EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.6, No.4, Jul–Aug 2000

West Nile Virus in New York Male-Killing Bacteria in Insects

DEPARTMENT OF HEALTH AND HUMAN SERVICES

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Migratory Birds and Spread of West Nile Virus in the Western Hemisphere

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West Nile virus, an Old World flavivirus related to St. Louis encephalitis virus, was first recorded in the New World during August 1999 in the borough of Queens, New York City. Through October 1999, 62 patients, 7 of whom died, had confirmed infections with the virus. Ornithophilic mosquitoes are the principal vectors of West Nile virus in the Old World, and birds of several species, chiefly migrants, appear to be the major introductory or amplifying hosts. If transovarial transmission or survival in overwintering mosquitoes were the principal means for its persistence, West Nile virus might not become established in the New World because of aggressive mosquito suppression campaigns conducted in the New York area. However, the pattern of outbreaks in southern Europe suggests that viremic migratory birds may also contribute to movement of the virus. If so, West Nile virus has the potential to cause outbreaks throughout both temperate and tropical regions of the Western Hemisphere.

The first known human case of West Nile virus infection recorded in the Western Hemisphere was reported in August 1999 (1). Eventually, 62 cases of the disease were confirmed; no new cases have been reported since October 16, 1999 (2). Of the human cases laboratory-confirmed as of October 8, 1999, 70% occurred within a circle 10 km in radius centered in the northern end of the New York City borough of Queens (1). Coincident in both space and time with the human outbreak was a substantial die-off of birds, particularly American Crows *(Corvus brachyrhynchos)* (3). Unusual bird deaths were noted around the Bronx Zoo in mid-August (4), approximately 8 km north of the epicenter of the human epidemic. Several thousand crows and other avian species are presumed to have died of the virus, mostly in and around the New York City area (5). In addition to wild birds, the die-off included specimens in the Bronx and Queens zoo collections (including Chilean Flamingos [*Phoenicopterus chilensis*], Guanay Cormorants [*Phalacrocorax bougainvillei*], Bald Eagles [*Haliaeetus leucocephalus*], Black-billed Magpies [*Pica pica*], Bronze-winged Ducks [*Anas specularis*], Impeyan Pheasants [*Lophophorus impeyanus*], Blyth's Tragopans [*Tragopan blythi*], and Snowy Owls [*Nyctea scandia*]) (6).

The spatial and temporal juxtaposition of avian and human infections in this instance and historically has led many epidemiologists to conclude that birds act as introductory hosts, perhaps by infecting ornithophilic mosquitoes, which in turn infect amplifying hosts and eventually humans (3,7). Despite the fact that migratory birds have long been suspected as critical agents in outbreaks of this and other arboviruses, the link remains conjectural because of the difficulty in determining the intensity and duration of viremia in naturally infected wild birds (8,9).

We present an overview of the association of West Nile virus with birds, focusing in particular on the advent and movement of the virus in the Western Hemisphere.

History of West Nile Virus

West Nile virus was first isolated and identified as a distinct pathogen from the blood of a woman in the West Nile region of Uganda in 1937 (10). Cross-neutralization tests have been used to classify the virus as a *Flavivirus* (Family

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Flaviviridae) closely related to Japanese encephalitis virus, eastern Asia; Kunjin virus, Australia and Southeast Asia; and St. Louis encephalitis virus, North and South America (11,12). Several genetic lineages of the virus have been identified in different geographic locations. The lineage associated with the New York outbreak has been identified as virtually identical with an Israeli strain of the virus by phylogenetic analysis of E-glycoprotein nucleic acid sequence data (13). West Nile virus was one of the most widespread flaviviruses, even before its recent entry into North America. Its distribution extends throughout Africa, the Middle East, and southern temperate and tropical Eurasia (14,15). During the 1950s, an estimated 40% of the human population in Egypt's Nile Delta was seropositive for the virus (16). The largest human epidemic occurred in Cape Province, South Africa, in 1974, when approximately 3,000 clinical cases of the virus were recorded (17).

Migratory Birds and West Nile Virus in the Old World

Migratory birds have long been suspected as the principal introductory hosts of West Nile virus into new regions for the following reasons: Outbreaks of the virus in temperate regions generally occur during late summer or early fall, coinciding with the arrival of large concentrations of migratory birds (and mosquitoes) (18-20); these outbreaks often occur among humans living in or near wetlands where high concentrations of birds come into contact with large numbers of ornithophilic mosquitoes (7,21,22); the principal vectors from which the virus has been isolated are mainly ornithophilic mosquitoes (*Culex univittatus* in the Middle East and *C. pipiens* in Europe) (7,13,14,18,22); antibodies to the virus have been found in the blood of many migratory bird species in Eurasia (14,15,22-24); migratory birds have been linked with transporting related viruses in the Western Hemisphere (9,25,26); West Nile virus has been isolated from some species of actively migrating birds (e.g., the Barred Warbler [*Sylvia risoria*] in Cyprus and the Turtle Dove [*Streptopelia turtur*] in Slovakia (20,23,24); viremia sufficiently long-term to infect vector mosquitoes has been documented in several bird species (18,19,27-29); and migration places substantial physiologic stress on birds. Stress has been shown to promote immunosuppression and enhanced replication of West Nile virus in rodents (30).

Further support for the possibility that migratory birds play a major role in virus transport comes from study of related viruses. For instance, both Eastern (EEE) and Western equine encephalomyelitis alphaviruses, ecologic relatives of West Nile virus, have been isolated from actively migrating birds in the United States (25,26). Evidence also indicates that the 1962 epidemic of EEE in Jamaica resulted from transport of the virus by birds from the continental United States (31).

Unlike the 1999 New York City epidemic, during which large numbers of dead and dying birds, especially crows, were observed concurrently with clinical reports of human infection with the virus (3), the Old World epidemics of West Nile virus had few concurrent reports of deaths of infected birds (7). This difference could indicate lack of both exposure and adaptation to the virus among New World avian populations compared with Old World species. Old World data indicate that susceptibility to fatal infection with the virus varies markedly for adult and young birds, with high death rates in juveniles and high incidence of circulating antibodies in adult birds (19). Susceptibility to infection also varies considerably among species. Hooded Crows (*Corvus corone*) had both a high death rate in young birds in laboratory experiments and high levels of circulating antibodies in adults, while Rock Doves (*Columba livia*) appeared to be much less susceptible to both infection and death from the virus (19).

Migratory Birds and West Nile Virus in the New World

As was the case for humans, the first birds documented as infected with West Nile virus in the Western Hemisphere were identified in August 1999 (6). Thereafter, large die-offs of wild and captive birds at the Bronx Zoo and other parts of the New York area coincided with the increasing number of human cases reported from the same region (1,4,6). As in several European outbreaks, the main vector in the New York City epidemic was identified as the ornithophilic mosquito *C. pipiens* (4). Furthermore, the outbreak in humans occurred at urban sites near wetlands where migratory birds, ornithophilic mosquitoes, and humans were concentrated. These circumstances, in conjunction with the

ecology of the virus in the Old World, support the conjecture that zoo, pet, domestic, or wild birds were responsible for introducing the virus to the New World. If so, birds could have served as the source by normal migration, displacement from normal range by storms, or importation (legal and illegal).

Normal Interhemispheric Migration

A small percentage of the populations of a few bird species migrate regularly in August and September from breeding grounds in the Old World to wintering grounds along the eastern seaboard of North America. An example of this group is the Eurasian Wigeon (*Anas penelope*), which breeds across the entire Palearctic region from Iceland to Siberia's Kamchatka Peninsula and winters primarily in the temperate and tropical zones of the Old World (32), where contact with West Nile virus is possible. However, a few Eurasian Wigeons, presumably from the Icelandic breeding population, winter regularly along the coast of eastern North America (32,33). These birds could contract the virus from vector mosquitoes infected by biting other members of the breeding population that winter in areas where West Nile virus is prevalent and bring infectious blood to the New World on their winter migrations.

Eurasian Wigeons are not the only species with such a migration pattern. Eurasian populations of several species in which evidence of exposure (e.g., antibodies) to the virus has been detected are rare migrants along the eastern seaboard of North America (Table 1). However, if normal migration were a likely pathway, the virus would likely have become established earlier in this hemisphere, since individual birds

Table 1. Trans-Atlantic migration of avian species from Eurasia to the eastern United States

Species	Possible mode(s) of entry	Documentation of exposure to West Nile virus
Cory's Shearwater, *Calonectris diomedea*	Vagrant (33)	
Manx Shearwater, *Puffinus puffinus*	Vagrant (33)	
Wilson's Storm-Petrel, *Oceanites oceanicus*	Vagrant (33)	
Band-rumped Storm-Petrel, *Oceanodroma castro*	Vagrant (33)	
Northern Gannet, *Morus bassanus*	Vagrant (33)	
Gray Heron, *Ardea cinerea*	Vagrant (33)	34
Little Egret, *Egretta garzetta*	Vagrant (33)	
Cattle Egret, *Bubulcus ibis*	Vagrant (33)	35
Greylag Goose (domestic), *Anser anser*	Pet and domestic bird trade	18
Falcated Duck, *Anas falcata*	Pet trade, zoos, vagrant (33)	
Eurasian Wigeon, *Anas penelope*	Migration (32), pet trade, zoos	
Mallard (domestic), *Anas platyrhynchos*	Pet and domestic bird trade	18,24
Garganey, *Anas querquedula*	Migration (32), pet trade, zoos	
Green-winged Teal, *Anas crecca*	Migration (32)	
Tufted Duck, *Aythya fuligula*	Migration (32)	
Eurasian Kestrel, *Falco tinnunculus*	Vagrant (33)	18,36
Jungle Fowl (domestic), *Gallus gallus*	Domestic bird trade	18,35
Quail, *Coturnix coturnix*	Domestic bird trade	36
Northern Lapwing, *Vanellus vanellus*	Vagrant (33)	22
Wood Sandpiper, *Tringa glareola*	Vagrant (33)	36
Little Stint, *Calidris minuta*	Vagrant (33)	36
Curlew Sandpiper, *Calidris ferruginea*	Vagrant (33)	
Ruff, *Philomachus pugnax*	Migration (33)	
Little Gull, *Larus minutus*	Migration (33)	
Black-headed Gull, *Larus ridibundus*	Migration (33)	22
Black-tailed Gull, *Larus crassirostris*	Vagrant (33)	
Yellow-legged Gull, *Larus cachinnans*	Vagrant (33)	
Common Tern, *Sterna hirundo*	Vagrant (37)	
Rock Dove (domestic), *Columba livia*	Pet trade	18,24,38
Oriental Turtle-Dove, *Streptopelia orientalis*	Pet trade	
European Turtle-Dove, *Streptopelia turtur*	Pet trade	16,22,35
Eurasian Collared-Dove, *Streptopelia decaocto*	Pet trade	35

of several species known to be susceptible to the virus migrate annually from Eurasia to the United States (Table 1). However, the numbers of migrants are so small that the probability of the cooccurrence of an infectious migrant, ornithophilic vector mosquitoes, and numerous avian amplifying hosts seems low. Furthermore, the most likely form of the virus carried by migrants would be that from West Africa, because that is where most western European-breeding populations of these species winter. The New York City strain of the virus was nearly identical to that found in the Middle East, which is different from the West African strain (13). Despite these considerations, normal migration remains a distinct possibility as the mode of entry for the disease.

Displacement of West African Birds to the New World by Tropical Storms

A very few birds, particularly seabirds, are carried by tropical storms across the Atlantic each summer from their normal environs on or near the coast of West Africa (39). A number of such storms form each summer and fall near the Cape Verde Islands off the western coast of Africa, travel across the Atlantic, and occasionally reach land along the East Coast of North America, depositing birds that were carried thousands of kilometers from their homes. Species known to have been infected by West Nile virus and whose habitat and distribution indicate that they might be affected by such displacement include the Gray Heron (*Ardea cinerea*), the Little Egret (*Egretta garzetta*), the Cattle Egret (*Bubulcus ibis*), the Black-headed Gull (*Larus ridibundus*), and the Yellow-legged Gull (*Larus cachinnans*) (Table 1). The same objections apply to this scenario for the introduction of the virus to the New World as for normal migration, i.e., low numbers and the likelihood that a storm-transported bird would be infected with the West African rather than the Middle Eastern form of the virus.

Legal and Illegal Importations of Domestic Birds

Although the legal importation of pet, zoo, and domestic birds (e.g., geese, ducks, turkeys, and chickens) has declined since enactment of the 1992 Wild Bird Conservation Act, 2,770 birds entered the country through John F. Kennedy International Airport in 1999: 323 pet birds and 2,447 commercial birds; an additional 12,931 birds passed through in transit (S. Kaman, USDA, Pers. Comm.). All legal importations are subject to U.S. Department of Agriculture (USDA/Animal and Plant Health Inspection Service) and U.S. Fish and Wildlife Service rules, regulations and procedures, and most birds undergo a quarantine of at least 30 days at USDA facilities located in the vicinity of three U.S. ports-of-entry (New York, Los Angeles, Miami). During quarantine the birds are isolated indoors in air-filtered isolation cages to prevent the transmission of communicable diseases, medicated for psittacosis, and tested for diseases affecting poultry (e.g., Newcastle Disease)(40). Even with such precautions, some birds infected with West Nile virus could be bitten by mosquitoes during transit to quarantine or could escape detection during quarantine; no tests specific for the virus are performed, and many Old World species remain asymptomatic if infected. After release from quarantine, viremic birds could transmit the virus to native birds if held outdoors in habitats (e.g., zoos) supporting both ornithophilic mosquitoes and concentrations of birds. Such a scenario is even more probable in the case of illegally imported birds, which would not be subject to quarantine or even cursory health examinations.

Animals Other Than Birds and Entry of West Nile Virus into the New World

Although birds appear to be the principal means by which the virus moves from site to site in the Old World, other modes of entry into the New World are possible. Humans, horses, and some other mammals are highly susceptible to infection by the virus (7), and not all become too sick to travel during periods of potential viremia. Furthermore, it is unlikely that all animal hosts and vectors have been identified. Intercontinental air travel could transport infectious animals from viral foci in Eurasia or Africa. An infectious mosquito may enter a plane in one of these areas, travel to New York, and infect a person, horse, or bird en route or after arrival.

Migratory Birds and West Nile Virus Distribution in the Western Hemisphere

Since identification of West Nile virus in the United States, a massive effort has been undertaken to determine when and where the

virus will next appear in the human population. The New York City area is thought most likely to be affected by the next epidemic. If transovarial transmission in mosquitoes or survival in overwintering mosquitoes were the principal means for persistence of this virus, annual recurrence of the virus might be expected at the same sites. However, while transovarial transmission of the virus has been documented, it occurs at low levels (< 1%) (15,41). In addition, public health officials have implemented mosquito-control measures that are likely to continue. Furthermore, although the virus may remain enzootic for years awaiting environmental conditions that favor an epidemic (36), the usual pattern in southern Eurasia is one of isolated outbreaks, apparently resulting from importation of active virus by migratory birds into an area with appropriate climatic, vector, and amplifying host conditions (7). This pattern suggests that future movements of the virus in the Western Hemisphere may depend on its persistent amplification in wintering avian populations in New World tropical, subtropical, and southern temperate regions, and subsequent importation into major avian concentration areas in the temperate region.

If this Old World pattern persists, the New York area is unlikely to be the site of the next human outbreak because the occurrence of optimal combinations of infecting host, vector, amplifying host, and susceptible human population depends on substantial annual variation based on stochastic environmental factors (e.g., rainfall and temperature) (7). The known ecology of West Nile virus indicates that the virus is more likely to persist in the Western Hemisphere if it is translocated by avian hosts to southern wintering sites. Old World data indicate that ideal over-wintering conditions for West Nile virus combine three key factors: a viremic, infectious host bird; active, ornithophilic mosquitoes to serve as vectors; and large numbers of one or more amplifying avian host species. This combination of numerous wintering birds and ornithophilic mosquitoes (e.g., in southern wetlands or wet agricultural or urban areas) could provide amplification and a permanent base for the virus from which it could be spread northward by migrating birds. Thus, understanding the major migration patterns of the potential infecting host species through the New York City region may hold the key to understanding the future of the virus in the Hemisphere.

Bird Migration in the New York Region

Four major routes are followed by birds that gather in and pass through the New York City area (defined as a circle 10 km in radius centered on north Queens) in late summer and early fall: the southeastern U.S. route, the circum-Gulf route, the trans-Gulf route, and the Caribbean island-western North Atlantic route.

Southeastern U.S. Route

Members of approximately 155 species of birds may originate in or pass through the New York area on their way to wintering grounds in the southeastern United States, following the route described by W.W. Cooke for the American Robin (*Turdus migratorius*) (42). Most of these species are not suitable hosts from the perspective of the virus, because they follow individual migration paths and do not gather in large numbers in habitats with high concentrations of ornithophilic mosquitoes. However, approximately 32 species of birds that follow the southeastern U.S. migration route would be likely to occur in high densities in or near wetlands on migration as they pass through New York and on to their wintering sites (Table 2). The European Starling (*Sturnus vulgaris*), normally considered to be "sedentary," is an example of a species a portion of whose northeastern populations follows such a migratory route (Figure 1). American Crows are another such species, as well as the Mallard (*Anas platyrhynchos*) (39). Mallards breed throughout the temperate and boreal regions of North America and, in late summer, members of the eastern Canadian populations begin to collect in flocks at wetlands preparatory for migration to wintering sites in the southeastern United States. Many Mallards from the northeastern United States also migrate to the southeastern United States, although some of the birds remain at their breeding grounds throughout the winter. Mallards migrating south gather in flocks along the migration route, as well as on the wintering grounds. However, between stop-over sites each bird follows its own migration path, so flock membership is changing continuously. As a result, birds found together as transients at a pond in Long Island or the Bronx are not likely to be members of the same winter flock in coastal

Table 2. Species that breed in temperate or boreal North America, by migration route

Species[a]	Southeast	Circum-Gulf	Trans-Gulf	Caribbean/ North Atlantic
Double-crested Cormorant (*Phalacrocorax auritus)*	+			
Cattle Egret (*Bubulcus ibis)*	+	+		
Black-crowned Night Heron *(Nycticorax nycticorax)*[b]	+	+		
Turkey Vulture *(Cathartes aura)*	+	+		
Gadwall *(Anas strepera)*	+			
American Wigeon *(Anas americana)*	+	+		+
American Black Duck *(Anas rubripes)*	+			
Mallard *(Anas platyrhynchos)*	+			
Blue-winged Teal *(Anas discors)*	+		+	+
Northern Shoveler *(Anas clypeata)*	+	+		+
Northern Pintail *(Anas acuta)*	+	+		+
Green-winged Teal *(Anas crecca)*	+			+
Canvasback *(Aythya valisineria)*	+	+		+
Redhead *(Aythya americana)*	+			
Ring-necked Duck *(Aythya collaris)*	+	+	+	+
Lesser Scaup *(Aythya affinis)*	+		+	+
American Coot *(Fulica americana)*	+		+	+
Red Knot *(Calidris canutus)*				+
Sanderling *(Calidris alba)*	+		+	+
Semipalmated Sandpiper *(Calidris pusilla)*			+	+
Western Sandpiper *(Calidris mauri)*	+		+	+
Least Sandpiper *(Calidris minutilla)*	+		+	+
Short-billed Dowitcher *(Limnodromus griseus)*	+		+	+
Laughing Gull (*Larus atricilla)*	+	+	+	+
Ring-billed Gull *(Larus delawarensis)*	+	+	+	+
Herring Gull *(Larus argentatus)*	+	+		
Common Tern *(Sterna hirundo)*	+		+	+
Black Tern *(Chlidonias niger)*				+
Rock Dove *(Columba livia)*	+			
Chimney Swift *(Chaetura pelagica)*				+
American Crow (*Corvus brachyrhynchos)*	+			
Fish Crow (*Corvus ossifragus)*	+			
Purple Martin *(Progne subis)*				+
Barn Swallow *(Hirundo rustica)*				+
American Robin *(Turdus americanus*)	+			
European Starling *(Sturnus vulgaris)*	+			
Red-winged Blackbird *(Agelaius phoeniceus)*	+			
Common Grackle *(Quiscalus quiscula)*	+			

[a]Species whose members potentially form large, dense flocks at feeding or roosting sites in or near wetlands during migration through the New York City area are listed according to migration route. Note that populations of the same species can follow different routes to different wintering destinations.
[b]Species documented as having been infected during the recent epizootic event are shown in bold (6).

Georgia or Alabama. After the wintering site has been reached or during northward movement in the spring, males search for mates. Once paired, a male follows his mate back to her natal area, which ensures wide dispersal of male birds throughout the Mallard's entire North American breeding range, a pattern seen in many duck species (43).

Circum-Gulf Route

Birds of some species that typically winter in Mexico and Central America avoid crossing large expanses of open water; these species include hawks, herons, and egrets, as well as some ducks and gulls. Although some birds may fly directly across the Gulf of Mexico to their winter quarters, others detour around the Gulf, passing along its

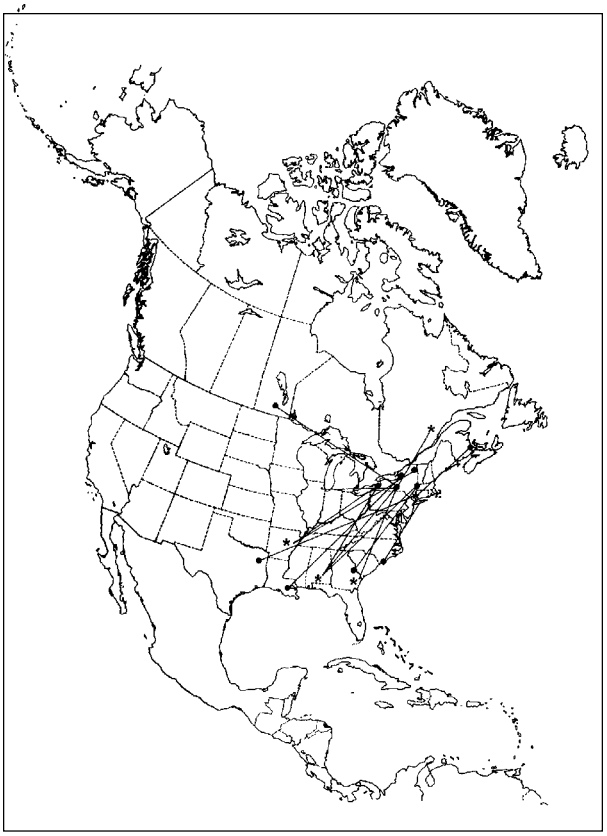

Figure 1. Southeastern U.S. migration pattern of the European Starling (*Sturnus vulgaris*), as shown by band returns. From Bull's Birds of New York State (37). Stars on the figure denote banding location; dots denote recovery location.

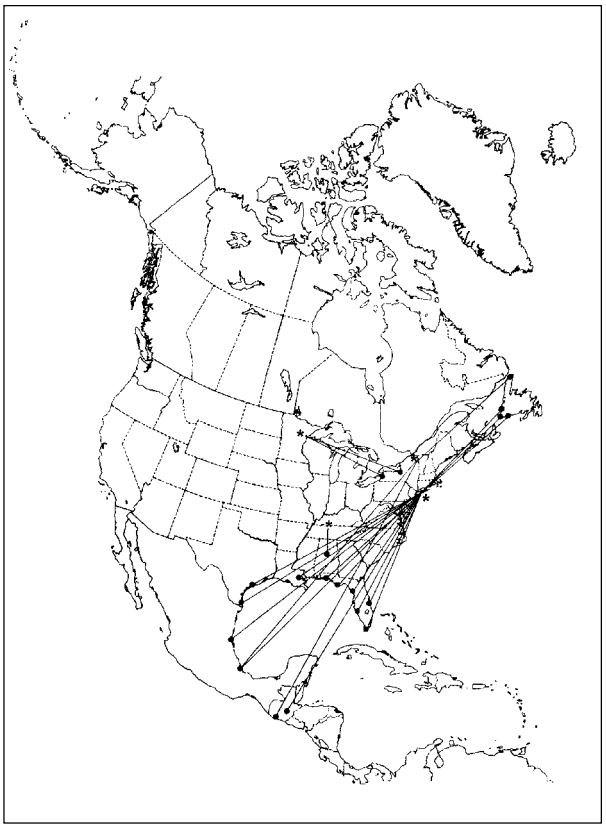

Figure 2. Circum-Gulf migration pattern of the Herring Gull (*Larus argentatus*), as shown by band returns. From Bull's Birds of New York State (37). Stars on the figure denote banding location; dots denote recovery location.

western coast during both south- and northward migratory journeys. Approximately 11 of these species might flock together in and around New York wetlands during migration and again on wintering grounds (Table 2). An example is the Herring Gull (*Larus argentatu*s) (Figure 2).

Trans-Gulf Route

Approximately 125 species of birds have populations that transit the New York region on their way to wintering grounds in Mexico and Central America following a trans-Gulf route in the fall (42,44). Distributional data indicate that most do not follow the same route north in the spring, but instead take a more westerly route over or paralleling the western Gulf coast (45). Also, many birds of these species, especially young birds or those from populations in the center of the continent, follow a circum-Gulf route both south and north. Although this group includes many migratory birds that breed in eastern North America, only approximately 12 species (Table 2) could serve as carriers for West Nile virus, because most migrate alone and do not gather in large roosting or feeding flocks in the winter or during migration (46).

Caribbean Island/Western North Atlantic Route

Approximately 70 species of birds have populations that pass through New York and cross the western North Atlantic or Caribbean Sea en route south to their wintering grounds on Caribbean islands or in South America. Like the trans-Gulf pattern, this route is elliptical, with

Figure 3. Caribbean/Western North Atlantic migration pattern of the Common Tern (*Sterna hirundo*), as shown by band returns. From Bull's Birds of New York State (37). Stars on the figure denote banding location; dots denote recovery location.

birds following a more westerly route across the Gulf of Mexico or along its western shore in the spring (44-46). Members of approximately 22 species of birds that gather in flocks during migration and during the winter follow this route (Table 2) (e.g., the Common Tern [*Sterna hirundo*]) (Figure 3).

