, includes gathering data and moving data beyond the electronic files of the laboratory to appropriate sites for analysis. A data management system should be able to perform these functions not only during an outbreak but throughout the period of surveillance as well.

The internal laboratory function, universally similar among most public health laboratories, includes data entry tailored for individual laboratories at the site; retrieval/query ability; and ability to add or delete tests, manage aliquots, share data input in different laboratories of the site, track the status of every specimen regardless of which laboratory tested it, develop reports for specimen submitters, and in some cases assign costs for laboratory tests performed and prepare invoices for submitters.

Requirements for the surveillance function include, in addition to certain critical laboratory data, the following facilities: to record clinical, exposure/risk factor, and demographic data about patients; to include data about multiple specimens and aliquots related to the same person, regardless of the interval separating the specimen dates; and to change questions or test results that are recorded for each specimen.

Although internal and surveillance functions are clearly separate, they are not independent. Data entered into databases for the internal function should be available without additional effort for the surveillance function. In fact, when the internal function is not electronic or when the internal electronic system is inadequate, the system performing electronic surveillance should also perform to some extent the internal functions. Good laboratory data management does not address the internal function at the exclusion of the surveillance function.

If a laboratory data management system is to be useful for emergency situations, it must provide mechanisms for adapting quic kly to the emergency situation. For example, it must provide a way to immediately create an electronic data collection instrument and to incorporate this new instrument into the system at all reporting sites electronically. For the surveillance function, these electronic features must include communications facilities to move data electronically from one location to another, mechanisms for sending messages or files, functions for simple analysis, and methods for preparing and printing reports. While some systems perform some of these functions, most systems do not provide all of them.

With appropriate systems in hand, data management plans for both urgent and routine events can be approached in a sequential fashion. With consensus among all participating investigators, epidemiologists must decide what data (both laboratory and epidemiologic) are needed so that data field characteristics can be defined. Consensus should be reached in the early phase of the outbreak investigation; otherwise participants in the investigation will of necessity begin developing ad hoc data management systems. The more thoroughly and carefully this task is performed, the more stable the data will ultimately become.

In a well-designed system, the initial definitions in an emergency situation can include projections about which data fields will be needed. However, for routine surveillance these can be more thoroughly planned. Thus, the data system should allow fields to be deleted if not needed and to be added if they become important. These modifications should 1) be handled without having to alter the system, 2) use simple menu-driven functions requiring no computer programmer intervention, 3) accomplish the changes

immediately, 4) be distributed to all investigators without disrupting their other functions during the investigation, and 5) be incorporated automatically.

Next, all known participants in the investigation must be identified. These should include local, state, and federal officials as well as academic or private participants who may provide reports to the central data repository. These participants must be identified to the system specifically by person and by site for system security. Appropriate state and federal offices should be informed concerning the computer system and the rules for its use well before an emergency occurs; therefore, sites will be on the system in advance of an urgent problem. However, the system must allow for additional sites to be added quickly. In an emergency, a temporary agreement must be drawn for all participants to cooperate with the demands of the situation, i.e., to use a particular software system and operate under a standard set of rules for collecting and reporting data for the emergency. This agreement may occasionally stipulate that participants share data temporarily in a common database for the sake of data integrity.

Entering clinical, epidemiologic/risk factor, and laboratory data about the same cases into the same database, rather than merging separate databases after the data are collected, provides such great payoffs in time savings and data integrity that the effort to obtain cooperation for a common database during an urgent situation is worthwhile. Although merging multiple databases during routine surveillance is feasible, emergency situations do not lend themselves to this type of data management. Therefore, the system to be used for these situations must accommodate a common database and provide a means of connecting the reporting sites to the database. When the reporting system is activated and data begin arriving at a central location, the system should facilitate analysis at every reporting site and provide a mechanism to export data (e.g., ASCII or .dbf files) for external analysis.

Emergency situations create unusual demands for epidemiologic and laboratory resources; therefore, data management should not disrupt or threaten to divert resources devoted to these other purposes. As the system is implemented, before emergencies occur, discussions of the resources required should be held with participants. Participants must devote some resources to data management, but these should be minimized. This is consistent with implementing a single system in the beginning of the outbreak investigation and continuing with it into the routine surveillance follow-up. Incorporating data into a second system for surveillance could waste resources.

Although, internal data management does not need to change to accommodate an outbreak, laboratories must implement systems that can directly feed data into the master reporting system database, either through an import function contained in the master system or by a direct interface between the internal laboratory system and the surveillance reporting system.

Data management considerations during outbreak investigations and surveillance in the United States include the political concerns of the participants. Political and legal constraints of all participants must be addressed before the need to deal with them arises. On a global scale, this consideration is equally important, especially in countries whose economies may be adversely affected by news of a dangerous disease situation. Individual country sovereignty must not be violated by data reporting, and the cooperation of each participating country or political entity (e.g., World Health Organization [WHO], Pan American Health Organization [PAHO]) must be obtained in an atmosphere of confidentiality. All attempts to obtain, share, or combine data on a regional or global basis must include a well-defined set of rules agreed upon by all participants. For example, data for scientific purposes might be received at an office of WHO or PAHO but not sent beyond these organizations.

Most often, for the sake of surveillance on a regional or global scale, data management considerations must focus first on establishing in-country data management infrastructures. This means that regional or global surveillance will first translate into establishing a master system, or at least compatible systems in individual participating countries. In most cases, data management systems available to developing countries do not provide the relational model needed by the laboratory. Therefore, efforts should be initiated to introduce and establish systems that can meet these needs in countries desiring to use them.

A plan for regional or global surveillance must include tools to respond to outbreaks and provide

for the computing equipment and modems or other means of transmitting the data electronically. Today's environment demands that most data management be done on personal computers located at critical sites where data can be input. However, data volume may ultimately require that the system provide for archiving data onto another medium. This does not preclude the use of personal computers for data management but simply recognizes that current technology limits the volume of data that can practically be managed and analyzed on personal computers.

The initial data management plan for a country should include a section on reporting procedures and the appropriate medium for archiving data. To handle an immediate, urgent situation the system should contain, at a minimum, a personal computer with large hard-disk capacity (at least 1-2 gigabytes at the central level and possibly 300-500 megabytes at each reporting site), large memory (at least 4 megabytes of RAM at every reporting site), adequate speed (at least 33 megahertz at every reporting site), and fast modems if appropriate. For sites located in areas with inadequate telephone lines, other provisions for electronic transmissions should be planned (e.g., diskettes). Until security can be assured on the Internet, we do not recommend using this medium for electronic tran smission of labo ratory clinical data for outbreak investigations and surveillance.

New Tools for the Management of Surveillance and Laboratory Data

The Public Health Laboratory Information System (PHLIS)

To address the need for a data management system for outbreak investigations and surveillance, the National Center for Infectious Diseases, CDC, in cooperation with the Association of State and Territorial Public Health Laboratory Directors in the United States, developed PHLIS. With this system, data entry screens (modules) are created and distributed to all reporting sites electronically, and data are input and reported within hours, without involving computer programmers. PHLIS provides the capacity for a hierarchical reporting scheme involving reports to multiple, successively higher reporting levels; a database is created at every reporting level so that all data reported to a site or input at the site are included in the database at that site.

The most recent version of PHLIS (Version 3.0), is a menu-driven system based on a relational data model sufficient for the needs outlined in the first part of this report. The system allows for a patient record to be input only one time and links multiple specimens for that patient record. This is true even if specimens for the same patient are entered in different disease modules, or if the patient's name is to be added into a module that contains only epidemiologic data (no laboratory specimens). PHLIS provides a core set of data to be collected on every patient. In addition, each disease module can be customized by adding additional fields to the core data if needed. The system can accommodate data for epidemiologic, laboratory, survey, and case-control studies, and for other public health needs.

Field staff can rapidly add their own data fields to existing disease modules to customize the data entry for special needs at each data reporting site. During an outbreak, a new module can be rapidly developed and electronically transmitted to all participating reporting sites.

The system, which includes data communication software, is configured so that data flow in a pyramid reporting structure: that is, data are reported from lower level reporting sites through higher level reporting sites and ultimately to a single central site. As data are passed to each successively higher level, they are automatically assimilated into that site's database. Thus, databases are built and updated at successively higher reporting s ites. Additional information about a case or specimen may be added at any reporting site; if desired, these additional data are also transmitted to the next higher reporting site.

To meet the need for feedback, PHLIS has a menu-driven option to transmit files or messages up and down the reporting chain, with these files and messages being transmitted automatically when connections are established for each data transmission. This facility is flexible enough to allow any valid user in the reporting chain to transmit files or messages to any other user in the reporting chain. For example, in the United States, a county health official who is included in the reporting system in one state can send messages or files to a participating county official in

another state. The feedback system does not mimic electronic mail because these files and messages are sent along the reporting chain in the same communications configuration as data reporting. Therefore, successful arrival of these messages at their destination(s) depends upon each member of the reporting chain between the sender and the receiver to establish a connection for reporting purposes. However, the system provides an alternative mechanism for sending files and messages directly to any other reporter having the capacity to receive them without going through the reporting chain.

PHLIS is used in all 50 state public health laboratories, as well as the District of Columbia and Guam. Disease modules included are animal rabies, *Campylobacter, Escherichia coli* O157:H7, Lyme disease, mycobacteria, respiratory and enteric viruses, human *Salmonella*, nonhuman *Salmonella*, *Shigella*, and drug-resistant *Streptococcus pneumoniae.*

PHLIS can be implemented independently: organizations can develop their own PHLIS pyramid reporting system. For example, PHLIS is currently being implemented at the Caribbean Epidemiology Center (CAREC) in Trinidad and in its member countries for the reporting of HIV/STD infections with the expectation that the reporting system will be expanded to accommodate other diseases. CAREC can receive reports from the member countries as each country is added to the reporting structure.

Laboratory Information Tracking System (LITS)

The second system, LITS, is a PC local area network-based system for tracking laboratory specimens. The system allows specimen information to be entered at a central specimen receiving site; additional information about the specimen can be entered into the system in any of the laboratories performing tests on that specimen. Although modules are customized for each laboratory's needs, laboratorians can add additional tests or delete obsolete ones. Furthermore, users can examine all the data about a specimen, including data from all laboratories that performed tests on the specimen. Other features in the system include cost billing, user defined reports, user defined query, and specimen or patient tracking and security. For emerging diseases, LITS provides a mechanism to standardize laboratory protocol across organizations and a mechanism to share data about specimens within an organization.

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Helicobacter hepaticus, a Recently Recognized Bacterial Pathogen, Associated with Chronic Hepatitis and Hepatocellular Neoplasia in Laboratory Mice

Gastric carcinoma, one of the most prevalent human cancers worldwide, is among the neoplasms for which epidemiologic evidence of environmental causes is strongest. The exact nature of these environmental causes was obscure until mounting evidence recently linked chronic infection of the gastric antrum mucosa by *Helicobacter pylori* (a microaerobic, gram-negative, spiral bacterium) with elevated cancer risk (1). It is now recognized that gastric B-cell lymphoma of mucosa-associated lymphoid tissue is also closely linked to gastric *H. pylori* infection, and eradication of the infection with antibiotics can result in regression of the lymphoma (2,3). This startling finding has stimulated intense interest in the genus *Helicobacter* and related organisms; as a result, additional species of *Helicobacter* are now frequently isolated and characterized from many non-human hosts. Until 1994, however, only *H. pylori* was known to be associated with tumor development, in humans or in any other animal species.

In 1992, at the National Cancer Institute's Frederick Cancer Research and Development Center (FCRDC) in Frederick, Maryland, a high prevalence of liver disease was observed among certain strains of mice; these mice were untreated controls in long-term chemical carcinogenesis experiments. Affected strains, notably A/JCr, had been bred at FCRDC under pathogen-free conditions and were free of known serologically detectable murine viruses and parasites; moreover, they had no histologically demonstrable hepatic abnormalities, except for a very low incidence (1% to 2%) of hepatocellular tumors in mice 15 months of age or older. Over a very short period, the prevalence of a histologically distinctive form of hepatitis increased to virtually 100% in male mice at 1 year of age (Table 1). The earliest demonstrable lesions were small, undistinctive foci of hepatic necrosis seen in young mice aged 2 to 6 months. In older mice, aged 6 to 10 months, there was a highly distinctive pericholangitis, consisting of abundant mononuclear cell infiltrates around bile ducts within portal triads. The biliary epithelium within affected ducts was focally swollen, and the luminal surfaces of damaged epithelial cells were poorly defined in hematoxylin and eosin-stained sections (4,5). In livers with extensive lesions, bile ductular (oval cell) hyperplasia was also prominent. Moreover, mice with hepatitis usually had hepatocellular tumors, often multiple, that included both adenomas and carcinomas (4).