Conclusions

Members of one or more avian species that pass through New York and gather in wetlands in large, dense groups potentially reach every part of the southeastern United States, Mexico and Central America, the Caribbean Islands, and South America during their migration south to wintering sites and nearly every part of North America during their migration north to breeding sites (Table 2, Figures 1-3).

Movement of the virus in the Old World seems to involve a set of conditions including infectious avian host, numerous ornithophilic mosquito vectors, and cross-species transmission to a numerous avian amplifying host (not necessarily the same species as the infectious host but the same location). Therefore, because outbreaks are dependent on a series of probabilities, the suitable wetland sites likely to receive the largest number of potentially infected hosts seem to be the most likely place for future outbreaks. Banding data, showing winter concentration areas for large numbers of birds (Table 2), indicate that the most likely place for a bird-spawned outbreak on the wintering ground would be along the coastal plain of Georgia, northern Florida, or Alabama—if the necessary ornithophilic mosquitoes are sufficiently active and abundant.

This information suggests that the best approach to minimizing effects of the virus on humans should involve intensive monitoring of fall and winter avian concentrations for abnormal die-offs; collecting and testing bird carcasses from such die-offs; and implementation of mosquito control measures at die-off sites. In addition, aviculturalists, poultry markets, zoos, and others involved with shipment of birds from one part of the country to another should test birds for West Nile virus during quarantine to ensure that they are not bringing in or sending out infectious birds (47).

Future research should define criteria for predicting where the virus will go next. Defining the duration of viremia or the frequency of cycling of active virus in the various mosquito and avian populations that have been exposed to the virus is critical. At present, nothing is known about long-term persistence of active virus in the blood of New World avian species, and little is known about long-term viremia in other avian species. As noted by Blaskovic and Ernek (48), "The role of birds in ecology of arboviruses depends upon whether the migrating vector finds favourable conditions in the new environment and whether the local vectors are capable of transmitting the appropriate virus. The presence of antibody for arboviruses in migratory birds indicates only a virus-host interaction but does not explain when and where the infection occurred." In addition, detection of antibodies for West Nile virus in avian serum does not show whether the bird could serve as a source for transmission of active virus by mosquitoes to humans. Determining the viremic potential of North American bird species

is critical to understanding the future of the virus in the New World and should be the focus of future research.

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References

1. Centers for Disease Control and Prevention. Update: West Nile-like viral encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:890-2.
2. Centers for Disease Control and Prevention. Guidelines for surveillance, prevention, and control of West Nile virus infection—United States, 2000. MMWR Morb Mortal Wkly Rep 2000;49:25-8.
3. Anderson JF, Andreadis TG, Vossbrinck CR, Tirrell S, Wakem EM, French RA, et al. Isolation of West Nile virus from mosquitoes, crows, and a Cooper's Hawk in Connecticut. Science 1999;286:2331-3.
4. Centers for Disease Control and Prevention. Outbreak of West Nile-like viral encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:845-9.
5. Rocke T. President's corner. J Wildl Dis 2000;36:Suppl.
6. Steele KE, Linn MJ, Schoepp RJ, Komar N, Geisbert TW, Manduca RM, et al. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City. Vet Pathol 2000;37:208-24.
7. Hubálek Z, Halouzka J. West Nile fever—a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis 1999;5:643-50.
8. Reeves WC. Overwintering of arboviruses. Prog Med Virol 1974;17:193-220.
9. Reeves WC, Hammon WM. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943-1952. University of California Publications in Public Health. Vol 4. Berkeley (CA): University of California Press; 1962.
10. Smithburn KC, Hughes TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med Hyg 1940;20:471-92.
11. Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 1989;70:37-43.
12. Wengler G, Bradley DW, Collett MS, Heinz FX, Schlesinger RW, Strauss JH. Family flaviviridae. VIth International Committee on Taxonomy of Viruses Report; 1999.
13. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7.
14. Karabatsos N, editor. International catalogue of arboviruses, including certain other viruses of vertebrates. 3rd ed., and Supplements 1986-98. San Antonio (TX): American Society of Tropical Medicine and Hygiene; 1985.
15. Peiris JSM, Amerasinghe FP. West Nile fever. In: Beran GW, Steele JH, editors. Handbook of zoonoses. 2nd ed. Section B: Viral. Boca Raton (FL): CRC Press; 1994. p. 139-48.
16. Smithburn KC, Taylor RM, Rizk F, Kader A. Immunity to certain arthropod-borne viruses among indigenous residents of Egypt. Am J Trop Med Hyg 1954;3:9-18.
17. McIntosh BM, Jupp PG, Dos Santos I, Meenehan GM. Epidemics of West Nile and Sindbis viruses in South Africa with *Culex (Culex) univittatus* Theobald as vector. South African Journal of Science 1976;72:295-300.
18. Taylor RM, Work TH, Hurlbut HS, Rizk F. A study of the ecology of West Nile virus in Egypt. Am J Trop Med Hyg 1956;5:579-620.
19. Work TH, Hurlbut HS, Taylor RM. Indigenous wild birds of the Nile Delta as potential West Nile virus circulating reservoirs. Am J Trop Med Hyg 1955;4:872-8.
20. Nir Y, Goldwasser R, Lasowski Y, Avivi A. Isolation of arboviruses from wild birds in Israel. Am J Epidemiol 1967;86:372-8.
21. Lvov DK, Klimenko SM, Gaidamovich SY. Arboviruses and arbovirus infections (in Russian). Medicina (Moskva) 1989:1-335.
22. Hubálek Z, Halouzka J. Arthropod-borne viruses of vertebrates in Europe. Acta Scientiarum Naturalium Brno 1996;30:1-95.
23. Watson GE, Shope RE, Kaiser MN. An ectoparasite and virus survey of migratory birds in the eastern Mediterranean. In: Cherepanov IA, editor. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Novosibirsk, Russia: Nauka; 1972. p. 176-80.
24. Ernek E, Kozuch O, Nosek J, Teplan J, Folk C. Arboviruses in birds captured in Slovakia. Journal of Hygiene, Epidemiology and Immunology (Prague) 1977;21:353-9.
25. Calisher CH, Maness KSC, Lord RD, Coleman PH. Identification of two South American strains of eastern equine encephalomyelitis virus from migrant birds captured on the Mississippi Delta. Am J Epidemiol 1971;94:172-8.
26. Lord RD, Calisher CH. Further evidence of southward transport of arboviruses by migratory birds. Am J Epidemiol 1970;92:73-8.
27. Semenov BF, Chunikhin SP, Karmysheva VY, Yakovleva NI. Studies of chronic arbovirus infections in birds. 1. Experiments with West Nile, Sindbis, Bhanja and SFS viruses (in Russian). Vestnik Akademii Medicinskikh NaukSSSR (Moskva) 1973;2:79-83.
28. Fedorova TN, Stavskiy AV. Latent infection of wild ducks with Omsk hemorrhagic fever and West Nile viruses (in Russian). In: Chumakov MP, editor. Aktualnye prolemy virusologii i profilaktiki virusnykh zabolevaniy. Moskva: Inst Poliomiel Virus Enc;1972. p. 226.
29. Chunikhin SP. Introduction to arbovirus ecology (in Russian). Med Virusol (Moskva) 1973;21:7-88.

30. Ben-Nathan D. Stress and infectious disease. Israeli Journal of Veterinary Medicine 1994;49:105-12.
31. Work TH, Lord RD. Trans-gulf migrants and the epizootiology of arboviruses in North America. In: Cherepanov IA, editor. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Novosibirsk, Russia: Nauka; 1972. p. 207-14.
32. Palmer RS, editor. Handbook of North American birds. Vol 2. New Haven (CT): Yale University Press; 1976.
33. American Ornithologists' Union. Check-list of North American birds. 7th ed. Washington: The Union; 1998.
34. Antipa C, Girjabu E, Iftimovici R, Draganescu N. Serological investigations concerning the presence of antibodies to arboviruses in wild birds. Revue Roumaine de Medecine, Serie de Virologie (Bucuresti) 1984;35:5-9.
35. Taufflieb R, Robin Y, Bres P. Preliminary findings on the role of birds in the ecology of arboviruses in Senegal. In: Cherepanov IA, editor. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Novosibirsk: Nauka; 1972. p. 296-302.
36. Hannoun C, Corniou B, Mouchet J. Role of migrating birds in arbovirus transfer between Africa and Europe. In: Cherepanov IA, editor. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Novosibirsk, Russia: Nauka; 1972. p. 167-72.
37. Bull J. Birds of New York State. Garden City (NY): Doubleday; 1974.
38. Work TH, Hurlbut HS, Taylor RM. Isolation of West Nile virus from hooded crow and rock pigeon in the Nile Delta. Proc Soc Exp Biol Med 1953;84:719-22.
39. Hurricane/tropical data for Atlantic. Unisys Weather. 1999. Available from: URL: http://weather.unisys.com/hurricane/atlantic/1999/index.html
40. Importing a pet bird. United States Department of Agriculture, Animal and Plant Health Inspection Service. Avaliable from: URL: http://www.aphis.usda.gov/oa/petbird.htm
41. Baqar S, Hayes CG, Murphy JR, Watts DM. Vertical transmission of West Nile virus by *Culex* and *Aedes* species mosquitoes. Am J Trop Med Hyg 1993;48:757-62.
42. Cooke WW. Bird migration. Washington: United States Department of Agriculture Bulletin 1915;185:1-48.
43. Bellrose FC. Ducks, geese and swans of North America. Harrisburg (PA): Stackpole Books; 1976.
44. Rappole JH, Ramos MA, Oehlenschlager RJ, Warner DW, Barkan CP. Timing of migration and route selection in North American songbirds. In: Drawe L, editor. Proceedings of the First Welder Wildlife Foundation Symposium. Sinton (TX): Welder Wildlife Foundation; 1979. p. 199-214.
45. Rappole JH, Ramos MA. Factors affecting migratory bird routes over the Gulf of Mexico. Bird Conservation International 1994;4:131-42.
46. Rappole JH. The ecology of migrant birds: a neotropical perspective. Washington: Smithsonian Institution Press; 1995.
47. Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD, et al. Limitations of captive breeding in endangered species recovery. Conservation Biology 1996;10:338-48.
48. Blaskovic D, Ernek E. Birds as hosts of arboviruses in Europe. In: Cherepanov IA, editor. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Novosibirsk, Russia: Nauka; 1972. p. 161-7.

Male-Killing Bacteria in Insects: Mechanisms, Incidence, and Implications

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Bacteria that are vertically transmitted through female hosts and kill male hosts that inherit them were first recorded in insects during the 1950s. Recent studies have shown these "male-killers" to be diverse and have led to a reappraisal of the biology of many groups of bacteria. *Rickettsia*, for instance, have been regarded as human pathogens transmitted by arthropods. The finding of a male-killing *Rickettsia* obligately associated with an insect suggests that the genus' members may be primarily associated with arthropods and are only sometimes pathogens of vertebrates. We examined both how killing of male hosts affects the dynamics of inherited bacteria and how male-killing bacteria affect their host populations. Finally, we assessed the potential use of these microorganisms in the control of insect populations.

Female insects commonly interact with bacteria they pass on to their progeny. These inherited bacteria are often beneficial symbionts that play a key role in host metabolism. In many cases (e.g., the aphid symbiont *Buchnera*), the bacteria are maintained in a special host organ, the bacteriome, with the host controlling transmission to progeny, and show evidence of cospeciation (1,2). In these cases, destroying the bacteria (e.g., through antibiotic treatment) causes a profound loss of host performance. In other cases, inherited bacteria are not integrated into host physiology and anatomy and do not show long-lived relationships with their host, as indicated by a lack of cospeciation (3). These bacteria may be broadly separated into two classes. First, bacteria maintained through a phase of horizontal transmission (e.g., *Rickettsia prowazekii*), with transmission to other arthropod hosts often occurring through a vertebrate or plant intermediate host (infection of the intermediate host and new acquisition of infection follow from host feeding); second, bacteria that rarely show horizontal transmission, but are maintained because they manipulate host reproduction. One set of manipulations manifested by these bacteria is increasing investment in daughters at the expense of sons. In these cases, particular host lines produce female-biased sex ratios, a trait that is inherited but curable with antibiotics. We considered one class of these, the male-killing bacteria, in which infection of a female results in the production of female-biased broods because male progeny die during embryogenesis.

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Systematics of Male-Killing Bacteria

Molecular systematic approaches have shown that male-killing bacteria derive from many different clades. In most cases, the data come from DNA sequencing of bacteria associated with the trait and confirmation of the trait association by polymerase chain reaction across infected and uninfected lines. Because inherited microorganisms are difficult to culture, Koch's postulates have been fulfilled formally in only two cases (4,5). Given this caveat, male-killing bacteria have been found within the genus *Spiroplasma* (Mollicutes) (4,6), the Flavobacteria-Bacteroides group (7), and the gamma and alpha subdivisions of the proteobacteria (5,8,9) (Figure).

Male-killing bacteria derive from arthropod-associated bacterial clades that are not themselves male-killers. The clades can be separated into two types according to the transmission mechanisms of bacteria within them: first, entirely horizontal transmission or a mix of horizontal and vertical transmission; and second,

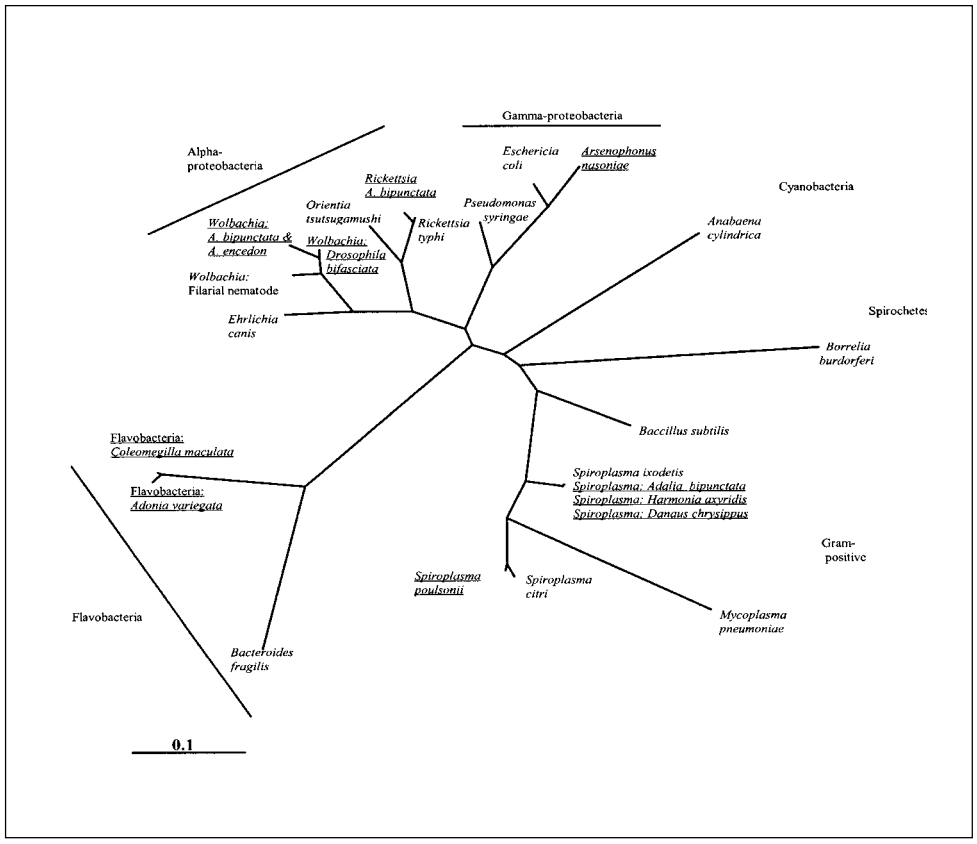

Figure. Phylogenetic relationship of male-killers and a selection of other eubacteria inferred from 16S rDNA sequences, using maximum likelihood implemented on PAUP*. The male-killing bacteria (underlined) have been labeled with the name of their insect host if a species name is not available. The relationships of the major bacterial groups are uncertain.

horizontal transmission that is not epidemiologically important. In the first type of clade are the genera *Spiroplasma* and *Rickettsia*. In *Spiroplasma*, most members have either horizontal transmission only (after feeding on a plant host) or a mix of horizontal and vertical transmission between arthropod hosts (10). *Rickettsia* most commonly have a mix of horizontal and vertical transmission, with horizontal transmission occurring after feeding on a vertebrate host. As recently as 10 years ago, *Rickettsia* was regarded as one of the vertebrate pathogens borne by arthropods. However, *Rickettsia* that show transmission after feeding on plant hosts are increasingly being recognized (11), and the finding of a male-killing *Rickettsia* in ladybird beetles (8) suggests that the group is associated with arthropods, some members of which cause disease in vertebrates. Other male-killing strains of *Rickettsia* will most likely be found. However, whether a bacterium from these groups could evolve male-killing yet retain horizontal transmission between females via feeding on a plant or vertebrate host has not been established. The fact that male-killers derive from such groups suggests this possibility.

In the second type of clade, vertical transmission rates far exceed those of horizontal transmission. *Wolbachia* and the flavobacterial lineage associated with arthropods are in this group. *Wolbachia* are usually maintained through manipulation of their host's reproduction (12). The closest relative of the flavobacterial male-killer is *Blattabacterium*, the beneficial inherited bacterium of cockroaches and termites (13).

Male-killing, a trait that evolves in bacteria already maternally inherited in arthropods, can occur if the ancestral agent is obligately vertically transmitted or a mix of vertical and horizontal transmission is present. Therefore, male-killing strains are likely to be common in the genus *Spiroplasma* and the alpha group of proteobacteria. Furthermore, the diversity of agents suggests that there is no taxonomic bar to where the transition to male-killing can take place. Thus male-killing strains are also likely to be found in the spirochetes and perhaps the beta

and delta divisions of the proteobacteria, as these groups are known to be vertically transmitted within arthropods.

Although vertical transmission of male-killing bacteria is the rule, transmission between host species has occurred. In *Spiroplasma*, the relatives of *S. ixodetis* cause male-killing in distantly related hosts (a butterfly and a ladybird beetle) (6,14). The evolutionary distance between beetles and butterflies indicates that the bacteria do cross between host species over evolutionary time.

Host Species Affected

The incidence of male-killing bacteria varies with host ecology and biology. The trait of male-killing is adaptive when the death of males promotes the survival of female siblings. If the bacteria can be transmitted only vertically, the death of male hosts can at worst be neutral (i.e., they cannot transmit the bacterium). Death of males is adaptive if it increases the survival of sibling females, who bear the same bacterium by virtue of common descent.

The features of host biology and ecology that increase the benefit to the bacterium of killing male embryos are sibling egg consumption (females eat their dead brothers), antagonistic interactions between siblings (male-killing may reduce both cannibalism of females and the intensity of competition between siblings), and deleterious inbreeding (15-17). These observations explain why male-killer hosts commonly lay eggs in clutches. Incidence is highest where there is also sibling egg consumption, as with coccinellid (ladybird) beetles. Approximately half of aphidophagous species bear male-killers, and one species (*Adalia bipunctata*) is host to at least three male-killing bacteria (6,8,9).

Male-killing bacteria have been recorded only in insects. However, the range of insect hosts is wide, with a variety of different sex determination systems. Given that close relatives of male-killing bacteria are found in noninsect arthropods (e.g., *Spiroplasma* and *Rickettsia* in ticks) and the conditions for the spread of male-killing strains are met outside insect hosts, cases of male-killing are likely to occur in species other than insects. Two examples merit particular examination. First, infection with *Orientia tsutsugamushi* is associated with production of all-female broods in the trombiculid mite, *Leptotrombidium fletcheri* (18,19); in this example, the nature of the resultant sex-ratio distortion (primary vs. secondary bias) needs to be assessed. Second, in the case of *Spiroplasma ixodetis* and its tick host *Ixodes pacificus*, the association of closely related bacteria with male-killing in insects needs to be assessed.

Prevalence of Male-Killers in Natural Populations

The prevalence of male-killers in natural populations varies with host species (Table 1). A prevalence value of 5%-50% might be "normal" among female hosts; however, in some cases prevalence is very low (e.g., 1% in *Drosophila*

Table 1. Prevalence of male-killers in natural populations of their insect hosts (proportion of females infected)

Bacterium	Host	Prevalence (%)	Ref.
Spiroplasma sp.	*Adalia bipunctata*	0-22	6
(*S. ixodetis* relative)	*Harmonia axyridis*	0-49	35
	Danaus chrysippus	40	14
Spiroplasma poulsonii	*Drosophila willistoni* group flies	0-3	20
Wolbachia	*Acraea encedon*	61-95	36
	Acraea encedana	95	21
	Adalia bipunctata	0-5	9
Unnamed Flavobacteria	*Coleomegilla maculata*	23	37
	Adonia variegata	13	38
Arsenophonus nasoniae	*Nasonia vitripennis*	4	15
Rickettsia	*Adalia bipunctata*	5-7	6,39
Unknown	*D. bifasciata*	0-7	26
	D. prosaltans	13	33
	Gastrolina depressa	0-81	22
	Epiphyas postvittana	4-7	40
	Hypolimnas bolina	0-61	41
	Spodoptera littoralis	24	42
	Lymantria dispar	9	43

willistoni [20]), and in some exceptional species > 90% of females are infected (e.g., the butterfly *Acraea encedana* [21]). However, there is likely to be study bias towards high-prevalence infections, and all very low-prevalence infections occur in drosophilids, where large samples can easily be bred. Infection prevalence also commonly varies between populations within a host, and prevalence can vary on a remarkably small scale. In the walnut leaf beetle (*Gastrolina depressa*) in Japan, male-killers are absent in populations at the north and south of the islands but present in 50%-80% of females in the center of the islands (22). Prevalence variation on a kilometer scale exists in *Acraea encedon* (21).

Prevalence is determined by the physiologic effect of infection on female host performance, the transmission efficiency of the bacterium from mother to progeny, and the level of advantage to male-killing (determined by host factors such as sibling egg consumption) (Table 2). Transmission efficiency may be influenced by the environment (e.g., high temperatures may lower transmission efficiency), the bacterium, and the host. Selection favors host genes that impede the transmission of the bacteria from mother to progeny. The spread of host resistance genes may prevent infections from commonly reaching the high prevalence achieved by other inherited bacteria.

Table 2. Factors affecting the prevalence of male-killing bacteria

Increase
Decreased rate of inbreeding suffered by female hosts
Increased access to early resources through consumption of dead sibling male hosts
Increased access to resources due to reduced competition, following death of sibling male hosts
Direct physiologic benefits of infection
Decrease
Inefficiency in vertical transmission
Direct physiologic costs of infection
Local extinction of groups having a high prevalence of male-killers

Mechanism of Male-Killing

Little is known about how male-killing is achieved. Neither the cue used to detect sex nor the mechanism by which death is brought about is known in any detail. Indeed, rather than two steps (detection then virulence) there may be only one (constitutive production of a factor that causes death in males only). What we know derives almost exclusively from study of the interaction between *Spiroplasma poulsonii* with *Drosophila*.

Studies of embryos from *D. willistoni* lines infected with *S. poulsonii* show that death occurs at two stages (23): 1) before gastrulation, associated with abnormal cleavage patterns; in particular, achromatic spindles, with other abnormalities of the mitotic process, which account for most embryonic deaths in male-killed lines. 2) After gastrulation, not associated with the normal brown coloration of necrotic embryos; rather, the embryo blackens as a result of breakdown of internal structures and pycnosis of nuclei.

The points of interaction between host and bacterium have been investigated in *D. melanogaster* lines transfected with *S. poulsonii*. In *Drosophila*, sex is determined by the ratio of the X chromosomes to autosomes. In females, which are 2X:2n, the peptide Sxl is produced. Sxl induces female development of the soma and the germ line. In males, which are X: 2n, Sxl is not produced. Absence of Sxl is associated with upregulation of genes on the single X chromosome (dosage compensation), male somatic development, and male germ line development. In *Drosophila*, the male-killer does not interact with any part of the somatic sex development pathway. Mutants of the *tra* gene bear two X chromosomes and produce Sxl but develop as somatic males. They are not, however, killed by *S. poulsonii* (24). Thus, the interaction between male-killer and host is not associated with somatic sex, so the target of detection and virulence is either before Sxl is produced, Sxl itself, or the dosage compensation or germ-line determination pathways.

Although the interaction between *Drosophila* and *S. poulsonii* is the only one studied in any detail, it appears that the mechanism of sex determination exhibited by different male-killer hosts varies widely. Male-killing bacteria have been observed in male heterogametic, female heterogametic, and haplodiploid hosts. Furthermore, members of the same clade of male-killers can be found in hosts of different sex determination systems. The same *Spiroplasma* kills males in ladybirds (male heterogametic) and butterflies (male homogametic). Similarly, male-killing *Wolbachia* have been observed in both male and female heterogametic species

(9). Given that male and female heterogametic systems count chromosomes in opposite directions and show different patterns of dosage compensation, the fact that male-killers operate in both these hosts suggests that the X:autosome counting mechanism and the dosage compensation pathway may not be the focus of male-killing activity; rather, somatic sex determination or germ-line sex determination may be the focus.

Experiments with *S. poulsonii* demonstrate that the somatic sex determination system is not the focus of male-killing behavior. In the case of the other male-killing *Spiroplasma*, the presence of the bacterium in species of different sex determination systems suggests that the focus is either the somatic sex determination or the germ-line determination system. Two conclusions are therefore possible: germ-line determination is the focus of male-killing in all cases, or male-killing has more than one basic mechanism. Further research is clearly warranted.