Hepatocellular tumors in mice are one of the most common endpoints in bioassays for chemical carcinogens. They were not, at that time, known to be associated with infectious agents. Accordingly, initial efforts to identify the cause of the hepatitis/hepatocellular tumor syndrome were directed toward possible sources of chemical exposure. The possibility of accidental exposure to experimental substances within the research animal facilities was ruled out when liver disease was identified in mice that had never left the breeding areas which are located in separate buildings. Extensive chemical analyses of food, bedding, water, and other possible sources of toxic substances had negative results.

Detailed pathologic examination by light microscopy of tissue sections from diseased livers was continued, and many special stains were used. One such stain, Steiner's silver impregnation procedure for spirochetes (6), revealed in hepatic tissue uniform bodies that were consistent in size and shape with bacteria. Homogenates of fresh liver tissue from diseased mice proved effective in transmitting hepatitis to A/J mice purchased from commercial sources outside FCRDC, when given by intraperitoneal injection (5). In addition, from these homogenates, a motile, spiral bacterium could be cultivated on blood agar plates incubated at 37 ^{o}C under anaerobic or microaerobic conditions.

This organism was subsequently characterized by ultrastructural morphologic examination, biochemical characteristics, and 16S rRNA gene sequence. Determined to be a new species related to *H. pylori,* it was given the name *H. hepaticus* (7). The bacterium is motile and gram negative, 0.2 to 0.3 μm in diameter, 1.5 to 5.0 μm long, and curved to spiral in shape, with one to several spirals; it has bipolar sheathed flagella (one at each end) but

Table 1. Increasing prevalence of hepatitis and hepatocellular neoplasia in control male A/JCr mice at the National Cancer Institute's Frederick Cancer Research and Development Center, 1989-1992[a]

Date killed	Number of mice	Age, in weeks	Mice with hepatitis (%)	Mice with liver tumors (%)
Jan-Mar 1989	48	47 ±3	0	0
May-Jul 1989	47	70 ±60	0	1 (2)
Jan-Apr 1992	6	36 ±4	2 (33)	0
Jul 1992	16	54	16 (100)	1 (6)
Aug-Oct 1992	6	64 ±3	5 (83)	3 (50)
Dec 1992	12	77	12 (100)	11 (92)

[a]Adapted from ref. 4.

lacks the periplasmic fibers that envelope the bacterial cells in other mouse *Helicobacter* species. *H. hepaticus* has strong urease activity, is oxidase and catalase positive, produces H_2S, reduces nitrate to nitrite, and grows microaerobically at 37 °C but not at 25 °C or 42 °C. It is resistant to cephalothin and nalidixic acid but sensitive to metronidazole. Photographs illustrating its morphologic structure by light (4,5) and electron (4,5,7) microscopy have been published. The species-defining characteristic of the organism, the nucleotide sequence of its 16S rRNA gene, has been used to develop a diagnostic assay based on polymerase chain reaction (8).

Systematic examination of rodents of all species and strains produced at FCRDC, especially retired breeders, showed that the characteristic hepatitis and associated bacteria were present in mice of several strains (A/JCr, DBA/2NCr, C3H/HeNCr) and that within these strains, the male mice were more severely affected than the female. Mice with severe combined immunodeficiencies were especially vulnerable. The precise location of organisms demonstrable by Steiner stain within infected liver parenchyma was shown by transmission electron microscopy to be invariably extracellular and characteristically within bile canaliculi (4,5). No liver disease was seen in some strains (e.g., C57BL/6NCr) or in F1 hybrids between sensitive and resistant strains (e.g., B6C3F1). Rodent species other than mice (e.g., rats, Syrian hamsters, and guinea pigs) were not affected.

In infected mice with severe combined immunodeficiency, cecal inflammation was histologically demonstrable (5), and organisms were isolated from the mucosa of the large intestine (7), which may mean that the usual ecologic niche occupied by *H. hepaticus* is that of a commensal colonizer of the intestinal tract (8). Since mice are coprophagic, it appears highly likely that natural transmission of the organisms is the oral-fecal route. Why and how *H. hepaticus* invades the liver in mice of certain strains remain to be determined. Hepatitis is also characteristic of certain other enteric pathogenic bacteria, such as *Campylobacter jejuni* (9) that, unlike *H. hepaticus,* have not been associated with liver tumor development. The tissue damage that accompanies persistent infection by *H. hepaticus, H. pylori,* and certain other *Helicobacter* species may be due, at least in part, to a soluble, trypsin-sensitive cytotoxin of high molecular weight produced by these organisms (10). There is no precedent for any direct role of such a toxin in carcinogenesis. On the other hand, chronic infections by viruses, bacteria, or certain parasites are recognized risk factors for human cancers at various sites. The hypothesis that chemically reactive, potentially genotoxic, substances of low molecular weight (including nitric oxide and active oxygen species) generated by inflammatory cells at the site of chronic infection may initiate or enhance carcinogenesis has been examined (11). The hypothesis is under active investigation in the context of *H. hepaticus* -associated liver disease.

H. hepaticus is susceptible to a number of antibiotics; treatment of susceptible, naturally infected 8- to 10-week-old strain A/JCr mice with single or combined antimicrobial agents has been evaluated for efficacy in eradicating established infections (12). Amoxicillin, metronidazole, and tetracycline administered singly failed to eradicate bacteria from the gastrointestinal tract, but either amoxicillin or tetracycline, in combination with metronidazole and bismuth, was effective in eradicating *H. hepaticus* from the liver, cecum, and colon when given by oral gavage for a period

of 2 weeks (12). The effect of antibiotic therapy on the carcinogenic process, or in older animals, remains to be established.

The importance of *H. hepaticus* to humans is not yet completely known. The organism clearly has the potential to confound bioassays for chemical carcinogens, but this potential has no direct effect on humans. Even though most *Helicobacter* species identified to date are characteristically associated with (and named after) specific mammalian host species in which they generally inhabit the gastrointestinal tract (with or without causing gastritis or other chronic inflammatory disease), the potential host range for some species is quite broad. *H. pylori,* originally isolated from humans, has recently been isolated also from the domestic cat; this raises the possibility that *Helicobacter pylori* may be a zoonotic pathogen that can be transmitted from companion animals to humans (13). Exploring the possibility of zoonotic transmission of *H. pylori, H. hepaticus,* or any other *Helicobacter* species would require isolation of the organism in question by culture methods. Serologic methods have not yet been refined to the level of species specificity. Humans infected with *H. pylori* mount a serum antibody response to the bacteria that is readily detected by enzyme-linked immunosorbent assays and is considered evidence of ongoing disease (1); mice infected with *H. hepaticus* similarly produce serum antibodies to that species that have been demonstrated by Western blotting (5). Antisera to *H. pylori* can be used to visualize *H. hepaticus* in mouse liver tissue sections stained by the avidin-biotin complex immunohistochemical procedure (5). The cross-reactivity between these two species precludes use of available serologic methods to establish whether *H. hepaticus* has infected humans.

Regardless of whether *H. hepaticus* is itself capable of infecting humans, it serves to demonstrate that liver tissue can be persistently infected by at least one member of the genus *Helicobacter*, and that liver cancer can be a long-term consequence of such infection. This discovery raises questions about the existence of a comparable relationship between liver cancer in humans and unrecognized bacterial infections. Reviews are under way of tissue blocks from pathology archives in search of organisms demonstrable by the Steiner stain in liver sections from human populations at high risk for liver cancer.

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Hemolytic Uremic Syndrome Due to Shiga-like Toxin Producing *Escherichia coli* O48:H21 in South Australia

Enterohemorrhagic *Escherichia coli* (EHEC) other than serotypes O157:H7 are increasingly recognized in association with hemolytic uremic syndrome (HUS) (1) and have been reported in Australia (2). While detecting strains of O157:H7 has become easier over the years, identifying the expanding number of other serotypes of EHEC also associated with HUS, with other conditions, and with healthy domestic animals is still very difficult.

Cases of HUS have been reported in Australia over a number of years. The most common serotype found was O111:H-, and Australia's recently reported first HUS outbreak (3) was caused by EHEC O111:H-. We wish to report a case of severe HUS due to serotype O48:H21, which, as far as we know, has not been previously reported as a cause of HUS. This case occurred in 1993, before surveillance of HUS had been initiated; after this case, between July and December 1994, 10 cases of HUS (from which four isolates were obtained; two were EHEC O111) were reported to the Australian Paediatric Surveillance Unit (E. Elliott, pers. comm.).

The patient in the 1993 case was an 8-year-old girl, living in a rural setting in the outskirts of Adelaide, South Australia. Her home was adjacent to a farm on which cows, sheep, and ducks were kept. A kelpie/healer cross puppy was in the house in November 1993. Also kept were a pet galah (Australian cockatoo) and pet fish. She was well until 23 December 1993, when she had diarrhea described as very smelly and watery "like the juice of tinned crab." The diarrhea became bloody on 2 January 1994 and was associated with severe abdominal pains which made the patient draw up her legs. She was having bowel movements six times a day, had become very weak, and was unable to stand. She was admitted to Adelaide Children's Hospital on 3 January 1994, and her condition progressed to anuric renal failure over the next few days. Serum biochemistry on 7 January showed a urea level of 23.3 mmol/L and creatinine level of 539 µmol/L. Her hemoglobin level fell from 157 g/L on 3 January to 86 g/L on 10 January. Her hematocrit fell from 48% to 24%, and her platelet count fell from 463 x 10^9/L to 47 x 10^9/L on these dates, respectively. The blood film showed microangiopathic hemolytic anemia with fragmented red cells. She required hemodialysis for 3 weeks and was discharged from the hospital on 31 January 1994.

Apart from the patient's 5-year-old brother, who had loose bowel movements for 1 day on 28 December 1993, no other family members were affected. An adequate dietary history was not obtained; however, no food had been eaten from commercial food outlets.

Stool samples were collected on 4 and 5 January 1994. The samples were probed for Shiga-like toxin (SLT)-I and SLT-II genes by polymerase chain reaction (PCR), and the results were positive. Approximately 80% of lactose-fermenting colonies on MacConkey agar were also SLT positive. No sorbitol-negative colonies were observed on sorbitol-MacConkey agar. In addition to being cultured for *E. coli*, the stools were also routinely cultured for *Shigella, Salmonella, Yersinia, Vibrio*, and *Clostridium*. In addition, stained concentrates were examined for *Giardia lamblia* and *Entamoeba histolytica* with negative results. Four typical *E. coli* strains were subjected to further tests. They were typical *E. coli*, positive in the indole and ONPG tests, negative in the Voges-Proskauer, citrate, TDA, malonate, urease, gelatine, and H2S tests. The strains fermented glucose, lactose, mannitol, xylose, rhamnose, arabinose, sorbitol, sucrose, and melibiose. They did not ferment inositol, adonitol, salicin, raffinose, or amylose. They decarboxylated arginine, lysine, and ornithine. All the strains produced enterohaemolysin (4). The strains were O and H serotyped (5, 6) and found to be serotype O48:H21. Supernatant preparations were tested on Vero cells (7) and found to give typical verocytotoxic reactions in titers of 10^3 to 10^4. The supernatants were also tested by enzyme-linked immunosorbent assay (ELISA) by using monoclonal antibodies 13C4 and 11E10 directed against SLT-I and SLT-II, respectively, and strong reactions with both antibodies were noted, confirming the presence of both SLTs.

Stool samples taken from the patient on 8 February 1994 were negative for SLT-I and SLT-II genes by PCR and were not cultured further. Stool samples from the patient's brother and local animals were not forthcoming.

That all four *E. coli* isolates tested were of serotype O48:H21 and demonstrated identical toxigenicity by both PCR and ELISA and the fact that SLT-positive organisms were not found in the stools collected during the patient's convalescence strongly suggest that this serotype was the causative organism. The toxicity, virulence, and part of the molecular structure of the SLT-II gene derived from the EHEC O48:H21 strain reported here (and whose novel serotype was discovered by the authors) have recently been described elsewhere (8).

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Does Treatment of Bloody Diarrhea due to *Shigella dysenteriae* Type 1 with Ampicillin Precipitate Hemolytic Uremic Syndrome?

Diarrhea-associated hemolytic uremic syndrome (HUS), the most common cause of acute renal failure in infancy and childhood, is often associated with infection by organisms producing Shiga toxin (ST) or Shiga-like toxin (SLT), mainly verocytotoxin-producing *Escherichia coli* (VTEC O157:H7) and *Shigella dysenteriae* type 1 (1,2). Although antibiotics are believed to be essential in treating shigellosis, treatment of *S. dysenteriae* type 1 patients with antibiotics to which the organism is resistant has been considered a risk factor for HUS (3,4).

Until 1993, HUS was rarely reported from Saudi Arabia. Four cases of diarrhea-associated HUS due to *S. dysenteriae* type 1 were identified in 1989 (J. Hibbs and A. Mishkas, unpublished report), and one case of HUS attributed to plasma transfusion was documented in 1988 (5).

In May 1993, four dysentery-associated HUS cases in two families were reported from northwestern Saudi Arabia (Tabuk). *S. dysenteriae* type 1 was isolated from the stool of each HUS patient. The organism was also isolated from 6 of the other 10 members of the two families who had dysentery. All isolates were resistant to trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, and ampicillin but sensitive to nalidixic acid. The two families had just returned from a 1-week visit to relatives in two neighboring villages in Gizan. This densely populated region in southwestern Saudi Arabia has about 1.2 million people living in more than 4,000 villages; the population is relatively poor and uneducated, and environmental sanitation is generally inadequate.

We defined a case of HUS as any case of bloody diarrhea (BD) that had all of the following: acute renal failure (serum urea nitrogen, 18 mg/dL (or 6.3 mmol/L); or creatinine, 1.3 mg/dL (or 115 mmol/L)); thrombocytopenia (platelet count 130,000/mm ^{3}); and hemolytic anemia (hemoglobin level less than 10 g/dL; or hematocrit less than 30%; or appearance of fragmented red cells on direct microscopy). We standardized the treatment of BD in the Gizan region as follows: No antibiotics were given for treatment of BD at the primary health care centers (PHCCs) before a stool specimen was taken for culture and sensitivity testing. After reviewing the preliminary results, we either recommended use of nalidixic acid for treatment of BD or were guided by the results of the stool culture. This protocol was followed for management of BD in the entire region.

Parasitologic, bacteriologic, and biochemical tests and drug treatment regimens were obtained for all patients admitted with BD or HUS to the regional referral hospital or five district hospitals in the outbreak area. BD cases were identified through hospital admission records, visits to PHCCs in the affected villages, interviews with family members of the identified patients, and school visits. We visited the houses of all HUS and BD patients and interviewed family members to ascertain which antibiotic was used to treat the BD patients; mothers were shown bottles and boxes of antibiotics and were asked to identify the antibiotic used for treating the children with BD.

We identified 233 cases of BD occurring from February through July 1993 among 79 families scattered over 19 contiguous villages. Affected villages were predominantly in southern Gizan region near the Yemeni border. One hundred ninety patients (81.5%) consulted PHCCs; of those, 97 (51%) were referred to hospitals, and 81 (43%) were admitted. Thirty-four other BD patients were admitted directly to hospitals (a total of 115 admissions). In nine BD cases patients did not seek medical care including seven (3%) who used traditional treatment (the Wicka plant). In 23 (10%) patients, 13 male and 10 female, BD developed into HUS. Four isolates of *S. dysenteriae* type 1 that showed the same antibiotic susceptibility described earlier were obtained from four patients with BD in different villages in the middle of the outbreak. We used Cary-Blair transport medium for transporting stool specimens collected before antibiotic treatment from newly recognized patients with BD. However, community- and hospital-based interviews showed that the sequence of symptoms was almost identical in all of the 233 BD cases: the condition started with colicky abdominal pain and tenesmus (69%), followed by watery diarrhea (60%), which rapidly became only

mucus and blood (83%) or blood-streaked (17%). Seven patients (3.0%) had rectal prolapse. *S. dysenteriae* type 1 was not isolated from any of the 23 HUS patients; however, all stool specimens were taken during antibiotic treatment.

Most BD cases (92.3%) were among Saudis; the remaining 7.7% were among Yemeni patients. No HUS case occurred among patients over 11 years of age. The male/female ratio for both BD and HUS was 1.3:1. Three boys and three girls with confirmed HUS died (case-fatality rate = 26.1%); none of the patients with uncomplicated BD cases died.

Of the 23 HUS patients, 18 (78%) became ill with the disease 2 to 14 days after hospital admission for uncomplicated BD. This compares with a hospital admission rate of 40 (27%) of 147 for children of the same age with BD from the same villages (odds ratio = 9.6, 95% confidence interval 3.1-35). Five children, aged 8 to 16 months, got HUS either before or on the day of hospital admission; all had received oral ampicillin at home for 5 to 7 days before their illness progressed to HUS. In comparison, two of nine children of the same age, with BD, who were not hospitalized, received ampicillin at home (OR = infinite, p value = 0.02, Fisher's exact test).

Eighteen HUS cases occurred after the patients were admitted to Samtah and Abu-Arish, two out of five district hospitals. The demographic, clinical, and laboratory profiles of BD and HUS cases are shown in Table 1A-C. Six different antibiotics were used in various combinations for treating BD

Table 1.

A. Profiles of children admitted to hospitals with bloody diarrhea (BD) or hemolytic uremic syndrome (HUS)

	Places of hospitalization of BD and HUS cases				
	Samtah	Abu-Arish	KFH	Bysh	Sabia
No. of BD cases	43	42	13	9	8
No. of HUS cases	8	14	-	-	1 [a]
Percentage of HUS cases	18.8	33.3	0	0	12.5
Median age in years: BD cases	4.0	5.0	7.0	4.0	4.0
HUS cases	1.8	2.8	-	-	0.8
Mean (±SD) of duration (in days) between onset of symptoms and admission to hospital:					
BD	3.8 (1.9)	5.5 (1.6)	4.5 (0.4)	3.7	3.9 (2.0)
BD complicated with HUS	5.2 (2.6)	4.5 (2.5)	-	-	-
HUS diagnosed on adm ission	13.0 (2.8)	7.5 (0.7)	-	-	6.0 (0.0)

B. Percentage of 110 BD patients treated with antibiotics[b,c]

Ampicillin	36.6	70.0	15.4	11.1	100.0
Metronidazole	14.6	55.0	7.7	0	100.0
Gentamicin	22.0	22.5	0	55.6	42.9
Nalidixic acid	70.7	57.5	61.5	66.7	0
Claforan	2.4	20.0	0	11.1	0
Amikacin	9.8	0	7.7	22.2	28.6

C. Laboratory values of children hospitalized with BD or HUS

Laboratory test made on the day of admission to the hospital[d]	Non-HUS cases		HUS diagnosed 2-14 days after admission to hospital		HUS diagnosed on admission to hospital	
	Mean (N) [e]	SD	Mean (N)	SD	Mean (N)	SD
Serum creatinine	60 (16)	46	63 (3)	79	279 (3)	94
Blood urea nitrogin (BUN)	5.4 (23)	5.8	15.3 (3)	12.0	23.0 (3)	6.1
Serum sodium	129 (33)	9	137 (3)	4.6	127 (3)	14
Serum potassium	3.8 (47)	0.8	3.9 (7)	1.1	4.6 (5)	1.1
(Leukocytes [WBC] count)	14.2 (48)	6.0	34.0 (6)	24.7	41.2 (5)	18.8
Hemoglobin	11.3 (50)	2.3	12.0 (1)	1.5	6.8 (4)	2.5
Hematocrit	36.1 (13)	3.9	NA	NA	15 (2)	7.1
Thrombocytes (platelets)	322 (5)	250	154 (1)	-	NA	NA
(Body temperature on admission)	37.8 °C (75)	0.8	37.9 °C (18)	0.8	37.9 °C (5)	0.9

[a] Community case of HUS. [b] Percent of cases receiving the corresponding antibiotics. A patient may receive more than one antibiotic. Totals do not add up to 100%. [c] This table does not include 5 cases diagnosed as HUS on admission and treated as cases of BD. [d] Creatinine in mg/dL, blood urea nitrogen in mg/dL, sodium in mmol/L, potassium in mmol/L, WBC (white blood cell count) in thousands/µm, hemoglobin level in g/dL, hematocrit, platelets in thousands/mm^3. Values shown are for children under 12 years of age only. [e] N = number of cases of BD diagnosed in the hospital. NA = not available.

Table 2. Risk of developing hemolytic uremic syndrome (HUS) by antibiotic combination used for treatment of bloody diarrhea (BD), Gizan, Saudi Arabia, 1993

Antibiotic combination group	Antibiotic combinations	Dysentery patients: Total (N = 110)	Dysentery patients: Developing HUS (N = 18)	BD cases admitted to Samtah and Abu-Arish Hospitals	HUS rate (%)	Risk ratio	95% confidence Interval[a]
Nalidixic acid with or without other antibiotics, but no ampicillan [b]	N(3/23), N+G (0/7) G+N+C+A (0/1), M+N (0/1), M+N+C (0/2), N+C (0/4)	41	3	28	7.3	1	0.23–4.34
No antibiotic	No antibiotic (0/12)	12	0	6	0.0	NC [c]	*p* value = 0.629 [d]
Antibiotic other than nalidixic acid or ampicillin	A (0/1), C (1/0), G (0/1). M+G (0/1)	4	1	4	25.0	1.58	0.22–11.58 [e]
Ampicillin with or without other antibiotics but no nalidixic acid		28	10	19	35.7	6.90	0.98–48.68
Ampicillin only	P (3/3)	6	3	5	50.0	6.11	1.31–28.54
Ampicillin with other antibiotics but no nalidixic acid	P+G (1/2), P+M (2/3), P+M+A (0/2), P+M+C (1/0) P+M+G (3/8)	22	7	14	31.8	10.50	0.54–205.39
Ampicillin and nalidixic acid with or without other antibiotics	P+M+G+N (1/1), P+M+N (0/10), P+M+N+C (1/0). P+N (1/8), P+N+A (1/2)	25	4	24	16.0	1.77	0.31–10.21

HUS rate (%) calculated from total number of BD in the five district hospitals using corresponding antibiotic .[a] Mantel-Haenszel weighted relative risk adjusted to hospital (Epi Info, version 6.02). Analysis restricted for data from Samtah and Abu-Arish hospitals. HUS was not reported from the other three district hospitals. [b] Reference group. A = amikacin, C = claforan, g = gentamicin, N = nalidixic acid, M = metronidazole, P = ampicillin. Numbers between parentheses in the second column (antibiotic combination) indicate the (number of patients with HUS who took the corresponding antibiotic combination number or patients with BD who took the same antibiotic combination but did not develop HUS in the five district hospitals). [c] NC = not calculated. [d] One-tailed Fisher's exact test. [e] Adjusted Mantel-Haenszel relative risk could not be calculated.

patients in these hospitals Table 1B. Treatment with ampicillin prescribed alone or with other antibiotics except nalidixic acid (Table 2), was associated with development of HUS. However, three BD patients who received nalidixic acid developed HUS. Of 12 BD patients (including eight children under 12 years of age) who received no antibiotic therapy, none got HUS.

These results support the implication from Bangladesh and from a parallel investigation in Saudi Arabia that inpatient antibiotic treatment of children with dysentery due to *S. dysenteriae* type 1 may precipitate HUS. We have extended these observations to show the same association for antibiotic treatment at home. Although Abu-Arish and Samtah hospitals received similar numbers of BD patients, more HUS cases were reported from Abu-Arish hospital, which used ampicillin to treat patients with BD. The various combinations of antibiotics used to treat BD patients could be explained by the presence of doctors from parts of the world that have different prescription practices. Three BD patients got HUS despite the use of nalidixic acid. Resistance to nalidixic acid among *S. dysenteriae* isolates was reported from Bangladesh; resistance increased from 2.1% in 1986 to 57.9% in 1990 (6).

We recommend a laboratory-based surveillance system to identify and promptly contain emerging outbreaks. Physicians need to be informed continuously about emerging resistant strains of bacteria and be cautious when using antibiotics to treat patients with dysentery unless the causative organism and the resistance pattern have been identified. Parents of children with BD need to be educated to take their children to the nearest health facility as soon as possible.

S. dysenteriae is a delicate bacterium that does not withstand adverse conditions (e.g., heat and dryness); prompt plating, preferably at bedside, is recommended (7). Failure to isolate *S. dysenteriae* type 1 from dysenteric stool specimens during this outbreak could be attributed to delayed plating of specimens, lack of appropriate transport media,

and treatment with antibiotics before stool specimens were obtained. Even with direct inoculation of stool specimens in pediatric wards, HUS resulted in a low yield of *S. dysenteriae* type 1 (8,9).