Direct Effects on Female Hosts

The interaction between male-killing bacteria and their female hosts is interesting. On the one hand, there is selection for a reduction in the number of bacteria present in the host (minimizing virulence) and for a direct physiologic contribution to host metabolism. On the other hand, their fitness is also associated with the fidelity of their transmission to progeny. There may be a trade-off between minimizing virulence and maximizing vertical transmission efficiency, especially if such efficiency is positively related to bacterial number. Thus these bacteria can be either detrimental (if the density of bacteria is high to ensure vertical transmission) or beneficial to the host (if the bacteria play a role in host metabolism).

Empiric studies have suggested that infection usually decreases the performance of female hosts (25,26). The one exception is the interaction between *Spiroplasma poulsonii* and members of the *Drosophila willistoni* group, in which larval development is accelerated by infection (27,28). However, infection is also associated with increased sterility and decreased longevity among adult females (28). Male-killing bacteria, unlike beneficial symbionts, are spread throughout host tissues, and the bacteria may be present in very high numbers. *Drosophila* are infected with extremely high titers of *S. poulsonii* within the hemolymph (29). *Adalia bipunctata* hemocytes are regularly infected with *Rickettsia* (30).

Beneficial effects of male-killing bacteria on host performance cannot yet be ruled out. However, positive effects may be fewer than those found in the "classical" beneficial agents, which typically perform a vital metabolic function that insects are unable to perform. Male-killers infect a minority of females and are rarely carried by larval or adult males. Thus, although they may add to host performance, they cannot substitute for any part of it. A host cannot be dependent on a male-killer for a physiologic function as it can on a beneficial symbiont.

Population and Evolutionary Effects on Hosts

Invasion of a host population by male-killing bacteria affects the dynamics of the host population and alters the pattern of selection on the population to ameliorate the effects of the parasite (Table 3). A high prevalence of male-killers may increase the proportion of female hosts that fail to mate (31), potentially reducing the population size of the host. A dearth of males can subtly alter the mating system of the host. Choice by females of male mates and competition among males for mating opportunities are the

Table 3. Population and evolutionary effects of invasion of a host by male-killing bacteria

Effects on population level	Evolutionary effects
Reduced population density at larval level due to death of male embryos	Selection for increased host clutch size
Failure of females to find mates where parasite prevalence leads to shortage of males, with potential effects on adult population size	Selection for genes that prevent transmission or action of male-killer
Altered epidemiology of sexually transmitted pathogens due to increased reproductive success of males	Alteration in host pattern of sexual selection due to alteration in population sex ratio

rule in insects. However, the biased population sex ratios that result from the spread of male-killing bacteria can reverse this pattern (31). Male choice of females and competition among females for males is expected, with a relaxation of selection on males to ensure paternity.

Male-killers that have invaded populations may cause changes to host biology. Theory predicts selection for an increase in the size of clutch produced (32). Most importantly, genes that prevent the action or transmission of the parasite will be favored. The presence of these genes has been reported (33), but their nature and mode of action are unknown. The means by which insects exclude bacteria is clearly of great import in our understanding of insect-borne diseases, and the nature of resistance genes is expected to be an important focus of future research.

One of the issues to be determined relates to whether male-killing bacteria can cause the extinction of their host. The case of the butterflies *Acraea encedon* and *A. encedana* is suggestive. The *Wolbachia* male-killer in these species is at high prevalence and clearly has some impact on the host population (21,31). If a male-killing bacterium showed perfect vertical transmission, host extinction would be likely. However, selection on the host acts to lower bacterial transmission efficiency, which may ultimately limit the frequency of extinction.

Conclusions: Implications and Uses of Male-Killing Bacteria

Male-killing is an adaptive trait that aids the spread of inherited bacteria through natural populations. The presence of male-killing strains in many bacterial taxa clearly indicates that male-killing should be considered in epidemiologic investigations of vertically transmitted bacteria. Male-killing is perhaps most important in interactions between arthropods and *Rickettsia* and *Spiroplasma*. Members of these genera frequently show horizontal transmission between arthropod hosts (after host-feeding), as well as vertical transmission in the arthropod host. Given that some bacteria in these groups induce male-killing, testing for the presence or absence of this trait should be a part of future investigations of their epidemiology.

The potential usefulness of male-killing bacteria in pest control has yet to be properly assessed. Male-killers may be used on their own to reduce host population size. Alternatively, they may be integrated into management schemes based on release of sterile males, so that they may amplify the effect of sterile releases on the population size of adult males. In addition, the recent discovery of male-killing in the clade *Wolbachia* adds an extra dimension to the use of this organism in direct and transgenic control of disease transmission.

The usefulness of male-killers in reducing pest damage on their own is debatable. Insect population size and population persistence are largely a function of female, not male, number. Thus, although the presence of a male-killer may reduce larval density, it is unlikely to decrease the population size of breeding females. Furthermore, the presence of density dependence during the larval stages is likely to reduce the effect of male death on numbers of larvae.

Perhaps a more realistic use of male-killing bacteria in pest management would be in conjunction with sterile male release systems of control. In sterile male release, control is achieved through release into the environment of mass-produced sterile males, which mate with females and lower their fertility (34). The success of sterile male release depends on maintaining a high ratio of sterile to normal males in the population. The presence of a male-killer in the host population lowers the number of fertile males and thus increases the effectiveness of any release. The effects of male-killing bacteria at different prevalences on sterile male release, in conjunction with the effects on host population dynamics, need to be investigated. However, direct use of male-killing bacteria as an aid to controlling host numbers is only achievable as a long-term stratagem. Following release of infected hosts into natural populations, spread will occur only in hosts with suitable ecologies and significant prevalence levels will be achieved over a period of years rather than weeks.

Another potential application of male-killing bacteria in the sphere of pest and disease vector control may occur indirectly through study of the virulence mechanisms of male-killers.

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References

1. Moran N, Baumann P. Phylogenetics of cytoplasmically inherited microorganisms of arthropods. Trends Ecol Evol 1994;9:15-20.
2. Baumann P, Lai C-Y, Baumann L, Rouhbakhsh D, Moran NA, Clark MA. Mutualistic associations of aphids and prokaryotes: biology of the genus *Buchnera*. Appl Environ Microbiol 1995;61:1-7.
3. O'Neill S, Giordano R, Colbert AME, Karr TL, Robertson HM. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatability in insects. Proc Natl Acad Sci U S A 1992;89:2699-702.
4. Hackett KJ, Lynn DE, Williamson DL, Ginsberg AS, Whitcomb RF. Cultivation of the *Drosophila* spiroplasma. Science 1986;232:1253-5.
5. Werren JH, Skinner SW, Huger AM. Male-killing bacteria in a parasitic wasp. Science 1986;231:990-2.
6. Hurst GDD, vd Schulenburg HG, Majerus TMO, Bertrand D, Zakharov IA, Baungaard J, et al. Invasion of one insect species, *Adalia bipunctata*, by two different male-killing bacteria. Insect Molec Biol 1999;8:133-9.
7. Hurst GDD, Hammarton TC, Majerus TMO, Bertrand D, Bandi C, Majerus MEN. Close relationship of the inherited parasite of the ladybird, *Coleomegilla maculata*, to *Blattabacterium*, the beneficial symbiont of the cockroach. Genet Res 1997;70:1-6.
8. Werren JH, Hurst GDD, Zhang W, Breeuwer JAJ, Stouthamer R, Majerus MEN. Rickettsial relative associated with male killing in the ladybird beetle (*Adalia bipunctata*). J Bacteriol 1994;176:388-94.
9. Hurst GDD, Jiggins FM, vd Schulenburg JHG, Bertrand D, West SA, Goriacheva II, et al. Male-killing *Wolbachia* in two species of insect. Proc R Soc Lond B 1999;266:735-40.
10. Whitcomb RF. The genus *Spiroplasma*. Annu Rev Microbiol 1980;34:677-709.
11. Davis MJ, Ying Z, Brunner BR, Pantoja A, Ferwerda FH. Rickettsial relative associated with papaya bunchy top disease. Curr Microbiol 1998;36:80-4.
12. Stouthamer R, Breeuwer JAJ, Hurst GDD. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. Annu Rev Microbiol 1999;53:71-102.
13. Bandi C, Damiani G, Magrassi L, Grigolo A, Fani R, Sacchi L. Flavobacteria as intracellular symbionts in cockroaches. Proc R Soc Lond B Biol Sci 1994;257:43-8.
14. Jiggins FM, Hurst GDD, Jiggins CD, vd Schulenburg JHG, Majerus MEN. The butterfly *Danaus chrysippus* is infected by a male-killing *Spiroplasma* bacterium. Parasitology 2000;120:439-46.
15. Skinner SW. Son-killer: a third extrachromosomal factor affecting sex ratios in the parasitoid wasp *Nasonia vitripennis*. Genetics 1985;109:745-54.
16. Werren JH. The coevolution of autosomal and cytoplasmic sex ratio factors. J Theor Biol 1987;124:317-34.
17. Hurst GDD, Majerus MEN. Why do maternally inherited microorganisms kill males? Heredity 1993;71:81-95.
18. Roberts LW, Rapmund G, Cadigan FCJ. Sex ratio in *Rickettsia tsutsugamushi* infected and non-infected colonies of *Leptotrombidium* (Acari: Trombiculidae). J Med Entomol 1977;14:89-92.
19. Takahashi M, Urakami H, Yoshida Y, Furuya Y, Misumi H, Hori E, et al. Occurrence of high ratio of males after introduction of minocycline in a colony of *Leptotrombidium fletcheri* infected with *Orientia tsutsugamushi.* Eur J Epidemiol 1997;13:79-86.
20. Williamson DL, Poulson DF. Sex ratio organisms (Spiroplasmas) of *Drosophila*. In: Whitcomb RF, Tully JG, editors. Sex ratio organisms (Spiroplasmas) of *Drosophila*; Vol. III. New York: Academic Press; 1979. pp. 175-208.
21. Jiggins FM, Hurst GDD, Dolman CE, Majerus MEN. High prevalence of male-killing *Wolbachia* in the butterfly *Acraea encedana*. J Evol Biol 2000;13:495-501.
22. Chang KS, Shiraishi T, Nakasuji F, Morimoto N. Abnormal sex ratio condition in the walnut leaf beetle, *Gastrolina depressa* (Coleoptera: Chrysomelidae). Appl Entomol Zool 1991;26:299-306.
23. Counce SJ, Poulson DF. Developmental effects of the sex-ratio agent in embryos of *Drosophila willistoni*. J Exper Zool 1962;151:17-31.
24. Sakaguchi B, Poulson DF. Interspecific transfer of the "sex-ratio" condition from *Drosophila willistoni* to *D. melanogaster*. Genetics 1963;48:841-61.
25. Hurst GDD, Purvis EL, Sloggett JJ, Majerus MEN. The effect of infection with male-killing *Rickettsia* on the demography of female *Adalia bipunctata* L. (two spot ladybird). Heredity 1994;73:309-16.
26. Ikeda H. The cytoplasmically inherited 'sex-ratio' condition in natural and experimental populations of *Drosophila bifasciata*. Genetics 1970;65:311-33.
27. Malogolowkin-Cohen C, Rodriguez-Pereira MAQ. Sexual drive of normal and SR flies of *Drosophila nebulosa*. Evolution 1975;29:579-80.
28. Ebbert M. The interaction phenotype in the *Drosophila willistoni*–spiroplasma symbiosis. Evolution 1991;45:971-88.
29. Sakaguchi B, Poulson DF. Distribution of "sex-ratio" agent in tissues of *Drosophila willistoni*. Genetics 1961;46:1665-76.
30. Hurst GDD, Walker LE, Majerus MEN. Bacterial infections of hemocytes associated with the maternally inherited male-killing trait in British populations of the two spot ladybird, *Adalia bipunctata*. J Invertebr Pathol 1996;68:286-92.
31. Jiggins FM, Hurst GDD, Majerus MEN. Sex ratio distorting *Wolbachia* causes sex role reversal in its butterfly host. Proc R Soc Lond B Bio Sci 2000;267:69-73.
32. Hurst GDD, McVean GAT. Parasitic male-killing bacteria and the evolution of clutch size. Ecol Entomol 1998;23:350-3.

33. Cavalcanti AGL, Falcao DN, Castro LE. "Sex-ratio" in *Drosophila prosaltans*—a character due to interaction between nuclear genes and cytoplasmic factors. Am Nat 1957;91:327-9.
34. Robinson AS. Sex ratio manipulation in relation to insect pest control. Annu Rev Genet 1983;17:191-214.
35. Majerus TMO, Majerus MEN, Knowles B, Wheeler J, Bertrand D, Kuznetsov VN, et al. Extreme variation in the prevalence of inherited male-killing microorganisms between three populations of *Harmonia axyridis* (Coleoptera: Coccinellidae). Heredity 1998;81:683-91.
36. Jiggins FM, Hurst GDD, Majerus MEN. Sex ratio distortion in *Acraea encedon* (Lepidoptera: Nymphalidae) is caused by a male-killing bacterium. Heredity 1998;81:87-91.
37. Hurst GDD, Hammarton TC, Obrycki JJ, Majerus TM, Walker LE, Bertrand D, et al. Male-killing bacteria in a fifth ladybird beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae). Heredity 1996;77:177-85.
38. Hurst GDD, Bandi C, Sacchi L, Cochrane A, Bertrand D, Karaca I, et al. *Adonia variegata* (Coleoptera: Coccinellidae) bears maternally inherited Flavobacteria that kill males only. Parasitology 1999;118;125-34.
39. Hurst GDD, Majerus MEN, Walker LE. The importance of cytoplasmic male killing elements in natural populations of the two spot ladybird, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). Biol J Linn Soc 1993;49: 195-202.
40. Geier PW, Briese DT, Lewis T. The light brown apple moth *Epiphyas postvittana* (Walker). 2. Uneven sex ratios and a condition contributing to them in the field. Austr J Ecol 1978;3:467-88.
41. Clarke C, Sheppard PM, Scali V. All female broods in the butterfly *Hypolimnas bolina* (L.). Proc Roy Soc Lond B 1975;189:29-37.
42. Brimacombe LC. All-female broods in field and laboratory populations of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Bull Entomol Res 1980;70:475-81.
43. Higashiru Y, Ishihara M, Schaefer PW. Sex ratio distortion and severe inbreeding depression in the gypsy moth *Lymantria dispar* L. in Hokkaido, Japan. Heredity 1999;83:290-7.

Communicating the Threat of Emerging Infections to the Public

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Communication theory and techniques, aided by the electronic revolution, provide new opportunities and challenges for the effective transfer of laboratory, epidemiologic, surveillance, and other public health data to the public who funds them. We review the applicability of communication theory, particularly the audience-source-message-channel meta-model, to emerging infectious disease issues. Emergence of new infectious organisms, microbial resistance to therapeutic drugs, and increased emphasis on prevention have expanded the role of communication as a vital component of public health practice. In the absence of cure, as in AIDS and many other public health problems, an effectively crafted and disseminated prevention message is the key control measure. Applying communication theory to disease prevention messages can increase the effectiveness of the messages and improve public health.

Although prevention has historically been part of medical intervention, communicating the threat of disease to the public has not always been in the forefront of public health efforts. Federal agencies have focused on the scientific accuracy of the laboratory research, disease surveillance, epidemiologic studies, and immunization efforts of their organizations. As scientific studies meticulously recorded the results of public health efforts in medical journals, reaching the public directly was, for the most part, left to the press and others who translated scientific journal information for the lay person. However, the era of AIDS, the emergence of new infectious organisms, microbial resistance to therapeutic drugs, and a new emphasis on prevention have expanded the role of communication as an important and necessary component of public health practice. At the same time, public interest in health information has soared (Figure 1) as the baby boom generation has aged and electronic publishing has broadened the audience and scope of the published word. At the crossroads of AIDS and the information age is the science of health communication, the means to disease prevention through behavior modification. In this article, we describe how communica-

Figure 1. Public interest in health information has soared in recent years.

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tion theory can be applied to the effective prevention and control of emerging infectious diseases.

Health Communication Methods

Health communication has been defined as the study and use of methods to inform and influence individual and community decisions that enhance health (1). Communication methods are used to create and increase public awareness of a disease; educate the public about a disease, its causes, and treatment; change a person's or group's attitudes about a disease; change individual behavior to prevent or control a disease; advocate for policy changes in favor of disease prevention and control; and create social norms that favor healthful living. Health communication theory utilizes four key elements of the communication process: audience, message, source, and channel. Effective health communication programs identify and prioritize audience segments; deliver accurate, scientifically based messages from credible sources; and reach audiences through familiar channels.

The Communication Process

Audience

Understanding the audience for which a message is intended is critical to the communication process. The clearer the understanding of the audience for which a message is intended, the better the chance of developing an effective message. The audience can be divided into smaller subgroups or segments of similar internal composition. Audience segmentation allows for more specific and individually tailored messages for each subgroup. An audience can be segmented on the basis of any number of criteria: demographics (sex, age, education); behavior (outdoors activities, food-handling practices, handwashing); and psychographic characteristics (values, attitudes, life-styles).

Once an audience is segmented, the subgroups are carefully assessed (through focus groups, in-depth interviews, demographic and other data) so that appropriate messages, sources, and channels can be formulated, first to inform the subgroup populations about a disease problem and then to propose acceptable behavior changes to prevent disease and promote healthful living. A specific example can clarify the segmentation process: campers, hikers, outdoor workers, and others who frequent wooded, brushy, and grassy places can be exposed to ticks involved in the transmission of Lyme disease. Suburban residents whose homes encroach on the habitats of deer and other animals infested by ticks are also at risk. Through audience segmentation, a message intended to increase awareness of Lyme disease can be directed at youth or middle-age, middle-income suburban men and women who frequently hike or camp. This subgroup, which is likely to read materials relevant to outdoors activities, could be reached through a brief leaflet (distributed at schools or physicians' offices) describing Lyme disease and simple ways to avoid it; through Internet resources on outdoors activities, where they may peruse the geographic areas likely to be infested with Lyme disease ticks and thus assess their personal risk; and through specialty shops, where along with insect repellent and hiking and camping gear, they may see posters urging them to check for ticks and may find light-colored clothes expressly marketed for ease of spotting ticks.

Audiences can also be segmented according to Prochaska's Stages of Change model (2), which suggests that behavior changes slowly, through a sequence of stages: precontemplation, contemplation, preparation, action, and maintenance. For example, the message, source, and channel may be different for an audience that has just heard of Lyme disease and is thinking about how likely it is to affect them (contemplation stage) than for an audience that has actively begun to take precautions (action stage) but needs reinforcement to make that behavior an integral part of their activities. Figure 2 portrays someone in the action stage who needs help integrating the message into normal life activities.

Message

Effective health communication messages follow some general principles (3): they are clear and simple, positive, and both emotional and rational; if they arouse fear, they show ways of alleviating the fear; and if they contain motivational appeals, the appeals follow established guidelines likely to produce the expected response.

On the individual or intrapersonal level, effective health communication messages often apply Prochaska's Stages of Change model and the Health Belief model to message design. The

Figure 2. Disease prevention messages are slowly integrated into life activities.

Health Belief model (4) addresses one's perception of personal risk for the disease and the behavior change recommended for decreasing the risk. Key variables in this theory include one's perceptions of the severity and susceptibility of the health threat, benefits from the recommended actions, barriers to taking action, cues to action, motivations for prompt action, and confidence in one's ability to take action; the message addresses one or more of these variables. For example, customers dining at a restaurant read the small print on the menu next to an entree with raw oysters: "Oysters may pose a health risk if eaten raw." In deciding whether to eat the raw oysters, customers would weigh the pleasure gained against the risk taken (benefits vs. barriers). They would consider the likelihood (susceptibility) and seriousness (severity) of illness and their capacity to prevent it.

On the interpersonal or interactive level, Social Cognitive theory is often used (5); its basic principle is that family members, friends, coworkers, family physicians, and other health professionals can influence a person's health behavior. One learns not only through personal experience but also by observing the actions of others and the consequences of these actions. Perception of risk and confidence in one's ability to take action are again key variables. If the family cook always uses separate cutting boards for raw meat and for fresh vegetables and washes the boards well after each use and the family stays healthy, the observers adopt the cook's behavior and associate it with a positive outcome.

On the community or organizational level, the Diffusion of Innovations theory applies (6). According to this theory, new ideas, products, and social practices follow a pattern as they spread within a society. Key variables include characteristics of the innovation itself (relative advantage, compatibility, complexity, observability), communication channels, and social systems (social networks, norms, structures).

Reaching culturally diverse groups with messages vital to disease prevention and trying to convince group members to alter their behavior to safeguard their health may sometimes require tools that transcend explanatory language. Explanatory language tends to isolate and fragment, to describe one event followed by another in linear fashion. Figurative language tends to synthesize and combine; it can unite different levels of thought, feeling, and behavior into a holistic picture that gives a rounded perspective; and it draws on such unusual vehicles as culturally specific metaphors, e.g., idiomatic sayings or proverbs, stories, or songs that express aspects of folk wisdom in plain but effective terms (7). The American Red Cross, the Centers for Disease Control and Prevention (CDC), and other health organizations have turned to such figurative language to create effective prevention messages (8) (Figure 3).

An intended audience that is motivated to change behavior may lack the skills or resources to do so. In a multicultural society, certain groups may resist changing risky behavior for fear that the change may strip them of their core culture or because behavior change is too stressful (9). One way to approach culturally based resistance is the use of metaphorical methods to ease fears and create a less threatening environment. A well-placed proverb—for example, "cada cabeza es un mundo" ("each head is a world" or "each person has his or her own thoughts, dreams, and aspirations and has a right to them as a unique person")—uses the cultural framework to balance a person's responsibility to cultural traditions with the need for well-being and good health (10).

Figure 3. Illustration from the American Red Cross African-American HIV/AIDS Program poster series. Used with permission of the American Red Cross, Copyright April 1992 (revised March 1997).

Regardless of the theory and the vehicle used, the only way to know if the audience will receive the intended message is to pretest the message with a representative sample of the audience. Focus groups, personal interviews, and other techniques similar to those used in audience segmentation are ways to pretest messages. For example, focus groups conducted to explore the causes of misuse of antibiotics in pediatric practice found that physicians felt under pressure from parents to prescribe antibiotics at every office visit; on the other hand, parents indicated that they would not insist on antibiotics if the reasons for not prescribing the drugs were explained to them. Educational efforts to narrow this communication gap would reduce the unnecessary use of antibiotics and perhaps the emergence of resistant strains of *Streptococcus pneumoniae* and other pathogenic bacteria (11).

Source

The source influences the effectiveness of the message. A source that is credible for one segment of the audience may completely miss the mark with another. While a scientist, physician, or other health-care provider may seem the ideal source of public health information, a community activist or a lay person affected by a disease may carry more credibility and have a greater public health impact. For example, a woman became a consumer activist and cofounded STOP (Safe Tables Our Priority) after her child died of hemolytic uremic syndrome, a complication of *Escherichia coli* O157:H7 infection, brought on by eating undercooked ground beef (12). The mother went on the road speaking out about her child's untimely death from eating a hamburger in a fast-food restaurant. Her moving testimonial (including poignant photographs) about the events surrounding the child's infection, illness,

and death had a direct and powerful effect on the audience most likely to eat undercooked hamburgers in fast-food restaurants. The testimonial also provided an excellent springboard for discussing *E. coli* infection and individual as well as public health prevention measures. The U.S. Department of Agriculture's Pathogen Reduction and Meat and Poultry HACCP (Hazard Analysis and Critical Control Points) rule, the first major change in meat safety regulation in the United States in 90 years, was put into place in large part through the efforts of STOP, which brought parents of O157 patients to Washington, D.C., to talk to legislators about the deaths of their children from eating undercooked ground beef (J.G. Morris, pers. comm.).

Channel

Even the best-crafted message is useless if it fails to reach the intended audience. The channel, or means by which the message is sent, is as important as the message. Mass media outlets (television, radio, magazines, newspapers, billboards, the Internet) provide ample opportunities, as do family, friends, health-care providers, and religious and other support groups (13). Other means such as telephone hot lines offer an opportunity for interpersonal communication anonymously and across geographic boundaries. Multiple channels can be combined to communicate a message more effectively. Mass media channels are most effective for increasing awareness and knowledge, but interpersonal channels work better in changing attitudes and behavior (6). A message delivered through the mass media can stimulate interpersonal discussions about a health issue. For example, a public service announcement about prevention of HIV and other sexually transmitted infections might prompt sex partners to discuss condom use (14). Computer-based communication channels (e.g., the Internet), which often provide interactive forums, are transforming and increasing channel options. By visiting various relevant web sites, a patient with chronic fatigue syndrome can locate scientific information about the disease, follow continuing research efforts, and connect with patient support groups. In 1997, during the avian influenza-like virus outbreak, the Hong Kong authorities set a new standard in communications about influenza by providing daily outbreak updates on a readily accessible Internet site. Information was also accessible on the FluNet World Health Organization Internet site (http://www.who.ch/flunet/).

Applying Health Communication Methods

Communication research provides demographic and other information that can be used in choosing the right channel to meet the needs of specific population groups. Such communication channels include mass media campaigns, news media stories, popular entertainment, media advocacy, and interpersonal communication.

Mass Media Campaigns

The mass media campaign, a traditional communication approach intended to produce a specific outcome within a specified period, is directed at large numbers of people through an organized set of communication activities (15). Research shows that different kinds of media affect audiences differently. For example, the news media inform and alert audiences to community developments and, in the process, shape community responses to these developments. The entertainment media fill leisure time and indirectly influence public beliefs (16,17). The business and advertising media stimulate interest in commercial goods and services and influence how and where we shop. In recent years, all these different media have also been used to disseminate health information, and for many people, they seem to have become the primary source of health information (18). The America Responds to AIDS campaign (14), which played a major role in AIDS prevention efforts, included television and radio public service announcements, printed materials (posters, booklets, brochures, billboards, bus ads), telephone hot lines, AIDS prevention messages integrated in movies and television shows, and specific AIDS information disseminated electronically through the Internet.