Treatment of BD due to *S. dysenteriae* type 1 with ampicillin may precipitate HUS. It would be valuable to retrospectively examine this association in other countries where both dysentery due to *S. dysenteriae* type 1 and HUS are reported.

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An Outbreak of Hemolytic Uremic Syndrome Associated with Antibiotic Treatment of Hospital Inpatients for Dysentery

Shiga toxin (ST) from *Shigella dysenteriae* type 1 is accepted as a cause of hemolytic uremic syndrome (HUS); however, the reasons why HUS develops in only some infected patients are not clear (1). The possibility that antibiotic therapy is associated with the development of HUS has been explored for *S. dysenteriae* type 1 and for *Escherichia coli* O157:H7 (2–4). In May 1993, during an outbreak of *S. dysenteriae* type 1 in Gizan, Saudi Arabia, an association between antibiotic treatment and HUS was also observed (5). The strain of *S. dysenteriae* type 1 was resistant to ampicillin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) and sensitive to nalidixic acid. We report here some of our preliminary observations for a concurrent outbreak.

In response to the Gizan outbreak, a circular was sent to all regions of Saudi Arabia requesting immediate reports of *S. dysenteriae* type 1. One region reported three cases of *S. dysenteriae* type 1 with the same antibiotic resistance pattern. The patients were visitors from the Najran region, on the Yemen border. The affected family reported that many other persons in their community of Barshash, home of about 6,000 Yemeni immigrants, had recently had dysentery. The regional hospital in Najran confirmed that several local children had been treated for HUS, and an investigation was initiated.

Beginning in February 1993, the numbers of dysentery patients at the Barshash Primary Health Care Center began to increase from 6 patients per week to 110 in mid May. After control measures were initiated, weekly incidence decreased to zero by late June. According to policy, the Primary Health Care Center treated dysentery patients with oral rehydration, whereas those admitted to the regional hospital received antibiotics. At the regional hospital, *S. dysenteriae* type 1 was isolated from four Barshash residents and one resident of another community.

Between March and May, the illness of 10 (of 42) children admitted to the regional hospital for dysentery progressed to HUS from 2 to 10 days (median 5) after admission. For all 10 children, physicians noted HUS onset as a sudden change in the clinical condition characterized by pallor, puffy face, peripheral edema, or oliguria. Within 2 days of clinical onset, blood urea nitrogen (BUN) levels of all 10 children were 10 mmol/L. In nine children, the hematocrit fell to below 75% of its value on admission. One child had microcytic hypochromic anemia on admission and was transfused with one unit of packed red cells; his hematocrit fell from 29.8% after the transfusion to 24.6% the day after HUS onset; his BUN level rose from 3.1 to 23.5 mmol/L; and his thrombocyte count fell from 529,000/mm ^{3} to 75,000/mm ^{3}. Red cell fragments were reported for seven patients. Thrombocytopenia, developed in seven patients; in two, thrombocyte counts decreased from admission values. A leukemoid reaction ($\geq$ 30,000 leukocytes/mm ^{3}) developed in seven patients.

HUS cases included six children from Barshash, one from a contiguous district, one with relatives in Barshash, and two visiting from southern Gizan region where the concurrent outbreak was occurring (5). No child was admitted from the community with HUS. Reasons given for admission were either dysentery or bloody diarrhea with mild or moderate dehydration. Two dysentery patients who developed HUS were admitted because no one was available to care for them at home. Characteristics on hospital admission of dysentery patients who did or did not develop HUS were similar with the exception of serum sodium level and BUN (Table 1). Elevations in creatinine (63 to 133 mmol/L) and urea (6.1 to 7.9 mmol/L) levels on admission were mild, and after intravenous rehydration returned to normal. No enteric pathogens were isolated in stool specimens from 35 patients.

All 42 dysentery patients received a variety of antibiotic combinations composed of ampicillin, TMP-SMX, nalidixic acid, gentamicin, erythromycin, and metronidazole. Higher rates of HUS were observed among patients receiving antibiotics to which the locally circulating *S. dysenteriae* type 1 was resistant (ampicillin or TMP-SMX) or antibiotics that are ineffective against *Shigella* (metronidazole, erythromycin, gentamicin) than

Table 1. Characteristics on hospital admission of dysentery patients whose illness did or did not develop into hemolytic uremic syndrome (HUS) during hospitalization, Najran, Saudi Arabia, March through May 1993

Characteristic	Dysentery patients		*p* value[a]
	Developed HUS (10)	Did not develop HUS (32)	
Age (yr mean)	4.4	4.8	0.71
Male sex	7 (70%)	23 (72%)	1.0
Percentile weight for age [b] (median)	15.4	16.3	0.57
Below fifth percentile	6 (60%)	12 (38%)	0.29
Days with dysentery (median)	3	3	0.70
Range	2–8	1–14	
Stools on first hospital day (median)	12.5	9.0	0.23
Range	2–26	2–43	
Temperature (mean)	38.2 °C	38.0 °C	0.64
Range	36.8 °C–39.4 °C	36.5 °C–39.4 °C	
Hematocrit (mean)	38.9%	38.8%	0.94
Below 35.5%	4 (40%)	7 (23%)	0.43
Thrombo cytes/mm ^{3}	555,000	501,000	0.62
Below 150,000	0	0	
Leucocytes/mm ^{3} (mean)	11,440	12,300	0.62
Above 12,000	7 (70%)	22 (69%)	1.0
Above 18,000	1 (10%)	4 (13%)	1.0
Serum sodium, mmol/L (mean)	130	134	0.02
Below 130 mmol/L	4 (40%)	7 (22%)	0.41
Serum potassium, mmol/L (mean)	3.4	3.4	0.86
Below 3.5 mmol/L	5 (50%)	16 (50%)	1.0
Serum creatinine, mmol/L (mean)	72	62	0.62
Above 62 mmol/L	5 (50%)	13 (41%)	1.0
BUN, mmol/L (mean)	4.1	3.2	0.06
Above 6.0 mmol/L	2 (20%)	2 (6%)	0.24

[a]Differences in means by Student's t-test, in medians by the Kruskal-Wallis test, and in proportions by Fisher's exact test (two-tailed). [b]National Center for Health Statistics.

among patients treated with nalidixic acid (with or without metronidazole) (Table 2). Stratification of this analysis by elevated creatinine level (62 mmol/L) or BUN (6.0 mmol/L) on admission or by weight for age above and below the fifth percentile had no effect on the magnitude or statistical significance of these associations. However, stratification by serum sodium on admission above and below 130 mmol/L yielded increased risk ratios of 15 (95% confidence interval, 1.6 to 147) for any ineffective antibiotic and 15 (95% confidence interval, 2.3 to 99) for ampicillin without nalidixic acid (Mantel-Haentzel analysis).

These strong associations of HUS with prior antibiotic therapy suggest that the antibiotics may be influencing the progression of *S. dysenteriae* type 1 to HUS (2,5). The Najran patients had milder disease on admission, and the risk ratios were higher than in the other two reports. The assumption that *S. dysenteriae* type 1 caused the dysentery is supported by the isolation of *S. dysenteriae* type 1 from community members during a concomitant dysentery outbreak and by the absence of other ST producing organisms. However, because culture results are lacking, it is possible that some hospitalized dysentery patients were infected with other organisms.

This investigation was retrospective, and without randomization, the selection of patients for treatment by severity of illness was managed with stratified analysis. All possible indicators of dehydration or severity of dysentery on admission were not available for stratification. However, these were also not ava ilable to the physicians for selection of patients. Moreover, nalidixic acid was considered effective therapy, and physicians did not indicate in the medical records that they were selecting nalidixic acid for less severely ill patients. Because of the variety of antibiotics prescribed, the effect of antibiotics given to most patients (metronidazole) in combination with other antibiotics could not be evaluated. The effectiveness of those given to a few patients (TMP-SMX) could not be assessed.

Table 2. Risk of developing hemolytic uremic syndrome (HUS) by antibiotic combinations used for in-hospital treatment of dysentery during a community outbreak of antibiotic-resistant *Shigella dysenteriae* type 1, Najran, Saudi Arabia, March through May 1993

Antibiotic combination	Dysentery patients: Developed HUS (10)	Dysentery patients: Total (42)	HUS rate/100	Risk ratio[a]	95% confidence interval[b]
Ineffective[c] antibiotics without nalidixic acid[d–j]	6	12	50	4.3	1.3–15
Ampicillin combinations without nalidixic acid[d–f]	5	7	71	6.2	1.9–20
Ineffective antibiotics without nalidixic acid or ampicillin[g–j]	1	5	20	1.7	0.22–14
Ampicillin and nalidixic acid with (2) or without (3) metronidazole	1	3	33	2.9	0.40–20
Trimethoprim-sulfamethoxazole, nalidixic acid and metronidazole	0	1	0	0	
Nalidixic acid with (24) or without (2) metronidazole (reference)	3	26	12	1.0	Reference

[a] Relative to the reference antibiotic combination (nalidixic acid with or without metronidazole). [b] Taylor series approximation standard. [c] Antibiotics to which the outbreak strain of *S. dysenteriae* type 1 was resistant (ampicillin, trimethoprim-sulfamethoxazole) or which are ineffective against shigella (metronidazole, gentamicin, or erythromycin). [d] Ampicillin and metronidazole (4 patients). [e] Ampicillin, metronidazole, and gentamicin (2 patients). [f] Ampicillin only (1 patient). [g] Trimethoprim-sulfamethoxazole and metronidazole (1 patient). [h] Trimethoprim-sulfamethoxazole, erythromycin, and metronidazole (1 patient). [i] Metronidazole only (2 patients). [j] Erythromycin and metronidazole (1 patient).

Antibiotic therapy may be associated with HUS in various ways. The antibiotics given may have been ineffective, so the infections were allowed to run their natural course to HUS. However, one would have expected at least a few cases to have developed at home among the hundreds of Barshash residents with dysentery. Another possibility is that ineffective antibiotics suppressed competing microbial flora, allowing less restrained proliferation of *S. dysenteriae* type 1. This does not account for the 12% HUS attack rate in patients treated with nalidixic acid. An in-vitro study of enhanced Shiga-like toxin I (SLT-1) production by *E. coli* O157:H7 suggests another explanation. Subinhibitory concentrations of antibiotics resulted in up to a 400% increase in SLT-I recovery relative to controls (6). This effect was maximal at maximum subinhibitory concentrations of antibiotics, and a quinoline antibiotic, ciprofloxacin, yielded the greatest increase of toxin. These findings are most consistent with the epidemiologic findings in this outbreak, including the occurrence of HUS in patients treated with nalidixic acid and the absence of HUS in untreated patients in the community. With these considerations, antibiotic treatment of dysentery from *S. dysenteriae* type 1 should be approached with caution.

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Epidemic Cholera in the New World: Translating Field Epidemiology into New Prevention Strategies

Cholera, a devastating diarrheal disease, has swept through the world in recurrent pandemics since 1817. The seventh and ongoing pandemic began in 1961 when the El Tor biotype of *Vibrio cholerae* O1 emerged in Indonesia. This pandemic spread through Asia and Africa and finally reached Latin America early in 1991 (1). After explosive epidemics in coastal Peru, it spread rapidly and continues throughout Latin America (Figure 1). Because of underreporting, the more than 1,000,000 cholera cases and 10,000 deaths reported from Latin America through 1994 (Table 1) (2) represent only a small fraction of the actual number of infections. Molecular characterization of *V. cholerae* O1 strains from Peru has shown that they do not match strains from anywhere else in the world; therefore, the source of the Peruvian epidemic strains remains unknown (3). Moreover, other strains have since appeared in Latin America. At least one of these, a strain resistant to multiple antimicrobial drugs, was first identified in Mexico and elsewhere in the world in mid-1991 and has since spread widely throughout Central America (4). The introduction of strains into new areas illustrates the rapid global transfer of pathogens. *V. cholerae* O139 Bengal, which emerged as a new cause of epidemic cholera in Asia in 1992, could also appear in Latin America (5).