Mass media campaigns can raise awareness of an issue, enhance knowledge and beliefs, and reinforce existing attitudes (19-23). They can also change attitudes and behavior, especially when the change is simple and of obvious benefit to the intended audience. An intermediate objective of many mass media campaigns is to stimulate the search for additional information on a given health issue. When information-seeking is a desired outcome, mass media campaigns include a hot line for people to call for additional

information. The hot line not only facilitates the search for information, it also provides a means of measuring the effect of the mass media effort. For example, a recent study found that gender, cultural values, and anxiety may affect the response of Spanish-speaking callers to HIV information over the hot lines; therefore, health educators and others who design disease prevention programs need to examine whether these programs should reinforce or challenge traditional gender roles, gender norms, or cultural values (24).

Peptic ulcer disease affects 25 million Americans and has a $6 billion impact on the nation's health-care costs. In 1994, a National Institutes of Health consensus development conference panel concluded that patients with ulcers caused by *Helicobacter pylori* infection require antibiotic treatment (25). A 1995 study showed that 72% of the general population did not know that ulcers were caused by an infection (26). The prevalent belief that ulcers were caused by stress could deter affected persons from seeking medical attention for peptic ulcer disease. Additional studies indicated that primary-care physicians were treating 50% of patients with first-time ulcer symptoms without testing for *H. pylori*. To remedy this lack of awareness of *H. pylori* infection and its relationship to peptic ulcers, Congress mandated that CDC inform consumers and health-care providers about the link between *H. pylori* infection and ulcers. CDC collaborated with other federal agencies, academic institutions, and private industry in designing and implementing an *H. pylori* educational campaign to 1) inform consumers that ulcers are caused by an infection that can be cured; 2) increase physicians' awareness of *H. pylori*, its link to ulcers, and methods for its diagnosis and treatment; and 3) improve communication between patients and health-care providers about peptic ulcer disease and the successful treatment of *H. pylori*. Focus groups (consumer, private and managed-care physicians, pharmacists) were used to determine the structure and direction of a campaign. Materials developed as a result of the focus groups included a consumer brochure, a fact sheet for health-care providers, a waiting-room poster, and television, radio, and print public service announcements. A toll-free phone number (1-888-MY-ULCER) and a web site (www.cdc.gov/ncidod/dbmd/hpylori.htm) were also established. All materials were produced in English and Spanish; copies were mailed to health-care providers and state public health agencies.

The *H. pylori* campaign was launched in October 1997 with a national media briefing in Washington, D.C. The briefing provided an overview of peptic ulcer disease and focused on diagnosis and treatment, economic impact, and research issues associated with *H. pylori.* Evaluation of media tracking records shows that extensive press coverage resulted in reaching a potential audience of more than 21 million persons since the campaign began. Qualitative research with consumers and providers is being conducted to evaluate the campaign's communication messages. Quantitative studies are ongoing to determine attitudes and behavior of ulcer patients and health-care providers.

News Media Stories

Media stories can deliver accurate information on disease prevention in a more in-depth way than brief (paid or free) disease prevention messages (advertisements). Media stories create awareness among the intended audience, place health on the public agenda, and frame the way the issue is reported. Media stories may include the following strategies: newspaper and magazine coverage, news press conferences, press releases, video news releases, modular television or radio programming, talk show appearances, and Internet forums. Media stories are usually proactive, e.g., Parade magazine's section on "The Virus Hunters" (27) (Figure 4) and Time magazine's segment on *E. coli* infection, "The Killer Germ," (28) (Figure 5), or reactive, e.g., media coverage of the 1997 hepatitis A outbreak (150 cases) associated with frozen strawberries in Michigan (29). In the 1997 coverage, the Michigan Department of Health held a press conference announcing the outbreak and the implicated source (frozen strawberries served in public schools). Public health officials stated that 13 lots of frozen strawberries distributed to six states through the federal lunch program were possibly contaminated. Responding to the stories in the news and in the absence of information about hepatitis A, the mayor of Los Angeles held a press conference requesting 9,000 doses of immune globulin to inoculate schoolchildren who might have eaten the contaminated strawberries. Hepatitis A experts from CDC and the U.S.

Food and Drug Administration stepped in with information on hepatitis A, the proper use of vaccine, and the disease risk to the communities involved. These experts appeared on local and national news shows and delivered accurate information about the outbreak, which (having a broad geographic range and affecting children) generated extensive public interest. Through stories in the media, the public learned about hepatitis A and its causes, treatment, and prevention.

Media stories were used to alert the public about the 1993 *E. coli* O157:H7 outbreak of bloody diarrhea and serious kidney disease; the contamination (also in 1993) of a municipal water supply with the intestinal parasite *Cryptosporidium*, which caused the largest outbreak of waterborne illness in the United States; and the 1995 outbreak of Ebola hemorrhagic fever in Kikwit, Democratic Republic of Congo (then Zaire). In the Kikwit outbreak, health communication measures—which included education of physicians to recognize (or at least suspect) viral hemorrhagic fever, to reinforce the use of universal precautions and handwashing, and to collect specimens for laboratory confirmation using a method adapted to the local infrastructure—were embedded in the surveillance effort and were instrumental in stopping the outbreak and preventing further cases (30). City of Kikwit surveillance also included a mission radio network, daily voice contact by short-wave radio with 23 Catholic missions functioning in the Diocese of Kikwit, an area comprising several health zones, to inquire about possible cases and obtain follow-up information on villages with known cases within the last 3 weeks. Persons from Protestant churches and others with radios were contacted as needed. Ebola patients were visited by members of the regional surveillance team from Kikwit or by local health-care workers who had been trained in Kikwit. While being monitored for secondary cases, family members and other villagers were educated about the disease (30).

Popular Entertainment

Popular entertainment (television shows, movies, popular songs) is effective in educating audiences about disease prevention. This strategy borrows from Albert Bandura's Social Learning theory—that most behavior is learned through modeling (31)—and involves persuading

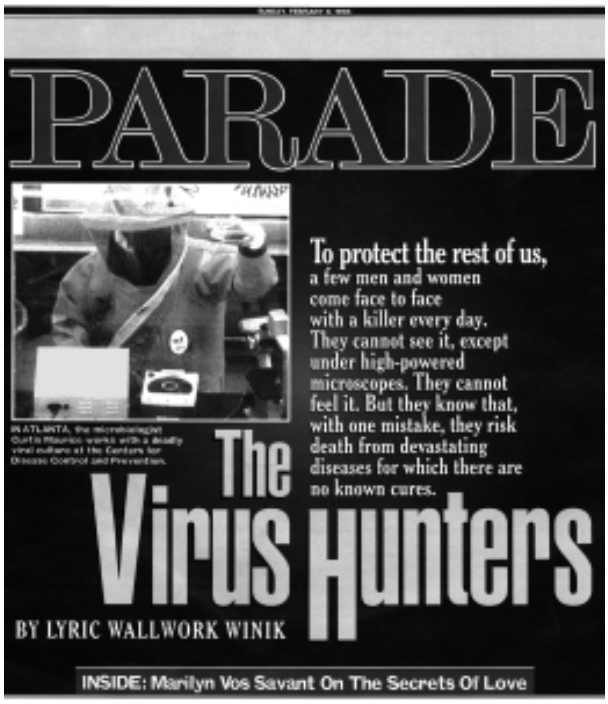

Figure 4. Cover of February 8, 1998, Parade magazine.
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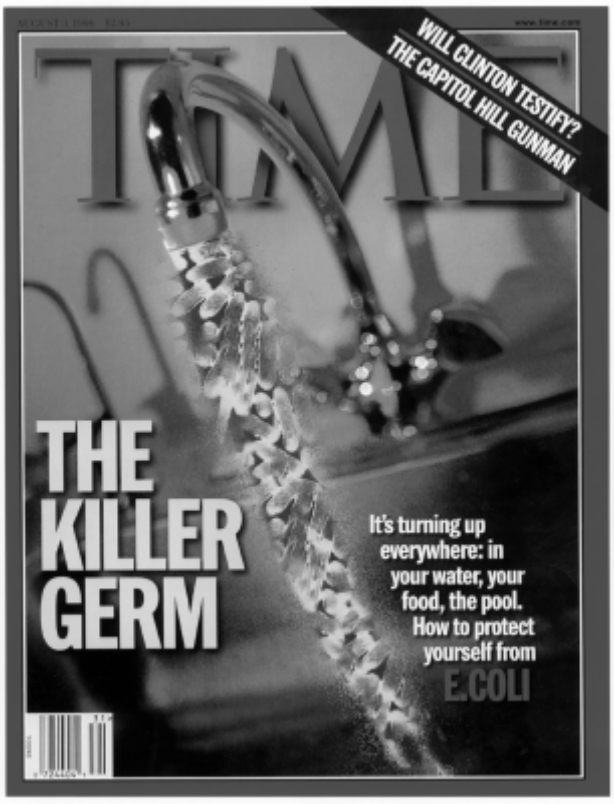

Figure 5. Cover of August 3, 1998, Time magazine
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scriptwriters, directors, and producers of popular shows, movies, and other entertainment to incorporate health issues into their programs (32). The audience learns new habits and behavior by seeing them on the screen or the stage and adapting them to their own situation.

The entertainment media not only attract attention, reinforce existing behavior, and demonstrate new behavior, they also tap into the audience's emotions. When the audience responds emotionally, the educational message is more likely to influence their behavior than when they respond only rationally (33). In psychotherapy, stories are often used to motivate patients to change behavior; patients who often resist direct statements and refuse to confront complex issues directly may consider such issues if they are presented through stories (2). The television series ER, which routinely carries safe sex, blood safety, handwashing, and other health messages as part of its series, provides a safe context for messages that might otherwise be viewed as threatening or intrusive.

In Puerto Rico, Head Start centers supported by the local Rotary Club stage dramas for preschool-age children to teach them about dengue prevention, and in Sierra Leone, puppet shows and songs have been used to deliver rodent-abatement messages to local villagers to prevent Lassa fever.

Media Advocacy

A relatively new communication approach, media advocacy, promotes changes in public health policy rather than in individual behavior (23) and stimulates media coverage to frame public debate and increase support for effective policies. Advocacy, which steers public health attention away from disease as a personal problem to health as a social issue, includes three fundamental steps: increasing media coverage and visibility of an issue, shaping the debate surrounding the issue, and advancing an effective policy (34). Media advocacy can influence public debate and put pressure on policymakers by increasing the voice of public health and visibility of values, people, and issues behind the voice (34). The goal of media advocacy is to empower the public to participate fully in defining the social and political environment in which decisions affecting health are made. This approach has been widely used in promoting the better use of antibiotics; as a result, a well-informed mother asks the pediatrician if her child's ear infection is viral or bacterial, thus relieving the physician from having to prescribe a drug that is unnecessary and ineffective. Disease information reaching the public through stories in the media during the 1997 hepatitis A outbreak in Michigan may have had broader implications for support of hepatitis A prevention programs and about policy decisions within the food industry regarding, for example, irradiation pasteurization of certain foods (35).

In the case of emerging infections, an unexpected by-product of media advocacy was the plethora of lay publications and films on infectious microbes. These microbes captured the artistic imagination of fiction writers and movie producers and brought about Outbreak, Pandora's Clock, The Hot Zone, The Coming Plague, and The Runaway Virus. In spotlighting an issue, the media also enhance the visibility, legitimacy, and power of the community. Media advocacy has played a key role in increasing awareness of emerging infections and advancing policies for their prevention and control.

In 1992, a report by the National Academy of Science's Institute of Medicine alerted the scientific community to a national (and global) infectious disease emergency: new infectious agents were emerging and old diseases thought to have been conquered were returning, often in more virulent forms; antibiotics were losing their effectiveness because infectious agents were fast becoming resistant to these drugs; and years of complacency had eroded the infectious disease infrastructure—all in an era of unprecedented travel and invasive environmental policies. This report, which might have had no impact outside the scientific community, became the center of a media campaign to raise awareness of the continued threat of infections and the need for public health action. Infectious disease experts from all over the United States drew up a strategic plan to address the threat of emerging diseases (36). Communicating the threat of these diseases and the resilient organisms that cause them was one of the plan's main goals. The plan was widely distributed to public health professionals around the world, and a summary was distributed to lawmakers. A flurry of media activity ensued. An Atlanta Journal and Constitution special segment on emerging microbes, When Bugs Fight Back (37), won the 1993 Pulitzer Prize for Explanatory Journalism.

Turner Broadcasting ran a series on infectious diseases based on J.B. McCormick's book Virus Hunters of the CDC. Media advocacy generated awareness among the public and lawmakers of the continuing threat of infectious diseases and the emergent crisis in global health.

Interpersonal Communication

Groups or individual citizens (family members, friends, co-workers, health-care providers) can effect behavior change and stimulate community involvement through interpersonal communication (one-on-one counseling, telephone calls, interviews, community forums, training, theater, web sites). As a result of such interpersonal communication and community involvement, safe food-handling techniques and use of meat thermometers are integrated in restaurant employee training; guidelines for safe kiddy pools are posted in pool areas attended by mothers of diapered children; and water filters that can prevent cryptosporidiosis are easy to find in local hardware stores.

Integrated Communication—Combining Strategies

Effective communication often involves multiple strategies and channels. The 1993 hantavirus outbreak in New Mexico generated an integrated communication effort involving scientific information, health-care delivery, public information to reduce panic, and dialogue between health workers and the affected Navajo community. Rapid response to the outbreak, collaboration with the affected community (including tribal medicine practitioners and elders), and sensitivity to cultural beliefs contributed to the success of the communication effort (38). Hantaviruses are rodent-borne agents spread to humans through infected rodent urine, saliva, or droppings. Campers, hikers, and others who take part in outdoors activities can become infected by breathing in the virus or (more rarely) by touching the mouth or nose after handling contaminated materials. A rodent's bite can also spread the virus.

Within hours of the first reported case of hantavirus, the Coconino County Health Department received telephone calls from the concerned public. Residents wanted to know how to recognize a deer mouse and what to do about rodents in and around their homes (38). Tourists wanted to know if it was safe to travel in and around the Four Corners area. The Coconino County Health Department, working with a public relations firm, distributed information to the city's hospitality industry. The communication link with the hospitality industry and tourists remained intact throughout the height of public concern. Because decreasing human contact with rodents was key to reducing the number of deaths, public education was critical to hantavirus prevention. Educational materials (brochures, slides, fact sheets, video tapes) in English and Navajo designed and distributed by the Indian Health Service were critical in the control of the outbreak and the prevention of additional cases (39).

Elephantiasis (lymphatic filariasis), a parasitic disease targeted for global elimination by the World Health Organization, affects 15 million people, mostly women, who become disfigured by debilitating secondary bacterial infections in the legs. The major challenge, and the key to success, in elephantiasis treatment is to convince affected women that progression of disease is not inevitable and that its outcome, to a large extent, rests in their own hands. In Brazil, acceptance of this message has led to profound and positive changes in the lives of affected women (40). In Haiti, where CDC investigators have worked on elephantiasis control for the past 10 years, the ability to effectively communicate such a message was initially limited by lack of understanding of the issues related to social stigmatization, physical ability, and barriers to changing health behavior among women with elephantiasis (41). Evaluation of patient education booklets and radio dramas broadcast locally (many developed for this project) showed that these interventions significantly improved patient motivation and compliance with the recommended treatment regimen. Further, the data showed that this regimen, which consists of good hygiene, elevation of the leg, exercise, bandaging, and treatment and prevention of entry lesions with topical antifungal and antibacterial creams, can be done at home and be integrated into the daily activities of the affected women (41). Formative research techniques, including focus groups and personal interviews, are being used 1) to determine the feasibility, effectiveness, and cost of an elephantiasis treatment program in a community setting in Haiti; 2) to understand the economic, health, and social impact of the disease in Haitian women; 3)

to develop public health messages that will enhance the effectiveness of the treatment program and evaluate patient compliance with the program; and 4) to understand the attitudes and practices of Haitian medical providers regarding disease treatment and develop educational programs for medical providers.

Conclusions

Communication theory and techniques, aided by the electronic revolution, provide new opportunities and challenges for the effective transfer of laboratory, epidemiologic, surveillance, and other public health data to the public who funds them. In what has been called the information age, health data are increasingly demystified by the communication media and are claiming their place in the marketplace as a public commodity. With health information readily available, the final decisions (and responsibility) about individual health are increasingly transferred from the health-care provider to the patient who is most profoundly and directly affected by treatment and prevention measures.

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References

1. Freimuth V, Cole G, Kirby S. Issues in evaluating mass mediated health communication campaigns. In: WHO monograph "Evaluation in health promotion: principles and perspectives." Copenhagen: WHO Regional Office for Europe. In press 2000.
2. Prochaska JO, Diclemente CC, Norcross JC. In search of how people change: applications to additive behavior. Am Psychol 1992;47:1102-14.
3. Freimuth V. Developing the public service advertisement for nonprofit marketing. In: Belk RW, editor. Advances in nonprofit marketing: a research manual. Vol 1. New York: JAI Press Inc.; 1985. p. 55-93.
4. Becker MH, Haefner D, Kasl SV. Selected psychosocial models and correlates of individual health-related behaviors. Med Care 1977;Suppl:27-46.
5. Bandura A. Social foundations of thought and action. Englewood Cliffs (NJ): Prentice-Hall; 1986.
6. Rogers EM. Diffusion of innovations. 3rd ed. New York: The Free Press; 1983.
7. Gordon D. Therapeutic metaphors: helping others through the looking glass. Cupertino (CA): Meta; 1978.
8. Potter P. Using cultural metaphors to deliver effective prevention messages. The American Journal of Health Communications 1996;4-8.
9. Littman SK. Forward. In: Barker P, editor. Using metaphors in psychotherapy. New York: Brunner/ Mazel; 1985. p. vii-viii.
10. Zuniga ME. Using metaphors in therapy: dichos and Latino clients. Soc Work 1992;37:55-9.
11. Barden LS, Dowell SF, Schwartz B, Lackey C. Current attitudes regarding use of antimicrobial agents: results from physicians' and parents' focus group discussions. Clin Pediatr 1998;665.
12. Heersink M. *E. coli* O157: the true story of a mother's battle with a killer microbe. New Jersey: New Horizon; 1996.
13. Miller MH. Seeking advice for cancer symptoms. Am J Public Health 1973;63:955-61.
14. Woods DR, Davis D, Westover B. America Responds to AIDS: its content, development process and outcome. Public Health Rep 1991;106:616-22.
15. Rogers EM, Story JD. Communication campaigns. In: Berger C, Chaffe S, editors.. Handbook of communication science. Newburg Park (CA): Sage Publication; 1987.
16. Brown JD, Childers KW, Waszak SC. Television and adolescent sexuality. Journal of Adolescent Health Care 1990;11:62-70.
17. Gerbner G, Gross L, Murgan M, Signiorelli N. Living with television; the dynamics of the cultivation process. In: Bryant J, Zillmann D, editors. Perspectives in media effects. Hillsdale (NJ): Lawrence Erlbaum Associates; 1986.
18. Freimuth VS, Stein JA, Kean TJ. Searching for health information: the cancer information service model. Philadelphia: University of Pennsylvania Press; 1989.
19. Alcaley R. The impact of mass communication campaigns in the health field. Soc Sci Med 1983;17:87-94.
20. Gandy OH. Beyond agenda setting. Norwood (NJ): Alblex; 1982.
21. Klapper J. The effects of mass communication. Glencoe (IL): Free Press; 1960.
22. McCombs ME, Shaw DL. The agenda-setting function of the media. Public Opinion Quarterly 1972;36:176-88.
23. Wallack L. Mass media and health promotion: promise, problem, and challenge. In: Atkin C, Wallack L, editors. Mass communication and public health. Newbury Park (CA): Sage Publication; 1990.
24. Scott SA, Jorgensen CM, Suarez L. Concerns and dilemma of Hispanic AIDS information seekers: Spanish-speaking callers to the CDC national AIDS hotline. Health Educ Behav 1998;25:501-16.
25. Sonnenberg A. Peptic ulcer. In: Everhart JE, editor. Digestive diseases in the United States: epidemiology and impact. Washington: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 1994:359-408; NIH publication no.94-1447.
26. American Digestive Health Foundation and Opinion Research Corporation. Familiarity with *H. Pylori* among adults with digestive disorders and their views toward diagnostic and treatment options. Bethesda (MD): American Digestive Health Foundation and Opinion Research Corporation; 1995.
27. Winik LW. The virus hunters. Parade 1998;Feb 8:6-9.
28. Kennedy M. The killer germ. Time 1998;Aug 3:56-62.

29. Centers for Disease Control and Prevention. Hepatitis A associated with consumption of frozen strawberries—Michigan, March 1997. MMWR Morb Mortal Wkly Rep 1997;46:288,295.
30. Khan AS, Tshioko K, Heymann DL, Le Guenno B, Nabeth P, Kerstiens B, et al. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. J Infect Dis 1999;179 Suppl 1:S76-86.
31. Bandura A. Analysis of modeling processes. School Psychology Digest 1975;4:4-10.
32. Center for Communication Programs, The Johns Hopkins University, The Annenberg School for Communication, University of Southern California, and Center for Population Options. Proceedings from the enter-educate conference: entertainment for social change. April 1989.
33. Center for Communication Programs, The Johns Hopkins University. Reaching young people worldwide: lessons learned from communications projects. 1986-1995. Working page No. 2, October 1995.
34. Wallack L, Dorfman L, Jernigan D, Thomba M. Media advocacy and public health: power for prevention. Newbury Park (CA): Saga Publications; 1993.
35. Osterholm MT, Potter ME. Irradiation pasteurization of solid foods: taking food safety to the next level. Emerg Infect Dis 1997;3:575-7.
36. Centers for Disease Control and Prevention. Addressing emerging infectious diseases threats: a prevention strategy for the United States. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 1994.
37. Toner M. When bugs fight back. The Atlanta Journal and Constitution 1993 Aug 23, Oct 16.
38. Graham G. Meeting the public's need for information during the hantavirus outbreak. Am J Public Health 1996;86:8.
39. Kreeger K. One year later, the hantavirus investigation continues. The Scientist 1994;8:14.
40. Eberhard ML, Walker EM, Addiss DG, Lammie PJ. A survey of knowledge, attitudes, and perceptions of lymphatic filariasis, elephantiasis, and hydrocele, among residents in an endemic area in Haiti. Am J Trop Med Hyg 1996;54:299-303.
41. Coreil J, Mayard G, Louis-Charles J, Addiss, D. Filarial elephantiasis among Haitian women: social context and behavioral factors in treatment. Trop Med Int Health 1998;3:467-73.

Reemergence of Pertussis in the Highly Vaccinated Population of the Netherlands: Observations on Surveillance Data

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We analyzed pertussis reporting, death, hospitalization, and serodiagnostic data from 1976 to 1998 to help explain the cause of the 1996 pertussis outbreak in the Netherlands. The unexpected outbreak was detected by an increase in pertussis reporting and by other surveillance methods. In 1996, according to reporting and serologic data, the increase in pertussis incidence among (mostly unvaccinated) children less than 1 year of age was similar to the increase in hospital admissions. Among older (mostly vaccinated) persons, the increase in hospital admissions was relatively small. The increase in pertussis incidence was higher among vaccinated than among unvaccinated persons of all ages. This resulted in lower estimates of vaccine effectiveness. The proportion of pertussis infections resulting in recognizable symptoms may have increased among vaccinated persons because of a mismatch of the vaccine strain and circulating *Bordetella pertussis* strains. The small immunogenicity profile of the Dutch vaccine may have resulted in greater vulnerability to antigenic changes in *B. pertussis*.

The incidence of pertussis has been greatly reduced by mass vaccination; however, even in countries with high vaccination coverage, the disease is reemerging (1-4). A sudden increase in cases reflecting a pertussis outbreak in the Netherlands in 1996 (5) could not be explained by a decrease in vaccination coverage, which remained stable at 96% for at least three vaccinations in the first year of life. Until January 1999, children were vaccinated at 3, 4, 5, and 11 months of age with a diphtheria, tetanus, pertussis, and inactivated polio vaccine. In 1999, the schedule changed, and vaccine was administered at 2, 3, 4, and 11 months of age. The vaccine used meets international standards; no sign of an abrupt or gradual deterioration of vaccine quality, as determined at product release by the mouse protection test, was found. The introduction of vaccination against *Haemophilus influenzae* type b in 1993 did not interfere with the immunoresponse to pertussis (6), and no cohort effect in children vaccinated for *H. influenzae* type b was observed (5). A mismatch between the vaccine and circulating strains of *Bordetella pertussis* (5,7-9) may have contributed to pertussis reemergence.

Determining the epidemiology of pertussis by case-reporting data is hampered by changes in case definitions, availability and interpretation of laboratory diagnostic tests, case-reporting rates, and diagnostic practice. To ascertain the current epidemiology of pertussis in the Netherlands and to try to determine the cause of the 1996 epidemic, we compared case-reporting data from January 1976 to September 1998 with other surveillance data (deaths, hospitalizations, and positive serodiagnoses).

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Methods

Surveillance Data

Case Reporting

Data from pertussis reporting (required by law since 1976) were obtained from 1976 to 1998 from the Inspectorate of Health. A case definition, introduced in 1988, included clinical symptoms and laboratory confirmation (or close contact with a person with laboratory-confirmed pertussis). The clinical symptoms are a serious cough >2 weeks, coughing attacks, or coughing followed by vomiting and at least one of the following: apnea, cyanosis, characteristic cough with whooping, subconjunctival bleeding, or leukocytosis. From 1988 to April 1997, laboratory confirmation was defined as either a positive culture of *B. pertussis* (or *B. parapertussis)* or positive two-point serology, in turn defined as a significant rise of immunoglobulin (Ig) G antibodies against pertussis toxin or IgA antibodies against *B. pertussis* in paired sera. In April 1997, a positive polymerase chain reaction (PCR) and positive one-point serology were also accepted as laboratory confirmation. Positive one-point serology was defined as high IgG or IgA antibody titers in a single serum sample.

From 1976 to 1988, only aggregated data were available on the total number of patients per year. These data included case-reporting date, number of vaccinated (at least three vaccinations) and unvaccinated or incompletely vaccinated patients, and number of patients with unknown vaccination status, with age at the time of report, by age-group (<1, 1-4, 5-9, and ≥10 years). From 1989 to 1992, the age (in years) and date of reporting were available for individual patients, while the aggregated data on vaccination status were similar to those of 1976 to 1988. Since 1993, the date of onset of symptoms, date of birth, age in years at the time of reporting, vaccination status, method of laboratory diagnosis, and contact with a person with laboratory-confirmed pertussis were included in the database. The method of laboratory diagnosis was differentiated as "microbiologic" (positive culture or PCR), "serologic," "epidemiologic" (i.e., contact with a patient with laboratory-confirmed pertussis), and "unknown."

The case distribution from 1976 to 1988 could only be assessed by date of reporting. From 1989 to 1992, the date of onset of symptoms was estimated by finding the median duration between date of first symptoms and date of reporting (1993 to 1994). This median duration (81 days) was then subtracted from the reporting date of the case at hand. The distribution of cases in 1989 to 1992 was based on this estimate, and age distribution was based on age at the time of reporting. For 1993 to September 1998, the distribution of cases was based on the date of first symptoms available in the database, and the age distribution was based on age at onset of symptoms.

Hospitalizations and Deaths

The number of hospitalizations with pertussis as the main diagnosis (ICD-9-CM 033) in 1976 to 1997 was obtained from the registry of the Foundation Information Center for Health Care. For 1989 to 1997, data were available by age group (<1, 1-4, 5-9, 10-14, 15-19, ≥20 years). The number of deaths (by age, in 5-year increments) caused by pertussis in 1976 to 1997 was obtained from the Central Bureau of Statistics.

Serology

Data on pertussis serology were obtained from the National Institute of Public Health and the Environment, the only laboratory in the Netherlands that performed serologic tests for suspected-pertussis patients from 1982 through January l, 1998. Other laboratories have performed an estimated 10% to 15% of serologic tests since 1998.

Serologic testing consisted of measuring IgA antibodies against a crude cell-wall preparation of *B. pertussis* (available since 1981) and IgG antibodies against purified pertussis toxin (available since 1984) in enzyme-linked immunosorbent assays, according to described methods that have not changed (10,11). The potency of the used reference sera was stable. Serologic interpretations have varied over the years. In 1982 to 1988, mostly single serum specimens were submitted. Since vaccination with the Dutch whole-cell vaccine only induces low levels of IgG against pertussis toxin (IgG-PT) and no IgA against *B. pertussis* (IgA-Bp), detection of IgA-Bp (sonicated mixture of the two strains included in the Dutch whole-cell vaccine) or moderate, high, or very high IgG-PT was reported as supportive of pertussis.

By 1987, it became clear that low and moderate IgG-PT and IgA-Bp levels were present

in a large proportion of the population and that the prevalence increased with age. Therefore, in 1988, serologic interpretation of single serum specimens was abandoned, and only significant increase of IgG-PT or IgA-Bp in paired sera was considered confirmation of pertussis. The same year, a strict case definition for pertussis reporting was introduced, which included "positive two-point serology" in the laboratory-confirmation criteria. However, in 1993, when serum specimens from the population, vaccinees, and pertussis patients were tested, high IgG-PT or high IgA-Bp levels (greater than an age-specific cut-off value) were found to be very rare in the population (<2.5%). Such levels were not induced by vaccination, were present in at least 90% of patients with PCR- or culture-confirmed pertussis, and decreased again within 6 months to levels below the cut-off value (12-14). Since 1994, the laboratory-reported detection of such high values in one or both samples of a serum pair indicates "possible pertussis," although the case definition for reporting remained unchanged. From April 1997, the detection of such high levels in patients' samples was formally defined as "positive one-point serology" and was included in the case definition for reporting as laboratory-confirmed pertussis.

From the serologic database, we retrieved data on patients whose date of disease onset was January 1989 to September 1998 and for patients whose date of serologic result was January 1986 to December 1987. Patients with positive two-point serology in 1989 to 1998 and patients with positive one-point serology in 1994 to 1998 were selected. The criteria we recently defined for positive one-point serology were retrospectively applied to the serologic data of 1986–1987 and 1989–1993. The distribution of cases in 1986 and 1987 was calculated based on the year of the test result and, in 1989–1998, based on the year of first symptoms.

Data Analysis

We used Epi-Info version 6.04 to estimate vaccine effectiveness[1] in persons ages 1 to 4 and 5 to 9 years (reporting data from 1976 to 1997), assuming an average vaccine coverage in the Dutch population of 96%. We compared completely vaccinated persons (at least three vaccinations) with incompletely vaccinated or unvaccinated persons (15).

Since positive serologic results are included in the case definition for reporting, the serodiagnosis and reported-case databases are not independent sources. For 1993 to 1997, the reported-case database and the database with records of serodiagnosis were linked at the individual-patient level to verify the type of serodiagnosis (positive two-point or positive one-point serology) on which the reporting was based. The completeness of the reported-case database was calculated from the proportions of reported patients with positive two-point and one-point serologic results. We used the statistical package SAS to analyze the data.

Results

Reported Cases, Hospitalizations, and Deaths, 1976 to September 1998

In the first years of mandatory reporting of pertussis cases, the case count was the lowest (Table 1). From 1983 to 1987, after immunoassays for pertussis serology became available, the number of reported cases increased yearly. In 1988, the year in which a case definition was introduced and positive serology was restricted to an increase in titer in paired sera, the number of reported cases declined sharply. Somewhat greater numbers of cases were reported in 1989-90 and 1993-94. In 1996, the number of cases was 12 times higher than in 1995, while a twofold decrease from the 1996 number was observed in 1997.

The number of reported cases from January to September 1998 (n = 1,582) was lower than that of the same periods in 1996 (n = 2,171) and 1997 (n = 2,004) but approximately six times higher than the average number of reported cases in the same months of 1989 to 1995 (n = 269).

The trend of hospitalizations was similar to that of case reports; however, the ratios varied by period (Table 1). This ratio was below one from 1976 to 1984, increased to 5.7 in 1987, and decreased sharply to 1.2 in 1988; it remained relatively stable from 1989 to 1995 but increased to 8.2 in 1996 and 6.1 in 1997.

From 1976 to 1997, seven deaths caused by pertussis were reported: one in 1981, two in 1993, two in 1996, and two in 1997. They occurred among children <1 year of age, except for one death in 1993, which occurred in the 5- to 9-year age group. According to the number of

[1]Vaccine effectiveness = 1 – (proportion of vaccinated cases/1 – proportion of vaccinated cases) X (1 – proportion vaccinated in the population/proportion vaccinated in the population).

Table 1. Reported cases, hospitalizations, ratios of reported cases to hospitalizations, positive 2-point serology and positive 1-point serology, 1976–1998

Yr	Reports	Hosps	Pos. 2-point test	Pos. 1-point test	Ratio of reports/ hosps	Ratio of reports/pos. 2-point test	Ratio of reports/pos. 1-point test
1976[a]	4	52	NA	NA	0.1	NA	NA
1977	25	93	NA	NA	0.3	NA	NA
1978	1	32	NA	NA	0.0	NA	NA
1979	26	43	NA	NA	0.6	NA	NA
1980	30	65	NA	NA	0.5	NA	NA
1981[b]	50	76	NA	NA	0.7	NA	NA
1982	80	98	NA	NA	0.8	NA	NA
1983	200	223	NA	NA	0.9	NA	NA
1984[c]	534	200	NA	NA	2.7	NA	NA
1985	1,522	313	NA	NA	4.9	NA	NA
1986	2,159	388	NA	2,717	5.6	NA	0.8
1987	2,709	474	NA	3,295	5.7	NA	0.8
1988[d]	112	92	NA	NA	1.2	NA	NA
1989	523	221	350	1,760	2.4	1.5	0.3
1990	397	157	278	1,204	2.5	1.4	0.3
1991	145	82	110	411	1.8	1.3	0.4
1992	160	101	238	861	1.6	0.7	0.2
1993	346	288	482	1,489	1.2	0.7	0.2
1994	519	276	498	1,867	1.9	1.0	0.3
1995	341	162	272	1,070	2.1	1.3	0.3
1996	4,231	513	1,885	7,854	8.2	2.2	0.5
1997[e]	2,671	436	924	4,107	6.1	2.9	0.7
1998[f]	1,582	NA	294	2,187	NA	5.4	0.7

[a]Mandatory case reporting introduced.
[b]IgA assay introduced.
[c]IgG assay introduced.
[d]Case definition for reporting (including strict criteria for interpretation of serologic tests) introduced.
[e]Formal acceptance of positive one-point serologic test.
[f]Data from January to September 1998.
1-point test = cases with one-point serology; 2-point test = cases with two-point serology; NA=not available.

hospitalizations among children <1 year of age, the case-fatality rates amounted to 0.1% on average in 1989 to 1995, 0.6% in 1996, and 0.7% in 1997.

Serologic Results

The cases with positive one-point serology followed a trend similar to that of cases reported in 1986 to 1987 and 1989 to 1998 (Table 1). In 1986 and 1987, the ratio of reported cases to cases with positive one-point serology was highest (0.8); it decreased in 1989, and remained relatively stable from 1989 to 1996 (0.2 to 0.4). In 1996 to 1998, this proportion increased from 0.5 to 0.7.

The trends of cases with positive two-point serology and reported cases were similar (Table 1). From 1989 to 1995, the number of cases in each database was similar (ratio 0.7 to 1.5); in 1996 and 1997 (ratio 2.2 and 2.9) and particularly in 1998 (ratio 5.4), the number of reported cases was higher than the number of cases with positive two-point serology.

Age-Specific Incidence, from Case Reporting and Hospitalizations, 1989–1997

In 1989 to 1995, the average annual incidence from case reporting was highest for infants <1 year of age. Such data for 1993 to 1995 show that the age-specific peak incidence occurred among ≤ 5-month-old infants. For infants <1 year of age, the incidence in 1996 was four times higher than the average incidence from 1989 to 1995 and 13 times higher than the incidence for older age groups (Figure 1). The age-specific peak incidence shifted to 4-year-old children in 1996 and 1997.

From 1993 to 1997, when the method of laboratory diagnosis was available for reported cases, similar shifts in age distribution were observed for cases confirmed by microbiologic

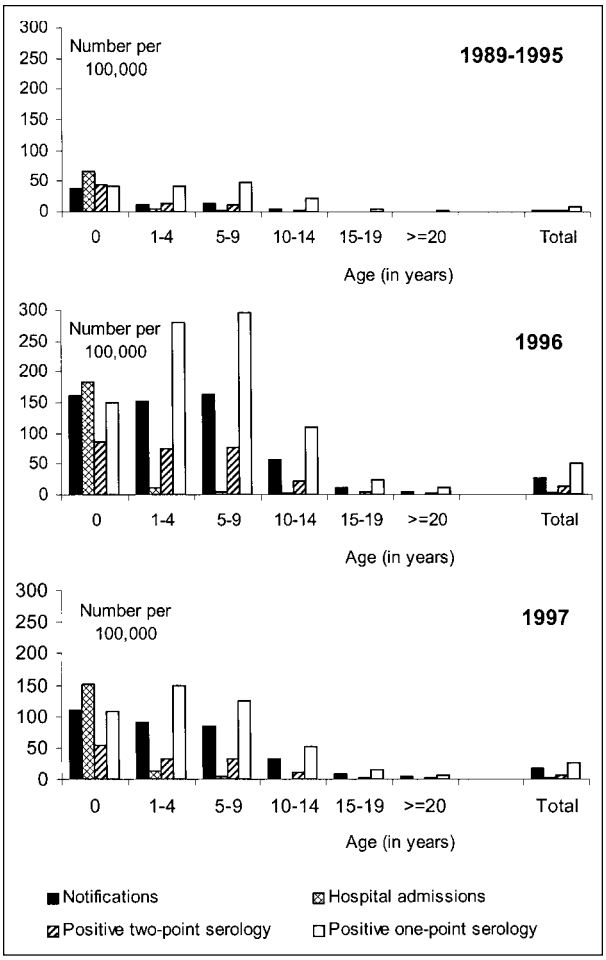

Figure 1. Average annual age-specific incidence (number per 100,000) in 1989 through 1995, in 1996, and in 1997.

method (i.e., culture or PCR), cases with positive two-point serology, and cases with positive one-point serology. However, reported cases confirmed microbiologically were those of the youngest patients; cases confirmed with one-point serology were those of the oldest patients. In contrast to reported cases, the greatest age-specific incidence of hospitalizations occurred among infants <1 year of age from 1989 to 1997 (Figure 1). The increase in incidence in 1996 was more stable for the various age groups.

Age-Specific Incidence, from Serologic Results, 1989-1997

As in case reports, because of a relatively greater increase of pertussis among older age groups, the peak incidence among cases with positive two-point serology occurred among ≤5-month-old infants from 1989 to 1995 and among 4-year-old children in 1996 and 1997. The incidence of cases with positive one-point serology was greatest among 7-year-old children in 1989 and shifted towards 4-year-old children from 1992 to 1997. As with case reports, the increase for infants <1 year old was smaller (fourfold) than that for older age groups (fivefold to sevenfold) (Figure 1).

Seasonal Trend

In March and April 1996, reported cases started to increase (Figure 2). The largest monthly number of cases occurred later in the year (October 1996) than in 1989-1995 and 1997-

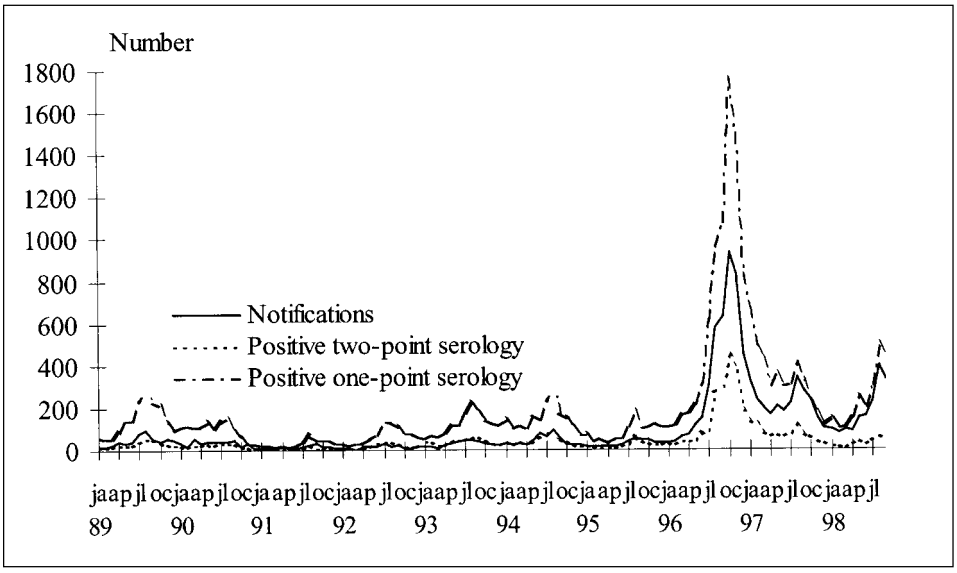

Figure 2. Reported pertussis cases, cases with positive two-point serology, and cases with positive one-point serology, per month, 1989–1998.

1998 (mostly in August, sometimes in July or September). The seasonal trend of positive two-point serology and positive one-point serology was similar to that of case reports (Figure 2).

Vaccination Status of Reported Cases

According to case reports, vaccine effectiveness was high in 1981 to 1984 (1 to 4 years of age: 94% to 99%; 5 to 9 years of age: 87% to 100%) and in 1988 to 1993 (1 to 4 years of age: 89% to 95%; 5 to 9 years of age: 78% to 89%). The estimates were somewhat lower in 1985 to 1987 (72% to 85% for children 1 to 4 years of age and 56% to 77% for children 5 to 9 years of age). The estimates decreased after 1993 (Table 2), were lowest in 1996, and could not be determined in 1997 since the proportion of vaccinated patients exceeded 96%.

Table 2. Reported cases according to vaccination status and estimate of vaccine-effectiveness according to method of diagnosis

Yr.	Method of diagnosis	1- to 4-yr-olds (no.)	Vaccine efficacy (%)[a]	5- to 9-yr-olds (no.)	Vaccine efficacy (%)[a]
1993	Microbiologic	14	93	14	94
	2-point serology	25	91	28	75
	1-point serology	24	79	26	50
	Other[b]	35	96	20	90
	Total	98	93	88	84
1994	Microbiologic	23	56	15	42
	2-point serology	36	67	36	83
	1-point serology	63	71	65	c
	Other[b]	37	89	29	91
	Total	159	77	145	72
1995	Microbiologic	14	85	9	67
	2-point serology	27	82	19	64
	1-point serology	39	64	45	c
	Other[b]	37	53	27	67
	Total	117	72	100	45
1996	Microbiologic	116	67	123	3
	2-point serology	245	c	288	49
	1-point serology	545	c	782	c
	Other[b]	290	66	355	40
	Total	1,196	34	1,548	16
1997	Microbiologic	63	51	68	57
	2-point serology	110	c	131	c
	1-point serology	283	c	345	c
	Other[b]	244	c	260	c
	Total	700	c	804	c

[a]Estimated vaccine effectiveness; a vaccine coverage of 96% was used to estimate the incidence and vaccine effectiveness.
[b]Epidemiologic, serologic (differentiation between positive two-point serology and positive one-point serology not possible), clinical, or method of diagnosis unknown.
[c]Vaccine effectiveness could not be estimated as the percentage vaccinated was more than 96%.

Vaccine effectiveness for almost all methods of diagnosis was greater in 1993 than in 1994 to 1997 (Table 2). The decreasing trend was not consistent with all methods of diagnosis. Estimates for cases diagnosed microbiologically tended to be the highest; estimates for cases confirmed by one-point serology tended to be the lowest.

Method of Diagnosis for Case Reports, 1993–1997

Linkage of the case-report and serodiagnosis databases for 1993 to 1997 showed that the proportion of reported cases confirmed microbiologically, epidemiologically, or by two-point serology, decreased, while the proportion of cases confirmed with positive one-point serology increased (Figure 3). The proportion of cases confirmed serologically but not matched with the serologic database also increased. Differentiating these cases by positive one-point and positive two-point serology was not possible. During 1996 and 1997, the proportion of cases confirmed microbiologically by quarter year was similar (8.7% to 10.2%), except for a smaller proportion (6.4%) in the last quarter of 1997. The proportion of cases confirmed by two-point serology was 12% to 19%. In the first two quarters of 1996 (34.9% to 35.6%) and in the fourth quarter of 1997 (34.3%), the proportion of cases confirmed by positive one-point serology was similar to the proportions for 1994 and 1995. The numbers increased to >50% in the fourth quarter of 1996 and the first quarter of 1997. The proportion of cases confirmed serologically that could not be matched with the serodiagnostic database was highest (35.3%) in the fourth quarter of 1997.

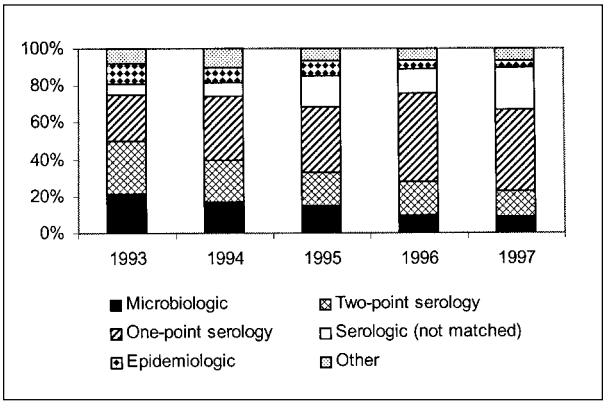

Figure 3. Method of diagnosis for reported pertussis cases from 1993 through 1997.

Estimated Rate of Case Reporting

The reported proportion of cases with positive two-point serology increased from 24% in 1993 to 26% in 1994, 28% in 1995, and 42%-43% in 1996 and 1997. A similar trend was observed for the various age groups. For positive one-point serology, the reported proportion increased from 6% in 1993, to 10% in 1994, 12% in 1995, and 27% to 29% in 1996 and 1997. The discrepancy was somewhat greater with increasing age. The increase in the reported proportions of cases with positive two-point and one-point serology was probably underestimated because the database of reported cases contained serologically confirmed cases that could not be matched with the serodiagnosis database. This proportion was highest in 1997 (Figure 3).

Discussion

The 1996 surveillance data show that the unexpected pertussis outbreak was detected not only by increased reporting of cases (5) but also by increased hospitalizations, cases with positive serology, and deaths. Vaccine effectiveness, which had already declined in 1994 and 1995, declined further in 1996 and 1997. According to case reports and serologic data, in 1996 the increase in pertussis incidence among (mostly unvaccinated) children <1 year of age was similar to the increase in hospitalizations. However, in older, mostly vaccinated persons the increase in hospitalizations was relatively small. Contrary to reports at the time, a somewhat smaller epidemic likely occurred in 1986 and 1987 (16,17).

The surveillance data for pertussis in the Netherlands were affected by changes in availability and interpretation of serologic tests, case definitions for reporting, and case-reporting rate. However, by relying on various surveillance sources, applying criteria for one-point serology used in recent years to serologic data of 1986 and 1987, and matching our database of reported cases with our serodiagnosis database, we gained a better understanding about whether observed changes in surveillance data represented true changes in the underlying incidence of pertussis.

The trend in hospitalizations likely reflects the incidence of severe pertussis; however, this trend is probably less sensitive to changes in availability and interpretation of serologic tests, case definitions, and case-reporting rate. Thus, increasing or decreasing reports of pertussis case and data on positive serology are likely to (at least partially) reflect true changes when they are accompanied by similar trends in hospitalizations.

We obtained more insight into the effect of changes in definitions of positive serology and case definitions for reporting on serologic and reported data. The current, more restrictive, criteria for positivity of one-point serology were applied to serodiagnostic data of 1986 and 1987. It was possible to study changes in the rate of reported cases with positive one-point serology and cases with positive two-point serology in 1993 to 1997 by linking the case-reporting and serodiagnosis databases. Furthermore, stratifying case reports according to method of diagnosis led us to conclude that the decrease in estimated vaccine effectiveness and the shift in 1996 and 1997 toward older age groups in the reported cases could only partly be explained by the enhanced application of positive one-point serology. Because of great variation in case definitions and types of laboratory confirmation, comparing numbers of reported cases in different countries is meaningless. Hospitalizations, although limited to severe pertussis cases, might be more useful for such international comparisons.

Our results clearly show that pertussis has remained endemic with epidemic peaks in the Netherlands, despite high vaccination coverage. Immunity after infection, as well as after vaccination, is not lifelong. Waning vaccine-induced immunity has been suggested as an explanation for the reemergence of the disease in other countries and probably has contributed to the pertussis epidemic in the 1980s and in 1996-97 (18-20). However, the outbreak in 1986-87 may also have been associated with the Dutch vaccine's temporary reduction in potency—from 16 to 10 opacity units per dose—in 1976 to 1984. The somewhat lower vaccine-effectiveness estimates in 1986 and 1987 might be explained by greater exposure to *B. pertussis* in epidemics than in interepidemic periods (21,22).

The remarkable increase in reported cases among vaccinated patients over a wide age range, starting 2 years before the 1996 outbreak, suggests a mismatch between circulating strains and vaccine strains (5,7-9). Antigenic divergence between vaccine strains and clinical isolates was observed for two important protective antigens, pertactin and pertussis toxin (9). Furthermore, data suggest that the whole-cell vaccine protects better against strains with the

pertactin vaccine type than against strains with nonvaccine types (9).

By analyzing serologic and hospitalization data apart from case-reporting data, we assessed the increase in pertussis incidence in 1996 among (mostly unvaccinated) children <1 year of age. The increase in incidence was accompanied by a similar increase in hospitalizations for pertussis in the same age group, which indicates that the virulence of *B. pertussis* for unvaccinated and unexposed persons did not change during the outbreak.

In contrast, for older, mostly vaccinated persons, the increase in hospitalizations was smaller than the increase found in other surveillance sources. While the incidence of hospital admissions was highest for infants <1 year of age, the incidence in other surveillance sources was highest in 4-year-old children. Therefore, a greater proportion of infected vaccinated persons may have had clinical symptoms because of antigenic shifts, which probably led to greater transmission of bacteria and thus a greater degree of infection in the population. This is shown by the increase of cases in unvaccinated infants.

Despite the findings of antigenic variants of pertactin and pertussis toxin in other countries, no outbreaks similar to those in the Netherlands have been observed (23-25). The vaccines in these countries may be potent enough to offset antigenic variation, or pertussis vaccines may protect less well against strains with pertactin profiles dominant in the Netherlands but less common elsewhere (23). The Dutch vaccine has been used in the National Immunization Program since 1953. No sign of an abrupt deterioration of vaccine quality, as determined for product release by the mouse protection test, has been found (5). However, the Dutch whole-cell vaccine induces low levels of antibodies against pertussis toxin and filamentous hemagglutinin and high levels of antibodies to agglutinogens and pertactin (6). This immunogenicity profile may have resulted in a greater vulnerability of the vaccinated Dutch population to antigenic changes in *B. pertussis,* especially with respect to pertactin. Since November 1997, the production process for the Dutch pertussis vaccine has been improved, resulting in a slightly higher expression of pertussis toxin. We have not yet studied the potential effect of this change.

Pertussis is also reemerging in Canada, where similar surveillance patterns may elucidate the role of the vaccine's immunogenicity profile (1,2,5). These similarities are the small proportion of infants and large proportion of patients 1 to 9 years of age affected, estimates of low vaccine effectiveness, and lower levels of antibodies against pertussis toxin after vaccination with the Canadian whole-cell vaccine (20,26). By contrast, the increase in pertussis cases in the United States is accompanied by greater proportions of affected infants and adults and more favorable vaccine-effectiveness estimates (22,27,28). The levels of pertussis toxin antibodies after vaccination were higher for an American whole-cell vaccine than for Canadian whole-cell vaccines (26).