Such introductions are not easy to prevent, because they may follow the arrival of travelers who are not aware of their infection or of ships carrying contaminated ballast water. The key to controlling epidemic cholera lies in limiting its spread by using measures that prevent sustained transmission. One measure might be using an inexpensive and effective vaccine to provide lasting protection; however, no such vaccine yet exists, although progress in vaccine development is being made (6-8). Another measure is interrupting transmission so that the causative organism never reaches the human h ost. This approach to prevention successfully controlled many epidemic diseases in the industrialized world, including cholera, typhoid fever, plague, and malaria, before vaccines or antibiotics were developed. Over the last century, a large engineering infrastructure, built in industrialized nations, has provided safe water and sewage treatment for nearly all people in these nations and has made sustained transmission of cholera in those countries extremely unlikely. Despite sporadic cases along the U.S. Gulf Coast and repeated introduction of the epidemic organisms by travelers, epidemic cholera has not occurred in the United States since the nineteenth century (9,10).

To prevent cholera by interrupting transmission of the organism to the host, it is important to understand precisely how the bacteria are transmitted. John Snow demonstrated waterborne transmission of cholera during a large epidemic in London in 1856 (11). He and many others since have suspected that other routes of transmission are also important. Epidemiologic investigations during the seventh pandemic have documented a variety of specific food and water pathways by which the bacteria reach the host, some of which were new and unsuspected (12). The El Tor biotype of *V. cholerae* O1, for example, multiplies rapidly in moist foods of neutral acidity (13). This bacterium also persists in the estuarine environment in niches that are poorly understood but may involve the plankton on which shellfish feed. This means

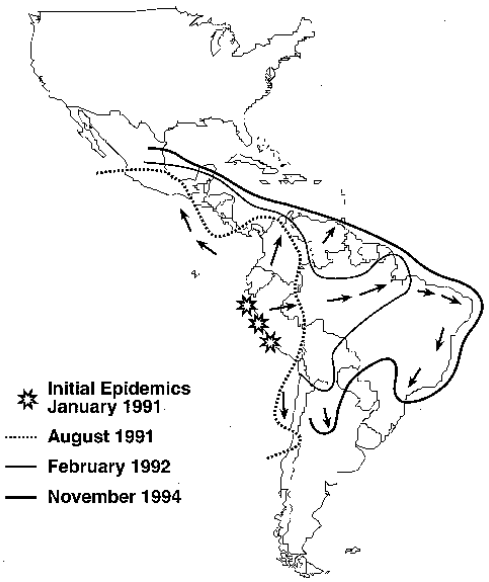

Figure 1. Geographic extent of the Latin American cholera epidemic over time. Lines represent the advancing front of the epidemic at different dates. As of mid-1995, all Latin American countries except Uruguay have reported cases; no cases have been reported from the Caribbean.

that raw seafood can be contaminated naturally before it is harvested. Understanding these pathways of transmission in detail has been central to devising successful control measures to block them. For example, advice to drink only boiled or bottled water would be of little use in outbreaks where the source was actually contaminated food, such as shellfish or leftover rice. On at least one occasion, such advice actually worsened the situation because the bottled water was itself contaminated (14).

When epidemic cholera appeared in Latin America, after an absence of more than 100 years, we conducted a series of eight rapid field investigations in collaboration with national public health authorities and the Pan-American Health Organization to define the pathways of disease transmission and the priorities for prevention. Conducted in various settings between February 1991 and August 1993, these investigations guided the initial emergency prevention efforts and the development of sustained pr evention measures (Table 2) (15-21).

The same case-control method was used for each investigation. We first interviewed a few patients in great detail about what they had ingested in the 3 days before they became ill, probing for known or potential vehicles of cholera. We then constructed a standardized interview questionnaire that asked about possible exposures. Using this questionnaire, we interviewed patients recovering from cholera as well as healthy persons of the same age and sex who lived in the same neighborhood. By comparing the frequency of positive and negative responses among the ill and well persons, we could identify the exposures most strongly associated with disease. For example, if 25 of 35 patients, but only 5 of 35 matched controls, reported eating sliced mango from street vendors in the 3 days before the illness began, the probability of observing this difference in proportions by chance alone is 0.000045, and the ratio of the odds of exposure is 15, a measure of the strong association between disease and consuming sliced mangos. When disease is statistically associated with more than one exposure, multivariate analysis can identify truly independent risk factors. In the above example, "eating food from a street vendor" would not be independent from "eating sliced mango" if one usually got sliced mango from a street vendor. This

Table 1. Cholera cases by country, as reported to the Pan American Health Organization, 1991 through 1994

Country	Date of first report	Number of reported cases			
		1991	1992	1993	1994
Argentina	Feb. 5, 1992	0	553	2,080	889
Belize	Jan. 9, 1992	0	159	135	6
Bolivia	Aug. 26 1991	206	22,260	10,134	2,710
Brazil	Apr. 8, 1991	2,101	30,054	56,286	49,455
Chile	Apr. 12, 1991	41	73	32	1
Colombia	Mar. 10, 1991	11,979	15,129	230	996
Costa Rica	Jan. 3, 1992	0	12	14	38
Ecuador	Mar. 1, 1991	46,320	31,870	6,833	1,785
El Salvador	Aug. 19, 1992	947	8,106	6,573	11,739
French Guiana	Dec. 14, 1992	1	16	2	NR [a]
Guatemala	July 24, 1991	3,652	15,686	30,605	4,227
Guyana	Nov. 5, 1992	0	5	66	0
Honduras	Oct. 13, 1991	17	388	2,290	4,965
Mexico	June 13,1991	2,690	8,162	10,712	4,059
Nicaragua	Nov. 12, 1991	1	3,067	6,631	7,821
Panama	Sept.10, 1991	1,178	2,416	42	0
Paraguay	Jan. 25, 1993	0	0	3	0
Peru	Jan. 23, 1991	322,562	210,836	71,448	23,887
Suriname	Mar. 6, 1992	0	2	0	0
United States	Apr. 9, 1991	26	103	22	34
Venezuela	Nov. 29, 1991	13	2,842	409	0
Total		391,734	352,300	204,547	112,612

[a] NR = no reports received.
Source: ref. 2.

case-control method can be rapidly carried out in the field at low cost.

Investigations showed that cholera was being transmitted by several distinct mechanisms. The predominant route of transmission in a given setting depends largely on the degree of sanitation already achieved. Therefore, a multifaceted approach to prevention is needed. Emergency measures, such as advice to boil drinking water or to heat all foods from street vendors, are difficult to sustain because of their inconvenience and high cost. Moreover, the cost of building large-scale water treatment and sanitation systems is extraordinary, estimated at $200 billion for all of Latin America (22). The challenge of cholera prevention lies in devising low-cost alternatives that are both effective and sustainable.

Waterborne transmission was identified in seven of the eight investigations. In three of these, the implicated water came from municipal systems or from tanker trucks that reportedly obtained water from municipal systems. Water delivered through poorly maintained municipal water systems can be contaminated by sewage because of leaky pipes, frequent pressure drops, and the lack of residual chlorine disinfectant in the water. In developing countries, water is rarely available 24 hours a day so it is usually stored in the home, where further contamination can easily occur. For example, when we measured the increase in contamination of the water as it was distributed and stored in Trujillo, Peru, fecal coliform counts, an index of sewage contamination, were 1/100 ml in water collected at the source well, 2/100 ml at public taps, and 20/100 ml in water stored in the home (13) In four investigations, the implicated water was collected from rivers or ponds, where direct sewage contamination was likely. Specific protective practices were also noted in these investigations, including treating water in the home by boiling it or by adding chlorine bleach, using a small-mouthed vessel to store water, pouring water out of the storage vessel rather than scooping it out with a cup, and having hand soap in the home. In one investigation on the Amazon, we found that the local common practice of adding citrus juice to water to improve its taste was protective because the acid in the fruit killed *Vibrio* bacteria (14). This observation gave local authorities a new, inexpensive, and immediately available emergency control measure.

The first stage of prevention, then, is providing safe drinking water. As a result of the above findings, we have developed and are testing simple and inexpensive methods of domestic water disinfection and storage that would also prevent other diseases transmitted by the same route. A pilot trial in a periurban area of Bolivia showed that disinfecting household water with a calcium hypochlorite solution and storing it safely in a special narrow-mouthed container was acceptable to a community of Aymara Indians (20). Compliance,

Table 2. Mechanisms of transmission of epidemic cholera in Latin America, as determined in eight epidemiologic investigations, 1991-1993[a]

	Peru			Ecuador	El Salvador	Bolivia	Brazil	Guatemala
	Trujillo Urban	Piura Urban	Iquitos Urban	Guayaquil Urban	Salvador Rural	Saipina Rural	Fortaleza Rural	Guatemala City Urban
Transmission mechanism	3/91 (15)	3/91 (16)	7/91 (17)	7/91 (18)	3/91 (19)	2/92 (20)	6/93 +[b]	7/93 (21)
Waterborne								
Municipal water	+	+		+				
Surface water			+		+	+	+	
Putting hands in water vessel	+	+						
Foodborne								
Street vendors' foods		+						+
Street vendors' beverages		+		+				+
Street vendors' ice/ices		+						+
Leftover rice		+	+					+
Fruits/vegetables			+					
Seafood								
Uncooked seafood				+				
Cooked seafood				+	+			

[a] Reference numbers are in parentheses.

[b] CDC Unpublished data.

as measured by chlorine residuals in stored water, was high among families using the intervention. The concentration of *Escherichia coli* bacteria in the stored water, a measure of fecal contamination, was significantly lower in households that used the intervention than in neighboring households that used traditional water handling methods (23). A field trial in rural Bolivia showed that villagers could generate their own disinfectant solution by using a simple electrolytic apparatus (24); with the disinfectant and the special water container the villagers provided clean water in their homes. Households using this intervention had 40% fewer diarrheal episodes than randomly selected neighboring families who used traditional water-handling methods. The combination of point-of-use disinfection and safer water storage containers could have broad effects, including local empowerment for production of potable water, safer infant foods, and new microindustries for the production of disinfectant solutions and water vessels (25). Cost-benefit analysis indicates that this strategy is cost saving if it prevents more than 20% of diarrheal illnesses (26). Inexpensive disinfectant generators are now being manufactured for this purpose in Ecuador. In Colombia, the number of cholera cases has dropped dramatically since late 1992 when chlorine disinfectant tablets were distributed for treating household water.

The second major route of cholera transmission is food contaminated in the market or home. This includes food and beverages sold by street vendors, leftover rice, and unwashed fruits and vegetables. This was an important route in four of the eight investigated areas, including Guatemala City, where there was no evidence of waterborne transmission (21). Foods and beverages sold by street vendors are a fixture of urban life throughout the developing world; they are often prepared in unhygienic ways and then held at ambient temperatures for hours, which permits rapid bacterial multiplication. Other problems associated with food and beverages from street vendors included using unsafe ice to chill beverages and selling homemade frozen drinks. Leftover rice is an excellent growth medium for *V. cholerae* O1 and eating leftover rice without reheating it was associated with illness in three investigations. In one investigation, illness was associated with eating unwashed produce that was probably splashed with river water while being transported to market in small boats.

Thus, the second stage of cholera prevention is to improve food handling, particularly for foods and beverages sold by street vendors. Many countries in Latin America have begun educating street vendors in fundamental food safety and linking this education to licensing (27). By itself, however, education may not improve food safety if clean water to prepare foods and beverages is not available and handwashing and dishwashing with soap and water are not routine. We are field testing a combined strategy of point-of-use disinfection, handwashing with soap, and use of a special water/beverage container to improve the microbial quality of beverages sold by street vendors. Because street vendors are responsive to customer demand, teaching consumers to look for street vendors that are visibly practicing better hygiene may reinforce more hygienic conditions. In addition to these efforts to improve food sold on the street, health authorities should advise the public to reheat leftover rice and wash fruits and vegetables before eating. In Santiago, Chile, suspicion that cholera was caused by vegetables irrigated with fresh sewage led to a ban on this practice; the ban not only prevented cholera transmission by this route, but also decreased the incidence of typhoid fever and hepatitis A dramatically (28).

Transmission through seafood (identified in two of the eight investigations) is a third major route of cholera transmission, distinct from other foodborne mechanisms, because it requires different prevention strategies. One investigation implicated both uncooked seafood and cooked crab, and another implicated cooked seafood eaten without reheating. Contaminated seafood also caused three outbreaks of travel-associated cholera in the United States. In the most dramatic, at least 75 persons contracted cholera after taking a flight from Latin America to California (29); illness was associated with eating cold seafood salad that was loaded onto the plane in Lima, Peru. Two other outbreaks followed the informal transport of cooked crabs from Ecuador to the United States in travelers' suitcases (30,31). Marine creatures may harbor *V. cholerae* O1 before they are harvested or may be contaminated by seawater used in seaside processing plants. In areas where raw and undercooked seafood are popular, seafood-associated cholera may occur even if the general level of sanitation and hygiene is high (32). Vibrios survive light cooking and can subsequently grow if

the seafood is held for many hours before eating (33).