Conclusions

The surveillance data support, even if they do not definitively explain, the hypothetical role of antigenic changes in *B. pertussis* during the 1996 pertussis outbreak in the Netherlands. The indisputable role of whole-cell vaccine in protecting against severe pertussis is clearly shown by the sharp decrease in hospitalizations among children >12 months of age; this protection is also shown by a much smaller pertussis incidence (in the past and at present) in the Netherlands than in countries with large unvaccinated populations (29). In such countries, 60% of unvaccinated persons have clinical pertussis before the age of 10. This incidence is at least 30 times higher than that in the Netherlands, even if we assume a case-reporting rate of 25% and an incidence similar to that observed during the Dutch epidemic in 1996.

Booster vaccination will be helpful in reducing the incidence of pertussis. However, some acellular vaccines do not contain the antigenic variants of pertactin and pertussis toxin that dominate in Europe (9,23,24). Furthermore, if pertussis infections are to be postponed until adulthood as a result of boosting, the probability of transmission from adults to young, unvaccinated infants might be greater. The effects of booster vaccination on the epidemiology of pertussis must be monitored carefully, and various surveillance sources must be used to distinguish surveillance artifacts and real epidemiologic effects.

Acknowledgments

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References

1. De Serres G, Boulianne N, Douville Fradet M, Duval B. Pertussis in Quebec: ongoing epidemic since the late 1980s. Can Commun Dis Rep 1995;21:45-8.
2. Milord F. Resurgence of pertussis in Monteregie, Quebec-1990-1994. Can Commun Dis Rep 1995;21:40-4.
3. Centers for Disease Control and Prevention. Resurgence of pertussis--United States, 1993. MMWR Morb Mortal Wkly Rep 1993;42:952-3, 959-60.
4. Andrews R, Herceg A, Roberts C. Pertussis notifications in Australia, 1991 to 1997. Commun Dis Intell 1997;21:145-8.
5. de Melker HE, Conyn-van Spaendonck MAE, Rümke HC, Wijngaarden JK van, Mooi FR, Schellekens JFP. Pertussis in the Netherlands: an outbreak despite high levels of immunization with whole cell vaccine. Emerg Infect Dis 1997;3:175-8.
6. Labadie J, Sundermann LC, Rümke HC, and the DTP-IPV-Hib vaccine study group. Multi-center study on the simultaneous administration of DTP-IPV and Hib PRP-T vaccines. Part 1. Immunogenicity. Bilthoven, The Netherlands: National Institute of Public Health and the Environment; 1996. Report No. 124001003.
7. van der Zee A, Vernooij S, Peeters M, van Embden J, Mooi FR. Dynamics of the population structure of *Bordetella pertussis* as measured by IS1002-associated RFLP: comparison of pre- and post-vaccination strains and global distribution. Microbiology 1996;142:3479-85.
8. Mooi FR, van Oirschot H, Peeters J, Willems RJL. Antigenic variation of the acellular vaccine component pertactin in the Dutch *B. pertussis* population. Bilthoven, The Netherlands: National Institute of Public Health and the Environment; 1995. Annual Report p. 74-5.
9. Mooi FR, van Oirschot H, Heuvelman K, van der Heide HGJ, Gaastra W, Willems RJL. Polymorphism in the *Bordetella pertussis* virulence factors P.69/pertactin and pertussis toxin in the Netherlands: temporal trends and evidence of vaccine-driven evolution. Infect Immun 1998;66:670-5.
10. Nagel J, Poot-Scholtens EJ. Serum IgA antibody to *Bordetella pertussis* as an indicator of infection. J Med Microbiol 1983;16:417-26.
11. Nagel J, de Graaf S, Schijf-Evers D. Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LPF antibody levels. Dev Biol Stand 1985;61:325-30.
12. de Melker HE, Boshuis HGL, Mommers MPTM, Sundermann LC, Labadie J, Rümke HC, et al. Serodiagnostiek van kinkhoest: interpretatie van hoge IgG/IgA concentraties in acute fase serum. (Her)evaluatie van eenpuntsserologie. Bilthoven, The Netherlands: National Institute of Public Health and the Environment; 1996. Report No. 128507003.
13. van der Zee A, Agterberg C, Peeters M, Mooi F, Schellekens J. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J Infect Dis 1996;174:89-96.
14. de Melker HE, Versteegh FGA, Conyn-van Spaendonck MAE, Elvers LH, Berbers GAM, van der Zee A, et al. Specificity and sensitivity of high levels of IgG antibodies against pertussis toxin in a single serum for diagnosis of infection with *Bordetella pertussis.* J Clin Microbiol 2000;38:800-6.
15. Farrington CP. Estimation of vaccine effectiveness using the screening method. Int J Epidemiol 1993;22:742-6.
16. van de Water HPA. Kinkhoestaangiften in Nederland in de periode 1976-1986 en de noodzaak van een uniforme ziektedefinitie. Ned Tijdschr Geneeskd 1988;132:821-8.
17. van de Water HPA, van de Laar MJW, Leentvaar-Kuijpers A. Wanneer is het kinkhoest? Verband tussen laboratoriumdiagnostische bevindingen en klachten. Ned Tijdschr Geneeskd 1988;132:828-33.
18. Bass JW, Stephenson SR. The return of pertussis. Pediatr Infect Dis J 1987;6:141-4.
19. Bass JW, Wittler RR. Return of epidemic pertussis in the United States. Pediatr Infect Dis J 1994;13:343-5.
20. Bentsi-Enchill AD, Halperin SA, Scott J, MacIsaac K, Duclos P. Estimates of effectiveness of a whole-cell pertussis vaccine from an outbreak in an immunized population. Vaccine 1997;15:301-6.
21. Ramsay MEB, Farrington CP, Miller E. Age-specific efficacy of pertussis vaccine during epidemic and non-epidemic periods. Epidemiol Infect 1993;111:41-8.
22. Guris D, Strebel PM, Tachdjian R, Bardenheier B, Wharton M, Hadler SC. Effectiveness of the pertussis vaccination program as determined by the use of the screening method: United States, 1992-1994. J Infect Dis 1997;176:456-63.
23. Mooi FR, He Q, van Oirschot H, Mertsola J. Variation in *Bordetella pertussis* virulence factors pertussis toxin and pertactin in vaccine strains and clinical isolates in Finland. Infect Immun 1999;67:3133-4.
24. Mastriantonio P, Spigaglia P, van Oirschot H, van der Heide HGJ, Heuvelman K, Stefanelli P, et al. Antigenic variants in *Bordetella pertussis* strains isolated from vaccinated and unvaccinated children. Microbiology 1999;145:2069-75.
25. Guiso N. Can new vaccines meet the challenge of novel pertussis strains? Abstract book of the 17th Annual Meeting of the European Society for Paediatric Infectious Diseases. Greece; 1999 May 19-21; p. 10.

26. Baker JD, Halperin SA, Edwards K, Miller B, Decker M, Stephens D. Antibody response to *Bordetella pertussis* antigens after immunization with American and Canadian whole-cell vaccines. J Pediatr 1992;121:523-7.
27. Centers for Disease Control and Prevention. Pertussis—United States, January 1992-June 1995. MMWR Morb Mortal Wkly Rep 1995;44:526-9.
28. Guris D, Strebel PM, Bardenheier B, Brennan M, Tachdjian R, Finch E, et al. Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990-1996. Clin Infect Dis 1999;28:1230-7.
29. Isacson J, Trollfors B, Taranger J, Zackrisson G, Lagergard T. How common is whooping cough in a nonvaccinating country? Pediatr Infect Dis J 1993;12:284-8.

Seroprevalence of West Nile, Rift Valley, and Sandfly Arboviruses in Hashimiah, Jordan

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We conducted a serosurvey among patients of a health center in Hashimiah, a Jordanian town of 30,000 inhabitants located near a wastewater treatment plant and its effluent channel. Serum samples from 261 patients ≥5 years of age were assessed for immunoglobulin G (IgG) and IgM antibodies against West Nile, sandfly Sicilian, sandfly Naples, and Rift Valley viruses; the seroprevalence of IgG antibodies was 8%, 47%, 30%, and 0%, respectively. Female participants were more likely to have been infected than male. Persons living within 2 km of the treatment plant were more likely to have been infected with West Nile (p=0.016) and sandfly Sicilian (p=0.010) viruses. Raising domestic animals within the house was a risk factor for sandfly Sicilian (p=0.003) but not for sandfly Naples virus (p=0.148). All serum samples were negative for IgM antibodies against the tested viruses. Our study is the first documentation of West Nile and sandfly viruses in Jordan and calls attention to the possible health hazards of living close to wastewater treatment plants and their effluent channels.

Arboviruses are transmitted by arthropods. Humans become infected through the bites of blood-sucking insects such as mosquitoes, ticks, and certain flies. The geographic distribution of the viruses varies with the presence and density of the appropriate vector. In Jordan, there have been no previous studies of arboviral infections and no clinical reports of the existence of such infections. We report the findings of a serosurvey of West Nile, sandfly Sicilian, sandfly Naples, and Rift Valley viruses. The study area has an abundance of *Culex pipiens* mosquitoes, which breed in the effluent channel of a nearby wastewater treatment plant (1). The presence of the above viruses in neighboring countries and the abundance of their vectors in the study area were the reasons the viruses were selected for this study.

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Methods

Study site

Hashimiah (32°7'N and 36°6'E) is a town of approximately 30,000 inhabitants in close proximity to a wastewater treatment plant and its effluent channel. The plant uses stabilization ponds, which receive more than double the amount of wastewater they were designed to treat. This overload results in insufficient treatment and poor-quality effluent. The effluent channel has many areas with excessive vegetation and stagnant water. Local residents have reported high mosquito and sandfly density and bad odor in the area. A baseline health assessment was undertaken as part of a comprehensive effort to solve the problem.

Study population

A total of 501 persons ≥5 years of age who attended the local health center from June 20 to July 30, 1998, were invited to participate. Of those, 261 (52%) agreed to undergo all the study procedures, including giving a blood sample.

Children <5 years of age were excluded because of the technical difficulties of drawing blood samples and obtaining their guardians' consents.

Data collection

Each participant was interviewed by use of a structured questionnaire, including questions about sociodemographic and other variables related to exposure to mosquitoes and domestic animals, and had a general physical examination focusing on pest-related problems. A 10-mL venous blood sample, obtained from each participant, was separated within 3 hours of collection and stored in an ice bag at -20°C until transported to Cairo, Egypt, for final laboratory analysis.

Samples were tested in U.S. Naval Medical Research Unit No 3 (NAMRU-3) laboratories. An enzyme-linked immunosorbent assay (ELISA) was performed for immunoglobulin G (IgG) and IgM antibodies against West Nile, sandfly Sicilian, sandfly Naples, and Rift Valley viruses. The West Nile strain was EG101, which was passed in mice (14 times) and in Vero cells (3 times). The two sandfly viruses were Sabin strain. Sicilian virus was passed in mice (37 times) and in Vero cells (4 times); Naples virus was passed in mice (49 times) and in Vero cells (4 times). The Rift Valley strain was ZH-501, which was passed in mice (6 times), in E-6 cells (once), and in Vero cells (3 times). A standard direct IgG ELISA was used for virus antigens. An IgM-capture assay employing anti-human IgM (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, USA) was used for the IgM assays. The viruses were grown in tissue cultures. At approximately 50% cytopathic effect, the viral proteins were extracted with Triton X (1%). These virus lysates were used for both IgG and IgM ELISAs. ELISAs for IgG used 96-well plates coated with antigens (viral infected and uninfected Vero cells) extracted with 1% Triton X (Sigma T-9284). Serum samples were added to the plates, and bound antibodies were detected by using goat anti-human IgG conjugated to horseradish peroxidase and detected with ABTS (2,2'-Azinopis [3-ethylbenzothiazoline-6-sulphonic acid], diammonium salt) substrate. For IgM, goat anti-human IgM antibodies were absorbed to ELISA plates and used to capture the patient's serum IgM. Anti-virus-specific IgM was detected by using the antigens and hyperimmune mouse serum. The antigen-antibody complexes were detected using goat anti-mouse IgG-horseradish peroxidase and ABTS substrate. All conjugates, capture IgM antibodies, and ABTS were from Kirkegaard & Perry.

Ethical Considerations

The study was undertaken in response to public concerns regarding potential health hazards of the wastewater treatment plant and its effluent channel on neighboring residents. The study protocol was approved by the Jordanian Ministry of Health. Verbal consent was obtained from all participants or their legal guardians. All identifying information was kept confidential.

Data Management and Statistical Analysis

Data entry and analysis used Epi-Info, version 6 software (2). Seropositivity was determined by a number of variables. Observed differences were assessed for statistical significance by chi-square, corrected for continuity.

Results

West Nile Virus

Approximately 8% of the study participants had evidence of past infection with West Nile virus (Table 1). Although information on travel

Table 1. Seropositivity of immunoglobulin G antibodies against West Nile virus, Jordan, 1998

Variable	Total	Seropositivity N (%)	p
Total	261	21 (8.0)	
Sex			0.202
Male	75	3 (4)	
Female	186	18 (9.7)	
Age			0.920
5-9 years	14	1 (7.1)	
10-29 years	158	12 (7.6)	
≥30 years	89	8 (9.0)	
Monthly family income			0.284
<100 JD[a]	76	9 (11.8)	
100-249 JD	156	11 (7.1)	
≥250 JD	29	1 (3.5)	
Presence of domestic animals			0.578
In house	86	9 (10.5)	
Near house	24	2 (8.3)	
None	151	10 (6.6)	
Distance from plant			0.016
Residence within 2 km	115	15 (13.0)	
More than 2 km	146	5 (4.1)	
Presence of mosquito bites on exam			0.660
Yes	156	14 (9.0)	
No	105	7 (6.7)	

[a]JD = Jordanian dinars.

was not collected, mobility of the study population is limited and thus unlikely to be the cause of infection with the virus. Cross-reactivity to other related flaviviruses is unlikely since no such viruses have been documented in Jordan. The infection rate among female participants (9.7%) was more than double that among male (4.0%), but it was not statistically significant (p=0.202). Although older age (≥30 years), lower family income (<100 Jordanian dinars), presence of domestic animals within the house, and presence of mosquito bites on examination seemed to be related to a higher prevalence of past infection with West Nile virus, none of these variables had significant effect. The only significant factor for past infection was distance between residence and treatment plant and its effluent channel. Study participants living within 2 km were approximately 4 times more likely to have been infected than participants living further away (p=0.016). No participants had evidence of acute infection with West Nile virus.

Sandfly Sicilian Virus

More than 47% of the study population had evidence of past infection with sandfly Sicilian virus (IgG seropositivity, Table 2). Female sex, presence of domestic animals within the house, and close residence to the treatment plant and its effluent channel were significantly associated with a higher prevalence of past infection with sandfly Sicilian virus. There was no evidence of acute infection (IgM positivity) with sandfly Sicilian virus.

Sandfly Naples Virus

More than 29% of the participants had IgG antibodies against sandfly Naples virus (Table 3). The only factor significantly related to past infection was age: participants ≥30 years of age were more likely to have been infected than those in the younger age groups (p=0.007); all were IgM seronegative, which indicates absence of acute infection with this virus.

Rift Valley Virus

All participants were seronegative for IgG and IgM antibodies against Rift Valley virus, which indicates that the study population had never been exposed to the virus.

Discussion

Our study is the first documentation that West Nile, sandfly Sicilian, and sandfly Naples

Table 2. Seropositivity of immunoglobulin G antibodies against sandfly Sicilian virus, Jordan, 1998

Variable	Total	Seropositivity N (%)	p
Total	261	123 (47.1)	
Sex			0.003
Male	75	24 (32.0)	
Female	186	99 (53.2)	
Age			0.284
<10 years	14	6 (42.9)	
10-29	158	69 (43.7)	
≥30	89	48 (53.9)	
Monthly family income			0.277
<100 JD[a]	76	41 (53.9)	
100-249 JD	156	71 (45.5)	
≥250 JD	29	11 (37.9)	
Presence of domestic animals			0.003
In house	86	53 (61.6)	
Near house	24	8 (33.3)	
None	151	62 (41.1)	
Distance from plant			0.010
Residence within 2 km	115	65 (56.5)	
More than 2 km	146	58 (39.7)	
Presence of mosquito bites on exam			0.804
Yes	156	75 (48.1)	
No	105	48 (45.7)	

[a]JD = Jordanian dinars.

Table 3. Seropositivity of immunoglobulin G antibodies against sandfly Naples virus, Jordan, 1998

Variable	Total	Seropositivity N (%)	p
Total	261	77 (29.5)	
Sex			0.165
Male	75	17 (22.7)	
Female	186	60 (32.3)	
Age			0.007
<10 years	14	1 (7.1)	
10-29	158	40 (25.3)	
≥30	89	36 (40.4)	
Monthly family income			0.68
<100 JD[a]	76	24 (31.6)	
199-249 JD	156	43 (27.6)	
≥250 JD	29	10 (34.5)	
Presence of domestic animals			0.148
In house	86	32 (37.2)	
Near house	24	7 (29.2)	
None	151	38 (25.2)	
Distance from plant			0.667
Residence within 2 km	115	36 (31.3)	
More than 2km	146	41 (28.1)	
Presence of mosquito bites on exam			0.485
Yes	156	43 (27.6)	
No	105	34 (32.4)	

[a]JD = Jordanian dinars.

viral infections are present in Jordan. The prevalence of sandfly viral infection was much higher than that of West Nile, but no acute sandfly infections were detected, possibly because 1) IgM positivity is short-lived and therefore the chance to be detected in a survey is much smaller and 2) children ≤5 years of age (who are more likely to be susceptible for acute infection) were excluded from the study.

Humans become infected with West Nile virus by the bite of an infected *Culex* mosquito. *C. pipiens* are abundant in the study area (1). Birds are the reservoirs of infection (3). The presence of the disease in Jordan is not unexpected as its known geographic distribution includes the Middle East, Africa, southern Europe, and Asia (4). In 1989, the seroprevalence of IgG antibodies to this virus among schoolchildren ages 8 to 14 years was 3% in an area in the Nile River Delta (5). In another report from Egypt, the seroprevalence of West Nile virus antibodies was 20% (6). In 1996, an epidemic of 393 cases of West Nile meningoencephalitis occurred in Romania (7). Recently, and for the first time in the United States, an outbreak of West Nile-like encephalitis occurred in New York (8, 9).

Infection with sandfly viruses is transmitted by the bites of infected *Phlebotomus pappatasi* sandflies. The principal reservoirs are humans and sandflies (10), though rodents are suspected to harbor them (3). Aseptic meningitis caused by Sicilian virus has been reported (11). Sandfly fever is present in the circum-Mediterranean area, extending to the east through the Balkans into China, the Middle East, and Southwest Asia (4). Travelers to disease-endemic areas and deployed troops are at high risk of contracting the disease (12). Among 298 Swedish United Nations soldiers who served in Cyprus, seroconversion, in a 6-month period, occurred in 7 (for Sicilian virus), 3 (Naples), and 1 (Toscana) (13). Cyprus seems to have a high prevalence of sandfly viruses, with a reported seroprevalence rate of 57% for Sicilian, 32% for Naples, and 20% for Toscana virus (14). Sandfly Sicilian and Naples virus infections have been documented in Egypt (5,6). Although these viruses have not been reported in Jordan, their vector (*P. pappatasi*) is ubiquitous (15-18), including in the Hashimiah area (Saliba EK, unpublished data). Sandflies breed mainly in dirt and garbage, but not in wastewater. The high prevalence of both sandfly fever viruses in Hashimiah may be attributed to the fact that immunity is serotype specific, i.e., infection with one serotype provides no protection for the other (12).

Possible explanations for the higher infection rates among women are their likelihood of spending most of their time at home and their caring for domestic animals in places not protected from mosquitoes and sandflies. These factors may also explain the higher prevalence of past infection among lower income people, who live close to the plant and its effluent channel, are more likely to raise domestic animals (mostly sheep and cows), and often keep the animals inside the homes. These circumstances create environmental conditions suitable for sandfly breeding.

Unlike sandfly Sicilian, the associations of sandfly Naples with gender, presence of domestic animals, and distance from the plant were not significant. The smaller number of seropositive samples for sandfly Naples may explain these inconsistencies. On the other hand, the higher endemicity of the Sicilian virus leads to exposure and subsequent immunity at an earlier age than for the Naples virus. Because young children (≤5 years) were excluded from the study, a weaker association between the Sicilian virus and age is not unexpected.

With the unprecedented increased population mobility in the form of tourism, business, and troop deployment, political borders are no longer barriers against the spread of infections. The West Nile-like encephalitis outbreak in New York, which is likely to have been transmitted from the Middle East (19), provides viable support for this notion. Therefore, our findings may be of interest outside, as well as within, Jordan. At the local level, the data should alert physicians to consider these viral infections in the differential diagnosis of conditions such as encephalitis, aseptic meningitis, and unexplained febrile illnesses. The data also highlight the need for preventive measures, such as educating people about self-protection and instituting public health programs directed against mosquitoes and sandflies. The study also calls attention to the possible health hazards of wastewater plants and, in particular, their effluent channels, on neighboring communities. Wastewater effluent channels, if not well maintained, provide potential breeding sites for *C. pipiens*. The absence of Rift Valley virus infection among the studied population does not

mean it is absent in other areas in Jordan. Further studies in different geographic areas are recommended.

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References

1. Wijeyaratne P. Initial investigation of insects and other pests in communities around the As-Samra Waste Water Treatment Plant. Environmental Health Project. Washington: U.S. Agency for International Development. Report for the file No. 140; 1997.
2. Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, et al. Epi-Info, Version 6: a word-processing, database, and statistics program for public health on IBM-compatible microcomputers. Atlanta (Ga):Centers for Disease Control and Prevention;1995.
3. Monath TP, ed. Arboviruses: epidemiology and ecology. Elkins Park (PA): Franklin Book Company, 1989.
4. Peters CJ. Infections caused by arthropod- and rodent-borne viruses. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, eds. Harrison's Principles of Internal Medicine. 14th ed. Vol. 1. New York: McGraw-Hill, 1998;1138.
5. Corwin A, Habib M, Olson J, Scott D, Ksiazek T, Watts DM. The prevalence of arboviral, rickettsial, and Hantaan-like viral antibody among school children in the Nile River Delta of Egypt. Trans R Soc Trop Med Hyg 1992;86:677-9.
6. Corwin A, Habib M, Watts D, Darwish M, Olson J, Botros B, et al. Community-based prevalence profile of arboviral, rickettsial, and Hantaan-like viral antibody in the Nile River Delta of Egypt. Am J Trop Med Hyg 1993;48:776-83.
7. Han LL, Popovici F, Alexander Jr JP, Laurentia V, Tengelsen LA, Cernescu C, et al. Risk factors for West Nile Fever virus infection and meningoencephalitis, Romania, 1996. J Infect Dis 1999;179:230-3.
8. Centers for Disease Control and Prevention. Outbreak of West Nile-like viral encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:845-9.
9. Centers for Disease Control and Prevention. Update: West Nile-like viral encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999; 48:890-2.
10. Tesh RB, Modi GB. Studies on the biology of phleboviruses in sandflies (Diptera: Psychodidae). 1. experimental infection of the vector. Am J Trop Med Hyg 1981, 33:1007-16.
11. Becker M, Zielen S, Schwarz TF, Linde R, Hofmann D. Pappataci fever (abstract). Klin Padiatr 1997;209:377-9.
12. Tesh RB. The epidemiology of *Phlebotomus* (Sandfly) fever. Isr J Med Sci 1989;25:214-7.
13. Eitrem R, Vene S, Niklasson B. Incidence of sandfly fever among Swedish United Nations soldiers on Cyprus during 1985. Am J Trop Med Hyg 1990;43:207-11.
14. Eitrem R, Stylianou M, Niklasson B. High prevalence rates of antibody to three sandfly fever viruses (Sicilian, Naples, and Toscana) among Cypriots. Epidemiol Infect 1991;107:685-91.
15. Oumeish OY, Saliba EK, Allawi TF. Cutaneous leishmaniasis, an endemic disease in Jordan. Jordan Medical Journal 1982;15:55-61.
16. Janini R, Saliba E, Kamhawi S. Species composition of sandflies and population dynamics of *Phlebotomus pappatasi* (Diptera: Psychodidae) in an endemic focus of cutaneous leishmaniasis: the southern Jordan Valley. J Med Entomol 1995;32:822-6.
17. Kamhawi S, Abdel-Hafez SK, Molyneux DH. A comprehensive account of species composition, distribution and ecology of phlebotomine sandflies in Jordan. Parasite 1995;2:163-72.
18. Saliba EK, Saleh N, Oumeish OY, Khouri S, Bisharat Z, Al-Ouran R. The endemicity of leishmania tropica (zymodeme MON-137) in the Eira-Yarka area of Salt District, Jordan. Ann Trop Med Parasitol 1997;5:453-9.
19. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Park Crise B, Volpe KE, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7.

Dual Captures of Colorado Rodents: Implications for Transmission of Hantaviruses

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We analyzed dual-capture data collected during longitudinal studies monitoring transmission and persistence of Sin Nombre virus in rodents in Colorado. Our data indicate that multiple captures (two or more rodents captured in a single trap) may not be random, as indicated in previous studies, but rather the result of underlying, species-specific social behavior or cohesiveness. In the pairs we captured, most often, rodents were of the same species, were male, and could be recaptured as pairs. Therefore, dual captures of rodents, which are unusual but not rare, tend to occur among certain species, and appear to be nonrandom, group-foraging encounters. These demographic and ecologic characteristics may have implications for the study of the transmission of hantaviruses.