Preventing seafood-associated cholera in the long term will depend on maintaining sewage-free harvest beds and improving sanitation in processing plants. In coastal areas where the organism persists in the environment, even in the absence of sewage contamination, education to discourage the consumption of raw or undercooked shellfish is also needed. Thorough cooking provides the greatest security, but it is sometimes resisted by local populations for cultural reasons. "Ceviche," a popular Latin American dish prepared from seafood that is marinated in citrus juice for variable lengths of time, is a case in point. Prolonged marination in acidic liquid is likely to inactivate vibrios if the acid can penetrate throughout the flesh and deep organs of the fish or shellfish (34). Further evaluation of this approach is needed, but for the present, encouraging the use of ceviche recipes that provide sufficient marination time may be a practical intervention.

In Latin America, as in other parts of the world, epidemiologic field investigations of cholera have defined the local routes of transmission, identified unsuspected and correctable control points, and quantified the effects of emergency measures. The results of investigations also have generated specific control strategies targeted to blocking the predominant routes. While this multistage portrait of transmission is complex, it is being translated into action and change. The longstanding deficits in basic urban infrastructure and the need for new efforts to correct them have never been more apparent (35,36). Workable prevention strategies include better domestic water storage containers, point-of-use water disinfection, attention to the education and hygiene of street vendors, and simple modifications of traditional recipes. Many other diseases are transmitted by these same waterborne and foodborne routes, so these control measures may prevent other infections in addition to cholera. If it becomes a catalyst for long overdue improvements in the safety of water and food, epidemic cholera can have a far-reaching impact on the public health of Latin America.

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Are North American Bunyamwera Serogroup Viruses Etiologic Agents of Human Congenital Defects of the Central Nervous System?

In 1941 Gregg provided the first evidence that rubella virus (family *Togaviridae*, genus *Rubivirus*) causes human congenital defects (1). Although rubella virus infection usually causes a mild disease comprising fever and rash, rubella epidemics have been associated with congenital defects in children of women who became infected during their first trimester of pregnancy (2). The risk of in utero rubella infection was reduced by the introduction of safe and effective vaccines for women of child-bearing age. Congenital abnormalities in fetal or neonatal ruminants also are related to exposure of pregnant dams to various viruses, including bovine viral diarrhea virus (family *Togaviridae*, genus *Pestivirus*), the arthropod-borne bluetongue viruses (family *Reoviridae*, genus *Orbivirus*), Wesselsbron virus (family *Flaviviridae*, genus *Flavivirus*), Rift Valley fever virus (family *Bunyaviridae*, genus *Phlebovirus*), Nairobi sheep disease virus (family *Bunyaviridae*, genus *Nairovirus*), and Akabane and Aino (family *Bunyaviridae*, genus *Bunyavirus*, Simbu serogroup) viruses (3-10). Infections of livestock with these viruses may produce low-titer viremia with no apparent clinical disease, or high-titer viremia and severe clinical illness in the dam. In utero infections may result in malformations of the developing fetus, fetal death with resorption, mummification, or miscarriage. Stillborn ruminants may show various musculoskeletal and central nervous system defects, including a syndrome of arthrogryposis with hydranencephaly (AGH).

Bunyamwera serogroup viruses (family *Bunyaviridae*, genus *Bunyavirus*) have been isolated from humans, and some, including Cache Valley (CV) and Tensaw (TEN) viruses, have been isolated from symptomatic and asymptomatic large mammals (11). Antibodies to CV virus and other viruses of the Bunyamwera serogroup are prevalent in livestock and large wild mammals and in humans in the Western Hemisphere from Alaska to Argentina (11). Viruses of this serogroup are isolated primarily from mosquitoes of the genera *Aedes* and *Anopheles*. These viruses have focal geographic distributions, although some are found over great expanses. CV virus, a common North American bunyavirus, has been isolated principally from mosquitoes of the genera *Culiseta*, *Aedes,* and *Anopheles*. The geographic distribution of this virus includes all North America, except the extreme southeastern states and southern Mexico (11). In the southeastern United States, TEN virus, also isolated from mosquitoes of the genera *Anopheles* and *Aedes*, is the only known representative of the Bunyamwera serogroup, probably because of the range of the principal vectors and vertebrate hosts; mutual exclusion of these two viruses likely occurs because of cross-protectivity between them (12).

Serologic and temporal associations of infection with CV virus and congenital malformations, including primarily AGH, were observed in sheep near San Angelo, Texas, between December 1986 and February 1987, suggesting that this virus causes AGH (13). Subsequent outbreaks of similar congenital defects occurred in sheep in Illinois in 1988 (J. Pearson, pers. comm.) and in North Dakota, Pennsylvania, Maryland, Michigan, and Nebraska in 1986 and 1987 (14). Antibody to CV virus (but not to other viruses) was found to correlate significantly with the occurrence of AGH and other congenital anomalies during the Texas outbreak (15, 16), and IgM antibody to CV virus was detected in colostrum-free neonates with AGH (C.H. Calisher, unpublished data). (Neither maternal IgM nor maternal IgG crosses the placenta in sheep; therefore, antibody in fetuses or in neonates before they received colostrum indicates fetal exposure to an infectious agent [17].)

In 1976, antibody to CV virus was detected in serum from cattle that had delivered calves with AGH in Saskatchewan, Canada, in 1975; however, the prevalence of antibodies to CV virus in the bovine population of that area was not investigated (R.E. Shope, pers. comm.). In Texas, in 1981, CV virus was isolated from a sick sheep and from a healthy cow in a herd with reproductive problems (18). This virus also was isolated in Texas in 1988 from a sentinel sheep in pasture where an outbreak of congenital defects had occurred in 1986 to 1987. These historical data suggest that

CV-virus-related congenital malformations may be more widespread than has been recognized.

Experimental infections have provided further evidence that CV virus causes embryonic death and multiple congenital malformations of sheep (19) and cattle (J. Edwards, pers. comm.). No association of TEN virus and disease in livestock, wild animals, or humans has been reported. To determine whether infection with selected Bunyamwera serogroup viruses, CV and TEN, is associated with certain human congenital defects, a serosurvey was done with serum samples from mothers of children with microcephaly or macrocephaly, and the results were compared with those from age- and location-matched controls. The results, reported here, provide the first evidence that these Bunyamwera serogroup viruses may be etiologic agents of certain congenital defects of the human central nervous system.

Two groups of 500 each human serum samples were selected at random from an archival collection stored at the National Institutes of Health (NIH), Bethesda, Maryland, and tested for neutralizing antibody. These samples were part of a collection of serum specimens obtained between 1959 and 1964, at delivery or post partum, (from about 50,000 women enrolled under defined protocols in a prospective study of congenital rubella syndrome. Specimens had been collected from all pregnant women cared for at the particular institution or from women randomly selected by using the last digit of their hospital registration number.

For a first series of tests, samples were selected from 200 mothers of children with macrocephaly (head size at least two standard deviations above the mean) and from 50 mothers of children with microcephaly (head size at least two standard deviations below the mean). The initial specimen from mothers with the respective defects was selected for testing. An equal number of mothers of babies without obvious central nervous system defects were selected as controls, which were age- (±2 years) and site-matched, were registered for the study in the same month, and were of the same race as mothers of children with either macrocephaly or microcephaly. Serum samples comprising this first group had been collected from pregnant women in Boston, Massachusetts (104), Providence, Rhode Island (20), New York, New York (72), Philadelphia, Pennsylvania (36), Baltimore, Maryland (38), Buffalo, New York (50), Minneapolis, Minnesota (42), Charlottesville, Virginia (38), Memphis, Tennessee (52), New Orleans, Louisiana (36), and Portland, Oregon (12); these were tested for neutralizing antibody to eight bunyaviruses (Table 1).

An additional 500 samples were tested; 250 paired samples, selected as above, from the same archival collection, two each from women in Boston (120), Buffalo (40), New Orleans (8), New York City (46), Baltimore (8), Charlottesville (12), and Minneapolis (16), including controls (selected as above). These were tested for neutralizing antibody to CV virus only, to follow up on results of tests with the first set of samples. One sample from each of these women had been collected in the first trimester of pregnancy, and the second had been collected at least 3 months later. Serum specimens were stored frozen at -20 ^{o}C until they were shipped on dry ice (-70 ^{o}C) to the Centers for Disease Control laboratory at Fort Collins, Colorado.

Twenty-nine of the first 500 serum specimens tested contained neutralizing antibody to CV, 29 had antibody to TEN, 29 to Jamestown Canyon, 26 to La Crosse, nine to Lokern, and six to Buttonwillow viruses. None had antibody to Main Drain or Mermet virus. No significant differences were observed in antibody prevalences to La Crosse, Jamestown Canyon, Lokern, and Buttonwillow viruses between mothers of microcephalic and macrocephalic infants and age- and location-matched controls (Table 1). Cases were not reviewed for other defects.

The prevalence of antibody to CV virus in mothers of microcephalic infants was not significantly different from the prevalence of such antibody in their matched control, but the presence of antibody to CV virus in mothers was significantly correlated with macrocephaly in their infants (χ^2, d.f. = 1, n = 400, 4.797 p <0.05) (Table 1).

None of the samples with neutralizing antibody to CV or TEN virus (titers ranged from 10 to 80) had IgM antibody. To determine whether there were statistically significant differences between prevalences of antibody to CV or TEN virus in mothers of macrocephalic infants and in their age- and site-matched controls, McNemar's chi-square was used. No significant difference was found for the presence of antibody to CV virus (p >0.05), but the presence of antibody to TEN virus (p < 0.05 or antibody to either CV or TEN virus (p <0.02) was related to the occurrence of macrocephaly in the infants of these mothers (Table 2).

Table 1. Antibody to Cache Valley, Tensaw, La Crosse, Jamestown Canyon, Lokern, or Buttonwillow viruses in mothers of microcephalic or macrocephalic infants and matched controls

Antibody to virus	Infant's condition	χ^2 (*p*)
Cache Valley	Microcephaly	3.840 (0.05)
	Macrocephaly	4.797 (<0.05)
	Either	0.915 (>0.20)
Tensaw	Microcephaly	4.891 (<0.05)
	Macrocephaly	4.071 (<0.05)
	Either	0.329 (>0.20)
Cache Valley or Tensaw	Microcephaly	5.983 (<0.02)
	Macrocephaly	4.806 (<0.05)
La Crosse	Microcephaly	0.211 (>0.20)
	Macrocephaly	1.481 (>0.20)
Jamestown Canyon	Microcephaly	0.709 (>0.20)
	Macrocephaly	0.037 (>0.20)
Lokern	Microcephaly	1.010 (>0.20)
	Macrocephaly	2.041 (>0.10)
Buttonwillow	Microcephaly	2.041 (>0.10)
	Macrocephaly	<0.001 (>0.20)

Bunyaviruses used for all tests were prototypes: (Bunyamwera serogroup) CV (strain 6V-633), TEN (A9-171b), Lokern (FMS-4332), Main Drain (BFS-5015), (California serogroup) La Crosse (Original), Jamestown Canyon (61V-2235), (Simbu serogroup) Buttonwillow (A-7956), and Mermet (AV-782). Samples were tested for antibody by serum dilution-plaque reduction neutralization (20). Briefly, diluted and heat-inactivated (56°C/30 min) serum was added to an equal volume of virus containing approximately 200 plaque-forming units (PFU), such that the final virus dilution was 100 PFU. Fresh human serum at a final concentration of 8% was added to all virus suspensions. Serum-virus mixtures were incubated at 4°C for 18 h, and 0.1-ml aliquots were dropped in the center of Vero cultures grown in 6-well plastic plates. After a 45-min period of adsorption, cells were overlaid with medium containing 2% agar and further incubated for 70 to 75 h at 37°C in 5% CO_2-95% air. A second overlay, containing medium, 2% agar, and 1:25,000 neutral red was then added, and the plates again were incubated until plaques were readable, usually 12 to 36 h later. A serum specimen was considered positive when it reduced the number of plaques 90% relative to control titrations, which ranged from 80 to 150 plaques. Samples that were positive in a screening (1:10) test were titrated for end-point, and the serum titer was taken as the highest twofold dilution of serum that reduced the number of plaques 90%.