The deer mouse, *Peromyscus maniculatus*, has been identified as the primary rodent reservoir of a newly identified virus responsible for the 1993 outbreak of severe respiratory disease in the southwestern United States (1). This virus, named Sin Nombre virus (SNV; family Bunyaviridae, genus *Hantavirus*) presumably is transmitted between rodents and to humans by inhalation of virus-contaminated aerosols of urine, saliva, and fecal material shed by subclinically infected rodents (2). Biting may also play an important role in rodent-to-rodent transmission (3).

We established longitudinal studies at three Colorado sites in 1994 to monitor SNV transmission and persistence in rodent populations and to assess factors that might influence virus transmission. On multiple occasions, two rodents were captured in a single trap. We summarize and analyze multiple-capture data and compare them with such data reported earlier (4-9). Our data indicate that rather than occurring as random encounters, dual captures may suggest underlying social behavior or cohesiveness that varies with species.

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Methods

Description of Sites

Study areas in western Colorado, at Fort Lewis (La Plata County, southwestern Colorado; N 37° E 13' 30.9" latitude, W 108° E 10' 51.1" longitude, elevation 2,438 m) and Molina (Mesa County, west-central Colorado; N 39°E 09' 45.8" latitude, W 108°E 03' 18.4" longitude, elevation 1,951 m) were chosen because they were near residences of case-patients during the 1993 hantavirus outbreak. We established trapping webs at both sites (10). At Piñon Canyon Maneuver Site in southeastern Colorado, four sites were trapped. Trapping webs were used in a pinyon-juniper–short grass prairie habitat (N 37° E 33.024', W 103° E 59.560', elevation 1,585 m) and at the head of a canyon (N 37° E 32.754', W 103° E 49.343', elevation 1,524 m); trapping grids were used within that canyon (N 37° E 32.193', W 103° E 49.125', elevation 1,341 m) and at a functioning windmill (N 37° E 31.327', W 103° E 53.545', elevation 1,585 m).

At Fort Lewis, the habitat is montane shrubland (11) superimposed on intrusive

igneous rocks forming laccoliths (12). Vegetation is predominantly composed of ponderosa pine (*Pinus ponderosa*), Gambel's oak (*Quercus gambeli*), a variety of grama grasses (*Bouteloua* spp.), and many other, more minor, floral components. At the Molina site, the habitat is semidesert shrubland (11) superimposed on Mancos shale (12). Vegetation includes juniper (*Juniperus* spp.), pinyon pine (*Pinus edulis*), and various shrubs and grasses. The Piñon Canyon site is managed by the Directorate of Environmental Compliance and Management, U.S. Department of the Army, Fort Carson, Colo. This site is a short grass prairie–pinyon-juniper community (13) with topographic features consisting of broad, moderately sloping uplands bordered by the Purgatoire River Canyon on the east, limestone hills on the west, and an extruded basalt hogback ridge on the south.

Sampling Methods

Under license of the Division of Wildlife, Colorado Department of Natural Resources, we sampled for 3 days each 6 weeks, as weather permitted. Webs were established as follows: Twelve 7.6-cm x 8.9-cm x 22.9-cm noncollapsible traps (H.B. Sherman Traps, Inc., Tallahassee, Fla.) were placed on the ground at 5-m intervals for 20 m and then at 10-m intervals for 80 m, in each of 12 rows; an additional trap was placed at the central point, for a total of 145 traps in each web. Each trap's location was marked with a construction flag. Traps were baited with a mixture of cracked corn, oats, and peanut butter (6:2:1) and allowed to remain open overnight and then throughout the remainder of the trapping period. When temperatures were expected to be <5C, cosmetic balls of nonabsorbent material were placed in each trap, so that trapped animals would retain heat until they were processed. Each morning, traps were examined, and rodents were taken to a central processing area, where they were identified, weighed, and bled by inserting a capillary tube into the retroorbital plexus, before release at the capture site. We followed standard methods for sampling rodents and minimizing hazards from potentially infected animals (14). The handling and processing of rodents by these methods do not have a substantial impact on the subsequent survival or probability of recapturing most species (15-16). Each trap in which a rodent was captured was washed thoroughly with a mild solution of detergent to sterilize virus-contaminated material, then rinsed thoroughly and air-dried before being replaced in the field. This cleaning removed or diminished scent cues deposited by former inhabitants that could influence successive captures.

Data Analysis

We first tested the null hypothesis that dual captures occurred at similar proportions across all sites. The observed number of dual captures per species was compared with the expected number of dual captures, obtained by multiplying the total capture events (single plus dual captures) for each species by the proportion of total trap events for all species that yielded dual captures (the total dual captures for all species [N=43] divided by the total capture events of all species [N=3972]). Four species (*Sigmodon hispidus* = hispid cotton rat, *Tamias minimus* = least chipmunk, *Chaetodipus hispidus* = hispid pocket mouse, and *Perognathus flavus* = silky pocket mouse) with fewer than five expected dual captures but one or more observed dual captures were not evaluated further. Twelve species with no dual captures and few total captures were excluded from most analyses:147 white-throated woodrats (*Neotoma albigula*), 142 white-footed mice (*P. leucopus*), 66 Ord's kangaroo rats (*Dipodomys ordii*), 65 northern grasshopper mice (*Onychomys leucogaster*), 38 northern rock mice (*P. nasutus*), 30 Mexican woodrats (*N. mexicana*), 9 southern plains woodrats (*N. micropus*), 9 brush mice (*P. boylii*), 8 house mice (*Mus musculus*), 3 Colorado chipmunks (*T. quadrivittatus*), and 3 meadow voles (*Microtus pennsylvanicus*) (Table 1). After testing (overall chi square), the observed proportion of dual capture events was compared with the expected value, after 95% Bonferroni confidence intervals (95% CI) for observed values were obtained (17). In these analyses, an upper standard normal table value corresponding to a probability tail of á/2$\underline{k}$ ($Z_{á/2\underline{k}}$) was selected, where á = 0.05 and $\underline{k}$ = four comparison groups (deer mouse, *P. truei* = pinyon mouse, *Reithrodontomys megalotis* = western harvest mouse, and other). Where expected values fell outside the derived 95% CI, significant differences among dual captures of individual or grouped species were indicated. We followed the method of Taulman et al. (9) and Slade (18) to distinguish rodents in dual captures according to gender and age (adult

Table 1. Rodents involved in dual captures from three Colorado study sites (two western, one eastern)

	Species[a]							
	P. man	P. truei	R. meg.	S. hisp.	T. min.	C. hisp.	Pe. flav.	Other[b]
Site (trap nights)	S (D)	S (D)	S (D)	S (D)	S (D)	S (D)	S (D)	S
Fort Lewis (10440)	505 (5)	6 (0)	0 (0)	0 (0)	71 (1)	0 (0)	0 (0)	2
Molina (9135)	566 (8)	217 (0)	2 (0)	0 (0)	132 (0)	0 (0)	0 (0)	12
Piñon Canyon (24375)	829 (10[c])	561 (0)	562 (15)	259 (2)	1 (0)	23 (1)	195 (1)	520
Total	1,900 (23)	784 (0)	564 (15)	259 (2)	204 (1)	23 (1)	195 (1)	534

[a]Numbers of single (S) and dual (D) captures are listed by species at each study site. P. man = *Peromyscus maniculatus*; P. truei = *Peromyscus truei*; R. meg. = *Reithrodontomys megalotis*; S. hisp. = *Sigmodon hispidus*; T. min. = *Tamias minimus*; C. hisp. = *Chaetodipus hispidus;* Pe. flav. = *Perognathus flavus.*
[b]534 rodents from 12 other species; no dual captures.
[c]One of these pairs was adult male deer mouse and an adult male pinyon mouse.

versus juvenile, based on weight). Animals were assigned to age classes according to body weight by using the values of Fitzgerald et al. (11).

Results

Dual Captures by Site

At Fort Lewis, 594 rodents belonging to two genera and three species were trapped in 10,440 trap nights between June 1994 and October 1997 (Table 1). Most single (86.4%) trap events and dual (5/6) captures were deer mice, but 71 least chipmunks, including one dual capture, were obtained. Two juvenile female deer mice were captured as a pair on successive days; one other female deer mouse captured as one of a pair had been captured alone the previous day (Table 2). One juvenile deer mouse was captured with an adult male deer mouse and then recaptured alone 5 months later. A dual capture of seropositive adult, male deer mice was made in October 1999 (data not included in these summaries). These two mice had been captured together in April 1999 and were seropositive at that time. Neither had been recaptured between April and October.

Table 2. Sex and age association for dually captured rodents

	No. of species (recapture pairs)[b]					
Age–Sex[a]	P. man.	P. truei	R. meg.	S. hisp.	T. min	Ch. hisp.
AM/AM	3	0	3	1	0	0
AM/AF	10 (1)	0	5 (2)[c,d]	0	0	1
AM/JF	2	0	2	0	0	0
AF/JF	1	0	1	0	0	0
JM/JM	2	0	2	0	0	0
JF/JF	3	0	1 (1)	1	1	0
JM/JF	2	0	1	0	0	0

[a]A = adult (deer mouse 18 g; western harvest mice 9 g; hispid cotton rats 125 g; least chipmunks 30 g; hispid pocket mice 50 g; silky pocket mice 7 g. J = subadult or younger; M = male; F = female. Source: Fitzgerald et al., 1994 (11).
[b]For definition of species see Table l.
[c]Adult male captured with an adult female 03/11/97 and with a different adult female 04/20/97.
[d]Adult male capture with an adult female 04/19/97 and with a juvenile female 04/20/97.

At Molina, 945 rodents belonging to four genera and seven species were trapped in 9,135 trap nights between October 1994 and October 1997 (Table 1). Most single (61.3% of trap events) and all dual captures were deer mice. Two of the pairs of deer mice dually captured had been trapped individually the previous day; three of the other six deer mice dually captured also had been trapped alone previously (Table 2). No dual captures of pinyon mice were obtained, although 217 mice of this species (many of them later recaptured) were sampled.

At the Piñon Canyon site, 3,008 rodents belonging to 11 genera and 18 species were trapped in 24,375 trap nights between January 1994 and November 1997. Deer mice were the dominant rodents for single and dual captures (28.2%), although dual captures were also recorded for western harvest mice (15 dual captures), hispid cotton rats (2 dual captures), hispid pocket mice (1 dual capture), and silky pocket mice (1 dual capture). No dual captures of pinyon mice were obtained, although 561 mice were trapped individually. In addition, no dual captures were made among 534 mice of 12 other species trapped (Table 1). An adult male deer mouse and an adult female deer mouse were captured as a pair on sequential days (Table 2). Two pairs of deer mice, two pairs of western harvest mice, four western harvest mice, and five deer mice had been captured singly the previous day.

Summary of Dual Captures

In all, 43 dual captures were made in 43,950 trap nights (Table 1), and the proportion of dual captures to all capture events (N=4,506) was 0.95% including all species and 1.08% excluding mice of the 12 species with no dual captures and too few total captures to be included (N=3,972). According to the overall proportion of dual captures to total capture events, the distribution of observed dual captures by species was different from that expected by chance, based on the overall proportion of dual captures to total capture events. Deer mice accounted for 42.8% of the 4,549 rodents captured, 42.7% of total capture events (48.4% when 12 species were removed from calculations), and 53.5% of the dual captures; western harvest mice represented 13.1% of the rodents, 12.8% of total capture events (14.6%), and 34.9% of the dual captures; pinyon mice represented 17.2% of all rodents captured and 17.4% of the capture events (19.7%), but no dual captures (one pinyon mouse was captured with a deer mouse); and mice of four other species included in further analyses represented 11.7% of all rodents captured and 11.9% of capture events (Tables 1 and 2; chi square = 21.67, df = 3, P<0.001). Comparisons of expected proportions of dual captures with Bonferroni 95% CIs of observed proportions indicated that pinyon mice were captured as pairs less frequently than expected by chance, while western harvest mice were captured as pairs more frequently than expected by chance (Table 3). Dual captures for deer mice and the category comprising four other species occurred at frequencies within the predicted range. The proportion of dual captures to total capture events by species (excluding the 12 removed) and across sites were consistent for deer mice (1.0%, 1.4%, and 1.2%) and pinyon mice (none at two sites).

Species Considerations

Of 43 dual captures, 42 (97.7%) were conspecific; the sole exception was a dual capture of an adult male pinyon mouse and an adult male deer mouse. Using data from the Piñon Canyon study site, and excluding the 12 removed species, we calculated the probability of obtaining only single species dual captures, using the proportion that each species contributed to the total captures as the relative expected availability of that species for being dually captured. When this method and the binomial distribution were used, same species dual captures could be expected to comprise 0.282 of all dual captures (849 deer mice captured, 0.34 of total, probability of dual capture = 0.116; 561 pinyon mice captured, 0.26, 0.068; 592 western harvest mice, 0.24, 0.058; 486 hispid cotton rats, least chipmunks, hispid pocket mice, and silky pocket mice caught, 0.20, 0.04), yet 15 (93.8%) of 16 dual captures were of the same species (chi square = 34.3, df = 1, P<0.001).

Sex Differences

Forty-five male rodents (52.3% of the dually trapped rodents) were involved in 34 (79.1%) of 43 dual captures; 41 female rodents (47.7%) were involved in 32 (74.4%) of 43 dual captures. Nineteen (44.2%) dual captures involved the same sex (11 pairs of male, 8 pairs of female rodents). Adult male and female deer mice and western harvest mice were caught more often as single-sex pairs than could be expected by chance (deer mice: chi square = 13.61, P<0.001; western harvest mice: chi square = 5.08, P = 0.02), although male and female deer mice (chi square = 0.00, P = 0.96) and western harvest mice (chi square 0.00, P = 0.95) were equally likely to be involved in dual captures.

Table 3. Numbers of dual captures among three rodent species

	Observed		Expected		
Species	No.	%	No.	%	Bonferroni[a] 95% CI
Peromyscus maniculatus	23	53.5	20.8	48.8	34.5%<P<72.5%
P. truei	0 (1)[b]	0.0 (2.3)[b]	8.5	19.7	0.0%<P<8.0%[c]
Reithrodontomys megalotis	15	34.9	6.3	14.6	15.8%<P<54.0%[d]

[a]Differences occur between the observed and expected percentage of dual captures when the expected falls outside the 95% Bonferroni confidence interval (CI) of the observed proportion.
[b]One *P. truei* was captured with a *P. maniculatus*. Values in parentheses and those generated for expected and Bonferroni 95% confidence interval categories assume observed dual captures = 1.
[c]P<0.05 observed less than expected.
[d]P<0.05 observed greater than expected.

Age Differences

A total of 36 (83.7%) dual captures involved animals of the same approximate age (23 pairs were adults, 13 pairs were juveniles, and 7 pairs were mixed; Table 2). Thirty-three juvenile rodents were involved in 20 (46.5%; 38.4% of the 86 rodents caught as pairs) of 43 dual captures but constituted only 17.5% of all captures (chi square = 25.8, df = 1, P<0.001). Adult deer mice were more likely than subadult deer mice (chi square 6.83, P = 0.009) to be involved in dual captures, but there was no significant difference between adult and subadult western harvest mice (chi square 1.61, P = 0.20). Adult females were never dually trapped with other adult females or with juvenile males, and adult males were not trapped with juvenile males. Of the 32 dual captures of rodents between October and April, the period in which 44.6% of the total trap nights and 49.1% of the rodents were captured, 12 (37.5%) involved juvenile rodents, compared with 8 (72.7%) of 11 rodents dually captured from May to September.

On three occasions, three deer mice were caught in a single trap. The first trio included two adult females and an adult male; one of the females and the male were captured together the next night and that female was captured almost 7 months later with a third female; none had antibody to SNV. An adult male, an adult female, and a partially cannibalized juvenile comprised the second trio; the male had antibody to SNV. An adult, seropositive female, an adult seropositive male, and a seronegative juvenile female comprised the third trio.

Discussion

Our findings indicate that, at these sites and these times, dual captures of rodents were unusual but not rare and that some species were more or less likely to be captured as pairs. Also, pairs most often comprised rodents of the same species, and males were more often captured as pairs than females. Certain animals were captured as one of a pair on multiple occasions, and pairs of rodents were recaptured as pairs. In previous analyses of dual captures of rodents, Bergstrom (7) and Bergstrom and Sauer (8) suggested that multiple captures occurred as "random nonsynchronous encounters" of pairs of small mammals, rather than as social traveling. Further, these researchers suggested that such events occurred under the following conditions: interspecific multiple captures in one trap; a higher spring weight in traps that capture more than one animal compared with traps with single captures; random sex-age associations of animals captured together; no recaptures of pairs once caught together; adults captured with juveniles; and increased numbers of double captures in areas with higher population density. A design using side-by-side traps or one using entry timers might provide collateral information, but the closing of a door on a trap might also frighten away a nearby rodent.

Our results discount some of the general statements listed above. Only once did we capture a pair of rodents of different species, and the number of same-species dual captures at the Piñon Canyon site far exceeded the expected number if rodents of each species were available in proportion to their capture frequencies and behaved randomly. In addition, locally abundant species, such as pinyon mice, were never captured as same-species pairs. Therefore, our findings do not support a null hypothesis of random species mixing among dual captures in these areas.

Getz (4), studying multiply captured *Microtus pennsylvanicus* in a Wisconsin marsh, found no indication of significant antagonism between adult and immature males, at least during the declining phase of the population cycle. In addition, he was able to capture many adult female-immature male pairs. Although he recorded more than 750 instances of dual captures, and some animals were captured together as many as six times, he concluded that no formal social structure was indicated within this meadow vole population and that movement and association of individual voles within this population were random. Analyzing multiple capture data on several rodent species in a mixed desert-shrub and mesquite-grassland in northern Mexico, Petersen (5) found that most (90%) of the multiple captures were made during the breeding season, that most (75%) of the intraspecific double captures were heterosexual, and that of 12 dual captures of *R. megalotis*, all were male-female; no indication was given of the ages of the rodents. Because certain species at relatively high population densities were not captured dually, Petersen concluded that some sigmodontine rodents are more social than are heteromyid rodents. Blaustein and Rothstein (6) reported multiple captures of *R. megalotis* and

concluded that the results of their studies (frequent capture as pairs, female-male combinations most prevalent, dual captures most common during the non-breeding season) suggested that this tendency may be adaptive with respect to predator avoidance and foraging.

Only once was the specific age-sex pairing of two adult females encountered in our study areas. Other researchers have also noted that this combination did not occur or occurred rarely (9). In contrast, adult male-adult female pairings occurred more often than expected by random assortment at our sites (Table 1) and those of Getz (4), Petersen (5), and Blaustein and Rothstein (6), suggesting that dual captures are not random among rodents of different sex and age classes in these communities. Different species communities sampled in other locations may show variant patterns. For example, although Fleharty and Mares's (19) data also indicated a single-sex predominance among dual captures of hispid cotton rats, these researchers obtained heterosexual pairs in only 14 of 50 double captures. Pairs of males were obtained on 26 occasions, pairs of females on 10, and a triple capture of males was obtained once. Obviously, specific age-sex pairing may vary by season, location, and species, and no general statement can be made about social traveling among rodents.

Odors of previous trap occupants can influence subsequent captures (11). Adult male and female deer mice may prefer traps baited with the odor of conspecifics during the breeding season, but outside the breeding season unscented traps are visited preferentially (20,21). Summerlin and Wolfe (22) suggested that dominant cotton rats are more susceptible to trapping than juveniles and that avoidance of traps visited by dominant rats may bias results toward adults. In our study, all traps in which rodents were captured were washed in dilute detergent before resetting and rinsed repeatedly and sequentially in buckets of fresh water to mitigate inhibition or attraction because of residual odors. Traps usually were not used for at least 3 weeks after they were washed. Such an interval likely would further serve to abate residual odors or disinfectant and mouse scents. In this regard, it has been shown that hypochlorite decontamination of traps does not influence trapping rates of rodents (23).

Although we did not test our trap spring weights, western harvest mice were captured as pairs more often than expected (Table 2). In Colorado, this species has an adult weight of >10 g, so that two adults weighed less than, or were similar in weight to, single adults of most other species caught at lower frequency. One would expect such an increase in the proportion of dual captures of smaller species if spring weight were a limiting factor. In addition, since western harvest mice (body length ca. 60 mm) are shorter than deer mice (body length ca. 90 mm), two western harvest mice entering in tandem would more likely be inside a trap when the treadle was tripped. This observation does not, however, rule out the possibility of social traveling but neither does it provide evidence to disprove such a hypothesis. These dually captured rodents might not have been traveling together, but group foraging certainly would put them in close proximity.

When our results were compared with those of Taulman et al. (9) (for their Sherman live traps only), the latter's ratio of double captures was 0.050/100 trap nights, with an overall trap success of 5.64 captures/100 traps. Our overall ratio of dual captures was nearly double, at 0.097/100 trap nights, but our trap success was substantially higher (8.94/100 traps). These proportions suggest that our higher ratio of dual captures was almost directly proportional to our higher overall trap results. Frequency of dual captures may increase with population density or trap success, as suggested by Bergstrom (7), Bergstrom and Sauer (8), and others. However, our findings indicate that dual captures do not occur randomly across species and demographic categories and support a hypothesis of nonrandom captures among the species and areas studied.

The influence of rodent behavior or spacing on hantavirus transmission has rarely been addressed (24). If dual capture trap success is an index of group traveling or cohesiveness, a reasonable first hypothesis is that species with higher degrees of social contact, or at least no aversion to it, have higher prevalences of infection with hantaviruses transmitted through close contact. Although data are fragmentary and alternative interpretations and caveats can be offered, in several studies on hantaviruses circulating in western rodent populations during nonepidemic periods, the prevalence of antibody to SNV was highest in western harvest mice (23%; infecting agent likely El Moro Canyon

virus) and lowest in pinyon mice (3%), while deer mice maintained a middle position (11%) (25). Dual capture data from this study are intriguingly consistent with these data, suggesting that further observations on behavior and spacing among species are warranted. In our continuing studies, we will sample tissues of dually captured deer mice and determine their familial relationship using DNA microsatellites, a recently developed technique (W. C. Black IV and G. A. Kaufman, manuscript in preparation).

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References

1. Childs JE, Ksiazek TG, Spiropoulou CF, Krebs JW, Morzunov S, Maupin GO, et al. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. J Infect Dis 1994;169:1271-80.
2. Tsai TF. Hemorrhagic fever with renal syndrome: mode of transmission to humans. Lab Anim Sci 1987;37:428-30.
3. Glass GE, Childs JE, Korch GW, LeDuc JW. Association of intraspecific wounding with hantavirus infection in wild rats (*Rattus norvegicus*). Epidemiol Infect 1988;101:459-72.
4. Getz LL. Social structure and aggressive behavior in a population of *Microtus pennsylvanicus*. Journal of Mammalogy 1972;53:310-7.
5. Petersen MK. An analysis of multiple captures in several rodents from Durango, Mexico. Journal of Mammalogy 1975;56:703-5.
6. Blaustein AR, Rothstein SI. Multiple captures of *Reithrodontomys megalotis*: social bonding in a mouse? American Midland Naturalist 1978;100:376-83.
7. Bergstrom BJ. An analysis of multiple captures in *Peromyscus* sp. with a critique on methodology. Canadian Journal of Zoology 1986;64:1407-11.
8. Bergstrom BJ, Sauer JR. Social traveling inferred from multiple captures: testing assumptions. American Midland Naturalist 1986;115:201-3.
9. Taulman JF, Thill RE, Williamson JH. Double captures of small mammals in single-capture traps: random encounters or social traveling? Southwestern Naturalist 1994;39:358-63.
10. Anderson DR, Burnham KP, White GC, Otis DL. Density estimation of small-mammal populations using a trapping web and distance sampling methods. Ecology 1983;64:674-80.
11. Fitzgerald JP, Meaney CA, Armstrong DM. Mammals of Colorado. University Press of Colorado. Niwot, Colorado, 1994.
12. Baars DL. Navajo country: a geology and natural history of the Four Corners Region. Albuquerque, New Mexico: University of New Mexico Press; 1995. p.255.
13. Costello DF. Vegetation zones in Colorado. In: Harrington HD. Manual of the plants of Colorado. Chicago: Swallow Press, Inc; 1954. p. iii-x.
14. Mills JN, Childs JE, Ksiazek TG, Peters CJ, Velleca WM. Methods for trapping and sampling small mammals for virologic testing. Atlanta, Ga.: U.S. Dept. of Health and Human Services; 1995.
15. Swann DE, Kuenzi AJ, Morrison ML, DeStefano S. Effects of sampling blood on survival of small mammals. Journal of Mammalogy 1997;78:908-13.
16. Parmenter CA, Yates TL, Parmenter RR, Mills JN, Childs JE, Campbell ML, et al. Small mammal survival and trappability in mark-recapture monitoring programs for hantavirus. J Wildl Dis 1998;34:1-12.
17. Byers CR, Steinhorst RK, Krausman PR. Clarification of a technique for analysis of utilization-availability data. Journal of Wildlife Management 1984;48:1050-3.
18. Slade NA. Analysis of social structure from multiple capture data. Journal of Mammalogy 1976;57:790-5.
19. Fleharty ED, Mares MA. Habitat preference and spatial relations of *Sigmodon hispidus* on a remnant prairie in west-central Kansas. Southwestern Naturalist 1973;18:21-9.
20. Daly M, Wilson MI, Behrends P, Faux SF. Seasonally variable effects of conspecific odors upon capture of deer mice, *Peromyscus maniculatus gambelii*. Behaviorial Biology 1978;23:254-9.
21. Daly M, Wilson MI, Behrends P. Factors affecting rodent's responses to odours of strangers encountered in the field: experiments with odour baited traps. Behavioral Ecology and Sociobiology 1980;6:323-9.
22. Summerlin CT, Wolfe JL. Social influences on trap response of the cotton rat *Sigmodon hispidus*. Ecology 1973;54:1156-9.
23. Yunger JA, Randa LA. Trap decontamination using hypochlorite: effects on trappability of small mammals. Journal of Mammalogy 1999;80:1336-40.
24. Korch GW, Childs JE, Glass GE, Rossi CA, LeDuc JW. Serologic evidence of hantaviral infections within small mammal communities of Baltimore, Maryland: spatial and temporal patterns and host range. Am J Trop Med Hyg 1989;41:230-40.
25. Mills JN, Ksiazek TG, Ellis BE, Rollin PE, Nichol ST, Yates TL, et al. Patterns of association with host and habitat: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the southwestern United States. Am J Trop Med Hyg 1997;56:273-84.