Although a human positive control for detecting IgM antibody by capture enzyme-linked immunosorbent assays was not available, serum specimens were tested by a modification of a published technique for detecting IgM antibody to California serogroup viruses (21). Mouse or sheep serum samples containing IgM antibody to CV virus or mouse serum with IgM antibody to TEN virus served as positive and negative IgM antibody controls. Statistical analyses were done by chi-square or McNemar's chi-square.

Table 3 summarizes the presence of antibody to CV and to TEN virus in women by hospital location and birth outcome. When prevalence of antibody to CV or TEN virus in these women was analyzed by location and birth outcome, no statistically significant differences were determined (comparative data not shown).

When the second set of 500 specimens (250 paired early- and late-pregnancy samples) was tested, specimens from eight women had neutralizing antibody to CV virus. No diagnostically meaningful change in titer was detected between six sample pairs, but fourfold rises in titer were detected in two others, (10 to 80, <10 to 40), indicating recent infections with CV virus or with a closely related Bunyamwera virus group. Six of the eight women in this group with antibody to CV virus delivered macrocephalic infants, including the two (one in New Orleans and one in New York City) whose specimens showed rises in titer to CV virus.

These analyses provide the first evidence that Bunyamwera serogroup viruses in North America are associated with congenital defects in humans: the occurrence of macrocephaly in infants was positively correlated with antibody to CV virus. Antibody to TEN virus and to either CV or TEN virus correlated with microcephaly and with macrocephaly. The presence of antibody to CV and to TEN viruses corresponded with the known geographic distributions of these viruses within the United States. Antibody to either of these viruses in a woman living in an area where that virus is not known to occur may reflect the close antigenic relationships and considerable cross-reactivity of these Bunyamwera serogroup viruses (22), differences between local virus strains and prototype viruses, or travel to an area in which the virus does occur (23). The two women with diagnostically significant rises in antibody titer to CV virus (one from New Orleans, where TEN virus has been isolated, and one from New York, where CV virus has been isolated) are, as far as we know, the first two persons with documented rises in antibody to a Bunyamwera serogroup virus in North America. Whether either of them had an associated illness could not be determined from the records. That among all the women tested they were the only ones with rises in antibody titer and that both gave birth to macrocephalic infants is, at the least, a fascinating coincidence. IgM antibody in human infections caused by other bunyaviruses may not persist for much more than a few months after

Table 2. Presence of antibody to Cache Valley and Tensaw viruses in mothers of macrocephalic infants and in matched controls

Mothers of macrocephalic infants	No.	Antibody-positive controls	Antibody-negative controls
Antibody to CV virus	16	3	13
No antibody to CV virus	184	5	179
McNemar's test [a]			
Antibody to TEN virus	15	1	14
No antibody to TEN virus	185	5	180
McNemar's test [b]			
Antibody to CV or TEN virus (or both)	19	3	16
No antibody to CV or TEN virus	181	5	176
McNemar's test [c]			

[a] $(13-5)^2/18 = 3.556$ (d.f. = 1), $p > 0.05$. [b] $(14-5)^2/19 = 4.263$ (d.f. = 1), $p < 0.05$. [c] $(16-5)^2/21 = 5.762$ (d.f. = 1), $p < 0.02$.

infection (21); IgM antibody has not been detected in humans with antibody to Bunyamwera serogroup viruses in North America. Therefore, the absence of IgM antibody in specimens with neutralizing antibody likely indicates that these infections were not acute, i.e., they occurred months before the specimens were collected.

A fundamental problem in establishing a relationship between infection of mammals with CV or TEN virus and attendant congenital anomalies is the inherent inadequacy of controls. The presence of antibody in humans or other animals with normal offspring does not necessarily argue against the hypothesis that CV virus causes congenital defects in humans because infection could have occurred before pregnancy. Additionally, even if this virus can be an etiologic agent of congenital anomalies, preexisting antibody to this virus could provide immunity for the mother and protect the fetus from viral infection. Thus, it cannot be determined with certainty whether the presence of antibody to a virus is coincidental to, or a cause of, the observed congenital anomalies.

Review of NIH records for this relatively small set of samples suggested that macrocephaly occurred somewhat more often when the first trimester of pregnancy included the months April and May for women living in New Orleans (4/16), Memphis (5/16), and Charlottesville (3/12) and in late summer-early autumn for women living in Boston (6/49), Minneapolis (3/11), Portland (2/6), and New York City (9/33). In each instance, these periods coincide roughly with the appearance of populations of *Culiseta* , *Aedes* , or *Anopheles* mosquitoes, the vectors of CV and TEN viruses. However, CV virus cannot be implicated in infections in New Orleans because this virus is not known to occur there, although TEN virus does.

The relatively small sample sizes in this study allow statistical interpretation but do not provide sufficient support to warrant statements as to the biological significance of the findings; therefore, we consider these data merely preliminary. Determining whether these data have merit awaits the results of additional studies of mothers of children with congenital defects and their offspring. More extensive studies are also needed to investigate the influence of gestational phase and fetal development on congenital defects; establish relationships between peak abundance of arthropod vectors and first trimesters of pregnancies; sequence the genomes of CV and TEN virus strains from various geographic areas and establish relationships between different gene sequences and virulence in livestock; and develop diagnostic capacity by using monoclonal antibodies, hybridization assays, polymerase chain reaction, and Western blotting techniques.

Table 3. Antibody to Cache Valley and Tensaw viruses in women, by location and birth outcome

	Antibody to CV virus		Antibody to TEN virus	
Location	In women with macrocephalic infants:: no. (%)	In controls: no. (%)	In women with macrocephalic: infants: no. (%)	In controls: no. (%)
Boston	2/49 (4.1)	1/49 (2.0)	1/49 (2.0)	1/49 (2.0)
Providence	0/7	1/7 (14.3)	0/7	1/7 (14.3)
New York City	2/33 (6.1)	1/33 (3.0)	1/33 (3.0)	1/33 (3.0)
Philadelphia	0/15	2/15 (13.3)	1/15 (6.7)	2/15 (13.3)
Minneapolis	1/11 (9.1)	0/1	1/11 (9.1)	0/11
Charlottesville	2/12 (16.7)	0/12	2/12 (16.7	0/12
Memphis	6/16 (37.5)	3/16 (18.8)	5/16 (31.3)	1/16 (6.3)
New Orleans	1/16 (6.3)	0/1	2/16 (12.5)	0/16
Portland	2/6 (33.3)	0/6	2/6 (33.3)	0/6

Given that many members of the family *Bunyaviridae* cause congenital defects in naturally and experimentally infected livestock, or may have such a potential (24,25), it would be worthwhile to continue investigations with domestic animals and to develop laboratory models to assess the teratogenic potential for humans of CV, TEN, and other viruses of the family *Bunyaviridae* .

It is also important to determine the roles of CV and TEN viruses in inducing human congenital defects and the relationships between prevalence of antibody to CV and TEN viruses, prevalence of congenital defects, and conception dates, all with respect to local environmental conditions.

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Lymphocytic Choriomeningitis Virus: An Unrecognized Teratogenic Pathogen

Lymphocytic choriomeningitis virus (LCMV), the first member of the arenavirus family to be isolated, is the causative agent of a zoonosis acquired from chronically viremic mice or hamsters (1). The clinical spectrum of acquired human LCMV infection ranges from inapparent and asymptomatic to, in rare instances, severely symptomatic, systemic, and fatal central nervous system (CNS) disease. Intrauterine LCMV infection has resulted in fetal or neonatal death, as well as hydrocephalus and chorioretinitis in infants (2-6). We have diagnosed congenital LCMV infection in three infants (7) and have collated published and unpublished data on three additional affected infants (8, G.R. Istre, pers. comm.). This report briefly summarizes the salient features of the infection in five of these six American infants and outlines the similarities between these and features observed earlier in Europe. We suggest that LCMV is a more frequent cause of CNS disease in newborns than previously recognized.

Congenital LCMV infection was first recognized in Great Britain in an infant who died at 12 days of age (3). Subsequently, fetal infection with spontaneous abortion (2) and congenital infection in liveborn infants with hydrocephalus and chorioretinitis were documented in Germany (4), France (6), and Lithuania (5). We have recently documented congenital LCMV infection in three infants from Arizona (7) and have obtained information regarding three additional neonates from Arizona, Nebraska (8), and Texas (G.R. Istre, pers. comm.). Detailed clinical and laboratory data are available for five of the six infants. All displayed nonobstructive hydrocephalus with periventricular calcifications, chorioretinitis, and psychomotor retardation. One of the five infants had sensorineural deafness. None of the infants had cardiac abnormalities. Two infants have had follow-up ophthalmologic and audiologic examinations which have shown neither the progression of chorioretinitis nor the development of new auditory deficits. *Toxoplasma gondii*, cytomegalovirus, *Herpes simplex* virus, rubella, enterovirus, and *Treponema pallidum* infections were excluded by culture or serology in all infants. The diagnosis of congenital LCMV infection was confirmed in all infants by immunofluorescence antibody (IFA) and enzyme-linked immunosorbent assays (ELISAs). In addition, serum, CSF, urine, and throat wash specimens from two infants were injected into Vero cell monolayers. Neither cytopathic effect nor LCMV antigens were detected after incubation. Because virus isolation was only attempted after the disease was first diagnosed when the children were 10 months of age, failure to isolate LCMV was not unanticipated.

Laboratory diagnosis of LCMV infection is generally made by serologic techniques. IFA is a more sensitive diagnostic method than either complement fixation or neutralizing antibody techniques (9,10). The newer ELISAs are now being used to evaluate congenitally infected infants. Testing the child's serum and CSF and a simultaneously obtained serum specimen from the mother yields the maximum information if done as soon after birth as possible.

The mothers of four of the five infants in this report had a history of febrile illness during pregnancy, in contrast to a minority of mothers of affected infants previously reported. Typical LCMV infection in adults is a biphasic disease with fever, malaise, myalgias, anorexia, nausea, vomiting, pharyngitis, cough, and adenopathy followed by defervescence and a second phase of CNS disease. However, CNS symptoms may appear without any prodrome or may never develop. Meningitis and meningoencephalitis are the most frequent neurologic manifestations of disease, although myelitis, Guillain-Barré syndrome, and sensorineural deafness have been reported (11). Between 1941 and 1958, 8% to 11% of viral CNS syndromes in hospitalized patients in a Washington, D.C., medical center were etiologically associated with LCMV (12). Arthritis, parotitis, orchitis, myocarditis, and rash have also been noted (13). Clinical interest in LCMV, however, has not been maintained, and the disease is rarely considered despite improved serodiagnostic methods.

Although a history of contact with rodents and their excreta is of diagnostic utility, it is not universally present. A maternal history of rodent exposure was elicited for three of our five infants. Wild mice (*Mus musculus*) and hamsters infected in utero with LCMV during maternal viremia develop both persistent viremia and viruria. The

virus is transmitted to humans by direct animal contact; by contact with infected rodent saliva, nasal secretions, urine, feces, semen, and milk; and by infectious aerosols (1). Human-to-human transmission has not been documented. The distribution of LCMV is highly variable within mouse populations. Seasonal, annual, and cyclical variations in rodent density and infection have been postulated but remain inadequately studied (14). LCMV spreads to humans in rural settings or when human habitats are substandard. Infected laboratory and pet rodents have also been associated with disease in humans (1). Serologic surveys and clinical studies have documented both epidemic and endemic human infection in Europe and the Americas. In Baltimore, 9.0% of house mice and 4.7% of residents have had measurable LCMV antibody (15,16).

We hypothesize that congenital LCMV infection is generally undiagnosed and may account for unexplained hydrocephalus with microcephaly or macrocephaly, deafness, blindness, and mental retardation (three of the five infants in this report were referred for infectious disease consultation by pediatric geneticists, and two were referred by pediatric neurologists). No accurate data are available regarding the prevalence and persistence of LCMV antibodies in unselected infants, children, and adults in diverse geographic locales or in children with unexplained visual and/or auditory deficits, microcephaly, and retardation. Increased recreational activities in rural environments, rehabilitation of and habitation in older rodent-infected domiciles, and acquisition of unscreened rodents for pets or laboratory use pose as yet undefined risks for LCMV infection to the fetus, child, and adult. The need for further research to define the frequency of LCMV infection in human and animal populations is clear. LCMV infection can best be prevented by educating the public and medical professionals on the hazards of contact with infected rodents.

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Hemolytic Uremic Syndrome

Along with a report of the first outbreak of hemolytic uremic syndrome (HUS) caused by Shiga-like toxin (SLT) producing *E. coli* in Australia (1), this issue of *Emerging Infectious Diseases* presents three papers detailing the investigations of pediatric HUS cases linked to Shiga toxin (ST) and SLT producing bacteria. Goldwater and Bettelheim present a case of pediatric HUS associated with SLT producing *Escherichia coli* (SLTEC) O48:H21 in South Australia; this strain has not previously been recognized as an SLTEC. Saeed et al. report on the increasingly common identification of HUS in Saudi Arabia, its association with multiple-antibiotic-resistant *Shigella dysenteriae* type 1, and the inherent dangers of treating such patients with ampicillin and nalidixic acid. Al-Qawari et al. report on the results of active surveillance for dysentery and HUS in Saudi Arabia and discuss a possibly elevated risk for HUS in patients with bloody diarrhea who are hospitalized and treated with nalidixic acid during an outbreak of *S. dysenteriae* type 1.

The three papers raise a number of important issues regarding HUS. First, it is clear that a large number of SLT producing bacteria have the potential to cause HUS, particularly among children. Current research has focused on *E. coli* O157:H7, which since the early 1980s has emerged as a major foodborne cause of bloody diarrhea, hemorrhagic colitis, and HUS since the early 1980s (5-7). However, the large outbreak of pediatric HUS in South Australia in 1995 caused by foodborne *E. coli* O111:HNM has demonstrated that minor pathogens can emerge as major causes of HUS (1). Goldwater and Bettelheim (5) and other researchers have identified a number of *E. coli* serotypes isolated from patients with HUS (6,7). A-Qawari et al. and Saeed et al. offer a timely reminder that *S. dysenteriae* type 1, a pathogen with a human-only reservoir, is an equally serious contender in HUS etiology and pathogenesis when conditions facilitate the person-to-person transmission of pathogens.

The second key issue raised by the latter two papers concerns treatment of bloody diarrhea. Both discuss the potential for antibiotic (ampicillin and nalidixic acid)-mediated HUS and conclude that this issue should be carefully evaluated before antibiotics are used to manage bloody diarrhea. Saeed et al. note that the wide variety of antibiotics used to treat bloody diarrhea in Saudi Arabia could be explained by the various prescription practices of doctors recruited from different parts of the world. Antibiotic resistance is a worrying component of the mechanisms of emerging infectious diseases. Inappropriate antibiotic use is a key factor in the development of resistance, and major efforts must be directed towards educating physicians on effective prescribing practices.

Central to all three papers is the need for surveillance of organisms that cause bloody diarrhea, hemorrhagic colitis, and HUS, as well as for knowledge of the local epidemiology of SLTECs, their potential sources, and the optimal way to investigate and manage outbreaks. Goldwater and Bettelheim discuss the characteristic disappearance of *E. coli* from patients' stools after the development of HUS and, therefore, the importance of early detection in cases of bloody diarrhea. Laboratory testing of bloody diarrheal specimens is clearly critical to understanding the epidemiology of toxin-producing organisms that relate to the development of HUS (8). However, testing can be difficult, time-consuming and costly. Not all laboratories routinely test for SLTECs or have the capacity to do so. In Australia, for example, using polymerase chain reaction (PCR) technology to detect SLT genes is expensive: a negative test result costs approximately $A15, but if the results are positive, the cost rises to around $A250 when the SLTEC is isolated and typed.

Human surveillance is essential to the early detection of outbreaks and to the critical assessment of the impact on public health of new approaches to food safety (2,8). We conservatively estimate that the South Australian HUS outbreak has cost around $A20 million in direct and indirect costs, with major impacts being felt by industry. This must surely be considered when contemplating the costs of surveillance. One approach may be to use PCR techniques on all samples of bloody diarrhea in children under the age of 16 because if surveillance is to be effective, it must be specific (9). Intermittent surveys, or the use of sentinel laboratories for all cases of diarrhea (8) could be undertaken and mandatory notification of HUS instituted.

Compulsory notification of *Shigella* infection is a requirement in Australia, and including SLTECs on the list of notifiable diseases is being

considered. A national surveillance scheme for HUS was established in 1994, although notification is not mandatory. Without formal notification requirements, good reporting of HUS has been associated with either clustering of cases or the fact that few hospitals in a region have the capacity to manage these cases (10).

Although more attention has been focused on *E. coli* O157:H7, S. *dysenteriae* is likely the most common cause of HUS in children worldwide and more attention needs to be given to this pathogen in terms of surveillance and control. A strong and healthy public health infrastructure is required to address the infectious disease issues raised by HUS (11).

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Guidelines on the Risk for Transmission of Infectious Agents During Xenotransplants

An increasingly critical shortage of human donors has limited the availability and benefit of organ and tissue transplantation. This chronic shortage, coupled with recent scientific and biotechnological advances, has been a catalyst for new therapeutic approaches directed at using animal tissues in humans. The use of xenogeneic tissues and organs for transplantation or perfusion has raised concerns about the potential of both recognized zoonotic pathogens and unknown xenogeneic agents to infect individual human recipients and then spread through human populations.

Public health guidelines intended to minimize the risk for transmission of known pathogens through human-to-human transplantation exist. Similar guidelines addressing the issue of infectious agents that may be associated with xenotransplantation are being jointly developed by Public Health Service working groups at the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration, and the National Institutes of Health. A provisional draft of these guidelines will be published in the Federal Register in late 1995. Public comment on the proposed guidelines is invited. Critical review by members of the transplant community is particularly sought. Publication of a final version of these guidelines in CDC's *Morbidity and Mortality Weekly Report* is planned for the spring of 1996.

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Emerging Infectious Diseases Featured at ICAAC/IDSA Meeting

Emerging infectious diseases were highlighted recently at a joint meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) and the Infectious Diseases Society of America (IDSA) in San Francisco. In his opening address, IDSA President Vincent Andriole stated that the topic of most pressing concern to both organizations was new and reemerging pathogens. Presentations were made on the following subjects: arenavirus hemorrhagic fevers; changing virulence of streptococcal infections; cryptosporidia, cyclospora, and microsporidia; dengue/dengue hemorrhagic fever; emerging fungal pathogens; epidemic diphtheria in the newly independent states; *Escherichia coli* O157:H7; *Helicobacter pylori* ; human ehrlich ioses; new lymphotropic herpesviruses; and rabies.

Common themes emanated from the presentations. The speakers noted that infectious diseases continue to occur throughout the world, both sporadically and as outbreaks, because of multiple factors. They observed that the incidence and prevalence of infectious diseases are increasing in certain populations, particularly among immunocompromised persons. Additionally, new infectious diseases and etiologic agents continue to be identified with remarkable frequency, and microorganisms are being identified as causes of chronic diseases, including cancer. Several presenters expressed concern about the migrations of animal reservoirs and arthropod vectors into new populations and geographic areas. The speakers also called for additional support for the public health infrastructure and for basic sciences that provide the foundation for infectious disease prevention and treatment.

Building a Geographic Information System (GIS) Public Health Infrastructure for Research and Control of Tropical Diseases

A course on using Atlas GIS software and associated peripherals, such as digitizing tablets and global positioning systems (GPS), to build a GIS public health infrastructure in Latin American countries was taught August 7 to 18, 1995, at the Centers for Disease Control field station in Guatemala City, which includes the Medical Entomology Research Training Unit housed at the Universidad del Valle de Guatemala. The course was funded by the Special Programme on Research and Training in Tropical Diseases and presented by the Latin American Tropical Disease Research Training Consortium. Course

instructors included statisticians and epidemiologists from the Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention in Atlanta, Georgia, and in Guatemala City, Guatemala, and staff from the National Aeronautics and Space Administration, Center for Health Application of Aerospace Related Technologies, Ames Research Center, Sunnyvale, California.

Objectives of the training included the following: mastery of the principles and general concepts of all GIS systems; use of Atlas GIS/DOS to associate map files with databases to produce thematic maps, manipulate various layers (rivers, highways, village locations) of the map files to produce customized maps, create buffers around geographic features, and use them in simple analyses; designing georeferenced data files that can be read by the GIS; digitizing paper maps to acquire new data for building a GIS; use of GPS to obtain latitudes, longitudes, and elevations of villages and other major landmarks and to use this information in the GIS; and mastery of importing/exporting databases and map files.

The course was designed to enable participants to set up and use a GIS for research, planning, or operational purposes. Participating were institutions from Mexico (two teams), Colombia (two), Puerto Rico, Costa Rica, Venezuela, Guatemala (two), Ecuador, and Brazil. Each team came to the course with ideas, maps, and data pertaining to an existing project that would be continued at their home institution. Student project areas included onchocerciasis, malaria, water sanitation, leishmaniasis, and public health and natural resource utilization/preservation. The students were taught digitizing and were asked to use Guinea worm surveillance data to create their own GIS.

A full day was devoted to geographic analyses. Topics covered included aggregating data from one geographic layer to another, combining geographic features with common database values, and combining selected features to form new map layers. A workshop on remote sensing, GIS, and image classification explained that satellite imagery and remotely sensed data are obtained by measuring reflectance on seven spectral frequencies and that ground cover can be partially deduced by the amount of reflectance at each band. Field exercises to practice GPS use in the Lake Atitlan area followed. Another workshop covered advanced digitizing and gave each team a good start on the digitizing part of their projects. Individual instructions were given on how to import map files from other GIS programs into Atlas GIS. Lastly, the Guatemalan onchocerciasis GIS system was presented as a case study.

In addition to the 2 weeks of training, each participating institution received a copy of all lecture notes, the critical hardware needed to continue the project at home, and the following software, complete with documentation: Atlas GIS/DOS, Import-Export, and Arcview 2. An ongoing Internet-based discussion group for class organizers and participants is providing a forum for dialogue and monitoring of participants' progress.

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APHA Session Features Emerging Infections

Emerging and reemerging infections will be the featured topic of a two-part session at the annual meeting of the American Public Health Association, October 29-November 2, in San Diego, California.

The session, titled "Emerging Infections: Solving the Mysteries in the Field and Laboratory," will focus on the worldwide impact of new and reemerging infections from both an epidemiologic and a laboratory perspective.

Eight speakers from national and international health organizations will discuss the following aspects of the public health threat of these diseases: public health strategies for controlling infectious diseases; social, geographic, ecologic, and environmental factors that have allowed these diseases to spread; the growing threat of antimicrobial resistance; the increased need for accurate and meaningful disease surveillance; and the challenge to apply the latest laboratory technology to rapidly detect and characterize new infectious agents.

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Southeast Asia Intercountry Consultative Meeting on Prevention and Control of New, Emerging, and Reemerging Infectious Diseases

An intercountry meeting to identify strategies and approaches for tackling the problems of new, emerging, and reemerging infectious diseases in the Southeast Asia region was held in New Delhi, India, August 21 to 25, 1995.

Nine countries (Bangladesh, Bhutan, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, and Thailand) participated. Experts from these countries, England, and the United States, as well as representatives from USAID, DANIDA, UNICEF, the World Bank, and the World Health Organization (WHO) also attended.

Meeting participants expressed serious concern at the global and regional spread of new, emerging, and reemerging infectious diseases, especially in the Southeast Asia region. Reports from various countries emphasized that these diseases not only have worldwide health implications but also can disrupt commerce and industry and set back important progress achieved in public health during recent years. The spread of these diseases also has major social and political implications.

Participants at the meeting underlined the importance of surveillance, prompt epidemiologic investigation, and the build-up of adequate laboratory capacities. The need for maintaining ecologic and environmental integrity in various developmental activities was also emphasized. Member countries were called upon to immediately review and strengthen capacities in epidemic surveillance and response and to formulate country-specific strategies and action plans to anticipate, quickly recognize, and rapidly respond to the threat of emerging infections.

To ensure program sustainability, participants stressed that strategies to combat emerging diseases should be an integral part of existing national infrastructures, particularly infectious disease control programs, and should build on the capacities that already exist. Four broad areas for tackling the challenge of emerging diseases were identified: strengthening communicable disease surveillance and response, strengthening the existing infrastructure, capacity building for prevention and control, and applied research.

A total of 12 actions were recommended. One, for example, was that each country create a rapid response team to react to epidemic situations. A second recommendation was that countries develop linkages between their national reference laboratories and WHO collaborating centers. This would be to strengthen diagnostic capacities, facilitate quality assurance, and promote national self-reliance in laboratory diagnosis.

Eight recommended actions for WHO to undertake were also enumerated. These included assessing and monitoring microbial susceptibility to antibiotics, and vector susceptibility to insecticides.

For the complete list of conclusions and recommendations of this meeting, contact WHO's Regional Office for South-East Asia.

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