The Role of Pathology in an Investigation of an Outbreak of West Nile Encephalitis in New York, 1999

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An outbreak of encephalitis occurred in New York City in late August 1999, the first caused by West Nile virus in North America. Histopathologic and immunopathologic examinations performed on human autopsy materials helped guide subsequent laboratory and epidemiologic investigations that led to identification of the etiologic agent.

In August 1999, the New York City Department of Health identified a cluster of eight patients with encephalitis, seven of whom had profound muscle weakness (1,2). These cases were initially thought to have been caused by St. Louis encephalitis (SLE) virus, a member of the genus *Flavivirus*, family Flaviviridae, on the basis of temporal, geographic, and epidemiologic similarities in the cases, clinical histories, identification of flaviviral immunoglobulin M (IgM) and IgG antibodies in serum and cerebrospinal fluid from suspected patients, and positive immunohistochemical staining for flaviviral antigens in tissue sections from the central nervous system of fatal cases.

Despite this early evidence, doubts remained as to whether SLE virus was the etiologic agent. Polymerase chain reaction (PCR) test results were negative when SLE virus-specific primers were used, and subsequent serologic testing using virus-specific plaque-reduction neutralization tests for SLE virus revealed only low-level SLE virus–neutralizing antibodies. Increased fatalities among crows and other birds, a phenomenon usually not associated with an SLE outbreak, were also being concurrently reported. Since the antibody used in immunohistochemical tests was known to cross-react with several members of the Japanese encephalitis virus serocomplex (which includes Japanese encephalitis virus, SLE virus, and West Nile virus) the initial serologic and immunohistochemical results could not exclude the possibility of another related flavivirus as the cause of the outbreak. Moreover, immunohistochemical testing of tissue from four fatal human cases indicated a high concentration of flavivirus in the brain stem and spinal cord. Subsequent reverse transcriptase-PCR tests, using several degenerate primer sets designed to detect conserved regions within a wide variety of flaviviruses, together with West Nile virus-specific primer sets identified West Nile virus as the etiologic agent of both the human outbreak and the equine and avian epizootics (3-5). This was the first reported outbreak of encephalitis caused by West Nile virus in North America.

Histopathologic examination of four fatal human cases showed varying degrees of neuronal necrosis in the gray matter, with infiltrates of microglia and polymorphonuclear leukocytes, perivascular cuffing, neuronal degeneration, and neuronophagia (Figure 1). Immunohistochemical staining demonstrated viral antigens in neurons, neuronal processes, and areas of necrosis (Figure 2). No immunostaining was seen in other major organs, including lung, liver, spleen, and

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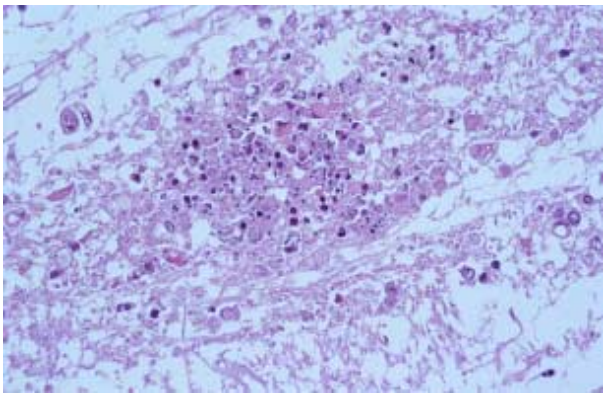

Figure 1. Neuronal necrosis with infiltrates of microglia and polymorphonuclear leukocytes. Hematoxylin-eosin staining. Original magnification, X100.

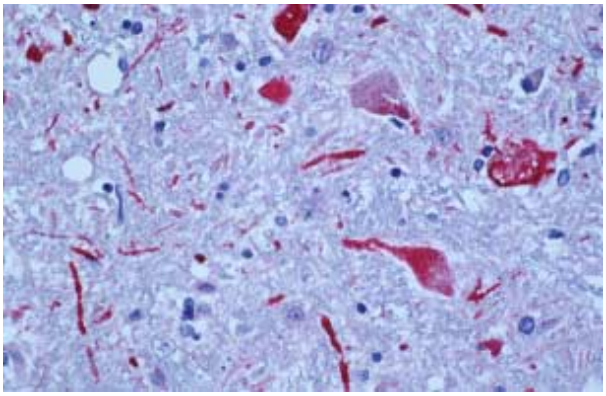

Figure 2. Positive staining of viral antigens in neurons and neuronal processes. Immunoalkaline phosphate staining, naphthol fast red substrate with light hematoxylin counterstain. Original magnification, X100.

kidney. The histopathologic lesions and immunostaining were more prominent in the brain stem and spinal cord, which may explain the clinical manifestation of muscle weakness in some patients.

These pathologic studies were critical to the eventual discovery of West Nile virus for several reasons. First, the initial examination of human autopsy specimens suggested a correlation between the histopathologic features of fatal cases and cases with serologic and immunohistochemical evidence of flavivirus infection. This correlation gave researchers increased confidence in the diagnosis in the early stage of the investigation. Second, infections of the central nervous system have important clinical significance because they can cause neurologic damage and death. Clinical manifestations, epidemiologic information, and patient history—while frequently suggestive of a diagnosis—are often unreliable for determining the specific cause of a central nervous system infection. A specific etiologic diagnosis is particularly important in this era of ever-changing environmental and ecological factors and increased awareness of atypical disease presentations. Localization of West Nile virus antigens by immunohistochemical analysis, in conjunction with classic histopathologic examination, is a prime example of how new pathologic techniques are being used in investigations of outbreaks caused by emerging infections (6). Third, the topographic distribution of histopathologic lesions and flaviviral antigens in the brain stem and spinal cord guide subsequent PCR testing. Such information also helps explain the clinical manifestations of illness and suggests that virus spread through neuronal processes plays an important role in the pathogenesis of human West Nile virus infection.

Autopsy rates are decreasing in the United States and worldwide (7). This decline has been influenced by escalating costs, jurisdictional and authorization uncertainties, and undue emphasis on antemortem diagnostic technologies. In this outbreak, autopsies were performed under the jurisdiction of New York City's Office of Chief Medical Examiner because of the obvious public health implications (8). Other notable recent examples in which autopsy contributed to the discovery or better understanding of emerging infectious diseases include investigations of hantavirus pulmonary syndrome, Ebola hemorrhagic fever, leptospirosis associated with pulmonary hemorrhage, new variant Creutzfeldt-Jakob disease, and Nipah virus encephalitis (9). These examples underscore the need to increase autopsy rates to detect infectious diseases (10,11).

Although it is not known when and how West Nile virus was introduced into North America, possible mechanisms include international travel of infected persons to New York City or transport of virus by mosquitoes, ticks, or imported infected birds. Many factors are associated with the geographic spread and emergence and distribution of zoonotic pathogens, including demographic and societal changes, modern methods of transportation, changing geoclimatic conditions, migration of animals and birds, and alteration of arthropod reservoirs and vectors. Collaboration

among scientists from diverse fields—such as epidemiology, clinical and veterinary medicine, entomology, microbiology, and pathology—will advance the recognition of emerging zoonotic infections (9,11,12). The investigation of this outbreak of West Nile encephalitis demonstrates the value of such a multidisciplinary approach.

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References

1. Centers for Disease Control and Prevention. Outbreak of West Nile-like viral encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:845-9.
2. Asnis DS, Conetta R, Teixeira AA, Waldman G, Sampson BA. The West Nile virus outbreak of 1999 in New York: the Flushing Hospital experience. Clin Infect Dis 2000;30:413-8.
3. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7.
4. Briese T, Jia XY, Huang C, Grady LJ, Lipkin WI. Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis. Lancet 1999;354:1261-2.
5. Centers for Disease Control and Prevention. Update: West Nile-like viral encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:944-6, 955.
6. Shieh W-J, Guarner J, Greer P, Morken T, Bartlett J, Ferebee T, et al. Histopathologic and immunohistochemical studies for acute viral encephalomyelitis in formalin-fixed human tissues. Lab Invest 1999;79:153A.
7. Hanzlick R, Baker P. Case of the month: institutional autopsy rates. Arch Intern Med 1998;158:1171-2.
8. Sampson BA, Ambrosi C, Charlot A, Reiber K, Veress JF, Armbrustmacher V. The pathology of human West Nile virus infection. Hum Pathol 2000;31:527-31.
9. Zaki SR, Paddock CD. The emerging role of pathology in infectious diseases. Emerging Infections 3. In: Scheld WM, Craig WA, Hughes JM, editors. Washington: American Society for Microbiology Press; 1999. p.181-200.
10. Schwartz DA. Emerging and reemerging infections. Progress and challenges in the subspecialty of infectious disease pathology. Arch Pathol Lab Med 1997:121:776-84.
11. Nolte KB, Simpson GL, Parrish RG. Emerging infectious agents and the forensic pathologist: the New Mexico model. Arch Pathol Lab Med 1996;120:125-8.
12. Brown C. Emerging diseases—what veterinarians need to know. J Vet Diagn Invest 1997;9:113-7.

Isolation of Two Strains of West Nile Virus during an Outbreak in Southern Russia, 1999

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From July to September 1999, a widespread outbreak of meningoencephalitis associated with West Nile virus (Flavivirus, Flaviviridae) occurred in southern Russia, with hundreds of cases and dozens of deaths. Two strains of West Nile virus isolated from patient serum and brain-tissue samples reacted in hemagglutination-inhibition and neutralization tests with patients' convalescent-phase sera and immune ascites fluid from other strains of West Nile virus.

From July to September 1999, a widespread outbreak of meningoencephalitis occurred in southern Russia (Volgograd, Astrakhan, and Krasnodar regions). Approximately 1,000 cases with at least 40 deaths were reported. Natural foci of arbovirus infections have been reported in southern Russia (1-5). Clinical and epidemiologic investigations indicated that this outbreak could be associated with West Nile virus; preliminary serologic testing of patient samples confirmed the presence of the virus (6). We report further virologic testing of patient isolates from this outbreak.

The Study

For virus isolation, we tested serum samples from 25 patients on days 4 to 6 of febrile illness, 18 samples of cerebrospinal fluid, and brain tissue samples taken from 5 patients at autopsy. The tissue and cerebrospinal fluid were analyzed for evidence of West Nile virus genome by reverse transcription-polymerase chain reaction (RT-PCR) primers on the basis of published NS5 and E genes (7,8). Virus was isolated by infection of 3- to 4-day-old suckling mice. Mice were injected intracranially with 0.01 mL of patient tissue, and blind passages were made on days 6 to 7 after inoculation. The suspension of brain tissue from previously injected, asymptomatic mice was inoculated intracranially into new mice. When mice began to show signs of illness, the brain tissue was examined for West Nile virus by hemagglutination (HT) and hemagglutination inhibition tests (HIT). A 10% suspension was prepared in 0.15 M NaCl and diluted fivefold with borate buffer solution to suppress nonspecific inhibitors. The suspension was then titrated at pH 6.4 with goose erythrocytes (9). Identification of the virus antigen in brain suspension of infected mice was also done by enzyme-linked immunosorbent assay (ELISA) with the direct sandwich method (9).

Immune ascitic fluids (IAF) of mice and convalescent-phase sera of patients in the current outbreak were used for identification of these strains and viruses by HIT, neutralization, and ELISA testing. Neutralization testing (NT) was done by the micro method in pig kidney cells with a single dilution of IAF in 10-fold dilutions of virus (Table 1). The results were assessed

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Table 1. Neutralization index of strain LEIV 27889 Vlg in neutralization test with immune ascitic fluid

Strain	Neutralization index
WNF 2266Ig (India)	6.5
WNF 22886G (India)	8.5
WNF Eg 101	8.5
Kokobera	6.0
Karshi	1.0
Apoi	3.0
Usutu	3.0
JE	3.5
Tyuleniy	2.0
St. Louis	0.5
TBE	0.5

according to a neutralization index calculated by the Reed and Mench method (9).

We examined brain tissue from five patients (63, 67, 71, 72, and 16 years of age) in the Volgograd region who died of meningoencephalitis. Flaviviruses and West Nile virus RNA were detected in all five samples by RT-PCR; however, virus was isolated only from the 16-year-old patient. In this case, suckling mice injected with brain tissue became ill on days 4 to 6. This incubation period decreased to 3 to 5 days on the second passage and to 3 days after subsequent passages. On the second passage, we detected hemagglutinins in mouse brain suspension of this virus at a titer of 1:128 at pH 6.4. The HIT for this isolate was inhibited by IAF to West Nile virus. Antigen of West Nile virus was also identified from mouse brain suspension by ELISA at titers of 1:80 to 1:160. This isolate was designated LEIV 22889 Vlg. All 18 samples of cerebrospinal fluid from patients were negative by RT-PCR and virus isolation in suckling mice.

Serum samples from 25 patients from the Astrakhan region were tested for virus isolation. Virus strain AST 986 was isolated in serum of one patient on days 7 to 8 after inoculation into suckling mice. The incubation period after the third passage was reduced to 3 days. Hemagglutinating antigen was identified in brain suspension of the mice on the second passage at titer 1:640, reciprocally with IAF of West Nile virus (Table 2).

Both strains LEIV 27889 Vlg and Ast 986 were reactive in HIT (Table 2). Antigens LEIV 27889 Vlg and strain LEIV Az-1640 of West Nile virus reacted in similar titers with IAF of all flaviviruses studied except yellow fever. When the strain LEIV 27889 Vlg was tested by NT (Table 1), virus was neutralized with IAF to all strains of West Nile and Kokobera viruses (Index of Neutralization 6.0-8.5). The identification of strains LEIV 27889 Vlg and Ast 986 was confirmed by HIT with convalescent-phase sera.

Table 2. Identification of the strains LEIV 27889 Vlg and Ast 986 by hemagglutination inhibition test with immune ascitic fluid and antigens of flaviviruses

	Viral antigens[a]							
	27889	AST	LEIV	LEIV	LEIV		YF	St.
IAF of viruses	Vlg	986	Az-1640	Az-72	Az-1628	JE	(Dakar)	Louis
LEIV Az-1640	320[b]	nt	1280	160	nt	nt	nt	nt
LEIV Az-72	80	nt	160	160	nt	nt	nt	nt
LEIV Az-1628	nt	640	nt	nt	1280	nt	640	nt
Japanese encephalitis	160	320	320	160	160	640	nt	nt
Kokobera	160	nt	320	160	nt	nt	nt	nt
St. Louis	160	160	320	160	80	0	0	160
Usutu	160	nt	160	320	nt	nt	nt	nt
Apoi	320	nt	320	160	nt	nt	nt	nt
Karshi	8	nt	80	80	nt	nt	nt	nt
Tyuleniy	160	nt	320	160	nt	nt	nt	nt
Kama	160	nt	160	160	nt	nt	nt	nt
TBE	20	nt	20	20	nt	nt	nt	nt
Yellow Fever (Dakar)	0	nt	nt	nt	0	nt	640	nt

nt = not tested; IAF = immune ascitic fluid.

[a]Isolates were identified by comparative testing with the following strains of West Nile virus: LEIV Az1640, Azerbaijan, 1967, from *Sitta europea* birds; LEIV Az1628, Azerbaijan, 1967, from *Turdus merula* birds; LEIV Az72, Azerbaijan, 1970, from *Ornithodorus capensis* ticks; 2269 Ig, Madras, 1956, from *Culex vishnui* mosquitoes; in Eg 101, 1951, from the serum of an Egyptian pediatric patient; and other flaviviruses: Japanese encephalitis (JE), St. Louis encephalitis (SLE), Yellow fever-Dakar (YF), tick-borne encephalitis (TBE), Kokobera (KOK), Usutu (USU), Apoi, Karshi (KSI), Kama, and Tyuleniy (TYU).

[b]quantity inverse IAF dilution.

Conclusions

According to virologic and serologic data from the Center of Ecology of Viruses, D.I. Ivanovsky Institute of Virology, and collaborating laboratories, the West Nile virus-endemic area in the former Soviet Union includes Moldavia, Ukraine, Bielorussia, the southern area of European Russia (regions of desert, steppe, and deciduous forests) and western Siberia-Altai territory (steppe and combined forest-steppe), Armenia, Azerbaijan, Kazakhstan, Tajikistan, Uzbekistan, and Turkmenia. For the last 20 years, illness has been observed in Kazakhstan and the republics of Central Asia, Astrakhan region (in Russia), Ukraine, and Azerbaijan (1). High risk for exposure to West Nile virus has been observed in the desert territories of the Volga basin, especially in the river valleys, where an outbreak occurred in 1999.

The ornithophilic mosquito species *Culex modestus* is of great importance for circulation of West Nile virus in natural foci of bird colonies in the Volga Delta and in populated areas. Both *Culex p. pipiens* and *C. p. molestus* feed on wild, sylvan, and domestic birds, as well as humans. In the Volga Delta, 56 species of birds are involved in virus circulation. In the coastal area of the delta, the most important hosts are shore birds, especially the Gressores order: the green heron (*Nicticorax nicticorax,* 45% of which had antibodies), great cormorant (*Phalacrocorax carbo*), coot (*Fulica atra*), waterhen (*Gallinula chloropus*), and great grebe (*Podiceps cristatus*), and to a lesser extent gulls and terns (10). In the agricultural region of the Volga Delta, 20 species of birds (particularly rooks, crows, and pigeons) are involved in virus circulation (11). Less virus circulation is seen in other areas of the delta, in semidesert region of Astrakhan, and Kalmykia. In the Kuban and Terek River deltas, the most important birds are herons, coots, and some species of ducks.

In light of these data, the occurrence of West Nile virus outbreaks is not surprising. However, the high death rates and a wide range of infected populations are unusual and probably result from factors such as high temperature, extended breeding places of mosquitoes, migration of some groups of populations, and perhaps change in the virus genotype. The ecology of West Nile virus in southern Russia is similar to that in northeastern Romania, in the Danube Delta (12,13). A different ecologic situation was observed during the West Nile outbreak in New York in 1999 (8,14-17). The virus may have been introduced to the American continent by infected mosquitoes (eggs or larvae) from disease-endemic areas in Africa, Asia, or Europe by ships or airplanes. The urban subspecies *C. p. molestus*, which can reproduce without bloodsucking, may have introduced the virus; these ornithophilic mosquitoes become infected when they bite infected birds. The high susceptibility of these mosquitoes to Karshi virus, which closely resembles West Nile virus, was confirmed experimentally (18). The results of genome sequencing of strains isolated in the last epidemic and other West Nile strains previously isolated in the former Soviet Union will be described in the future.

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References

1. Lvov DK, Klimenko SM, Gaidamovich SY. [Arboviruses and arboviral infections.] Moscow: Meditsina; 1989. p. 1-333. (In Russian)
2. Lvov DK. Ecological soundings of the former USSR territory for natural foci of arboviruses. In: Lvov DK, editor. Arboviruses. Sov Med Rev E: Virology. New York: Harwood Inc.; 1993. p. 1-47.
3. Lvov DK. Arboviral zoonoses of Northern Eurasia (Eastern Europe and the Commonwealth of Independent States). In: Beran GW, Steel JH, editors. Handbook of zoonoses. 2nd ed. Boca Raton: CRC Press; 1994. p.237-60.
4. Lvov DK, Deryabin PG, Myasnenko AM, Skvortsova TM, Aristova VA, Butenko AM, et al. Atlas of natural foci of virus infections in the Russian Federation, Federal Department of Medico-Biological and External Problems at Ministry of Public Health of RF. Moscow: D.I. Ivanovsky Institute of Virology RAMS; 1995, p. 1-187.
5. Lvov DK. West Nile Fever (Survey). Voprosi of Virology 2000;45:4-9. (In Russian).
6. Lvov DK, Butenko AM, Gaidamovich SY, Larichev VPh, Leschinskaya EV, Lozarenko VV, et al. Epidemic outbreak of meningitis and meningoencephalitis in Krasnodar territory and Volgograd region provoked by West Nile fever virus. Vopr. Virusol 2000;45:37-8. (In Russian).
7. Savage HM, Ceianu C, Nicolescu G, Karabatsos N, Lanciotti R, Vladimirescu A, et al. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996 with serologic and molecular characterization of a virus isolate from mosquitoes. Am J Trop Med Hyg 1999;61:600-11.

8. Briese T, Jia XY, Huang C, Grud LJ, Lipkin WI. Identification of Kunjin West Nile-like flavivirus in brains of patients with New York encephalitis. Lancet 1999;354: 1261-2.
9. Clarke DH, Casals J. Techniques for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. Am J Trop Med Hyg 1958;7:561-73.
10. Berezin VV, Semenov BF, Reschetnikov IA, Baschkirtsev VN. Importance of birds in the natural cycle of arboviruses transmitted by mosquitoes in the Volga delta. In: Transcontinental connections of migrating birds and their role in arbovirus distribution. Novosibirsk: Nauka; 1972. p.310-3. (In Russian).
11. Butenko AM, Chumakov MP, Bashkirtsrev VN. Isolation of West Nile virus in the Astrakhan region from mosquitoes and crows. In: Aetiology, epidemiology and clinics of CHF and West Nile fever, Astrakhan. Astrakhan: Institute of Poliomyecitis and Viral Encephalitis, Astrakhan Sanitary-Epidemiological Station;1969. p.39-40. (In Russian).
12. Yarovoj PI. Antibodies to some flaviviruses in sera of habitants of north-Moldavian forest-steppe landscape. In: Gaidamovich SY, editor. Arboviruses. Moscow: D.I. Ivanovsky Institute of Virology; 1981. p.120-1. (In Russian).
13. Vinograd IA, Beletskaya GV, Chumachenko SS, Omelchenko GA, Lozinsky IN, Yartus DS, et al. Ecological aspects of arboviruses in the Ukraine SSR. In: Vinograd IA, Beletskaya GV, Chumachenko SS, Omelchenko GA, Lozinsky IN, Yartus DS, et al. Ecology of viruses and foci of arbovirus infections. Moscow: D.I. Ivanovsky Institute of Virology; 1989. p. 21-7. (In Russian).
14. Anderson JF, Andreadis TY, Voshbrinck CR, Tirrell S, Wakem EM, French RA, et al. Isolation of West Nile virus from mosquitoes, crows and a Cooper's hawk in Connecticut. Science 1999;286:2331-3.
15. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the Northern United States. Science 1999;286:2333-7.
16. Centers for Disease Control and Prevention. Update: West Nile virus encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:890-2.
17. Centers for Disease Control and Prevention. Update: West Nile virus encephalitis—New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:944-55.
18. Khutoretskaya NV, Aristova VA, Rogovaya SG, Lvov DK, Karimov SK, Skvortsova TM. Experimental study of reproduction of Karshi virus (Flaviviridae, Flavivirus) in some species of mosquitoes and ticks. Acta Virol 1985;29:231-6.

Subacute Sclerosing Panencephalitis, a Measles Complication, in an Internationally Adopted Child

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A healthy 13-year-old boy who had spent the first 4.5 years of his life in an orphanage in Thailand before adoption by an American couple became ill with subacute sclerosing panencephalitis and died several months later. The boy had most likely contracted wild-type measles in Thailand. Measles complications are a risk in international adoptions.

Undiagnosed infections in internationally adopted children have been receiving increasing attention throughout the past decade. HIV, hepatitis viruses, *Treponema pallidum*, *Mycobacterium tuberculosis*, and intestinal parasites frequently complicate such adoptions (1,2). Subacute sclerosing panencephalitis (SSPE), a postinfectious neurologic complication of measles, can also occur. We describe a fatal case of SSPE in an internationally adopted child 9 years after he arrived in the United States.

Case Report

A 13-year-old boy of Thai descent was referred to the pediatric neurology clinic at the University of Iowa Hospital with cognitive difficulties and a progressive movement disorder. The boy was born in 1984 and spent the first 4.5 years of his life in an orphanage in Thailand before being adopted by an American couple from Dubuque, Iowa. His adoptive parents were told by the adoption agency that the boy's medical history was unremarkable. No history of measles was reported. At adoption, the child appeared healthy and well nourished, and at no time afterwards did he have an illness suggestive of measles. Shortly after arrival, he displayed a short attention span and easy distractibility, for which he was eventually diagnosed with attention deficit hyperactivity disorder. He was treated with low-dose methylphenidate for several years with good results.

The child remained healthy throughout childhood until the age of 13 years 2 months, when his mother noted personality changes of irritability and worsened attention. Several months later, he developed intermittent, random, low-amplitude, lightning-like jerking movements of the extremities. The abnormal movements (thought to be tics) improved moderately, but transiently, after the methylphenidate was discontinued.

During the next several months, the boy became increasingly withdrawn and emotionally labile. He was treated for depression, but fluoxetine induced a marked worsening of the movement disorder and was discontinued. He was next treated with valproic acid, again with worsening in the movement disorder and no improvement in the psychiatric symptoms. Although the boy's academic performance had previously been average, he began to fail academically. He lost previous mathematics and language skills, and his teachers and parents noted progressive memory deficits. The movement disorder evolved from random myoclonic jerks of all four extremities to drop attacks many times a day, during which, while walking or standing, he would suddenly fall to the floor.

On examination at 13 years 9 months, the boy appeared healthy. He was alert and cooperative, but produced little spontaneous or prompted speech. He followed simple verbal commands, but had difficulty with more complex ones and appeared confused by simple written commands;

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his adoptive mother indicated that at earlier times, he could have easily understood and followed such commands. Cranial nerve examination was notable for saccadic pursuit movements of gaze, hypometric saccades, and mild facial diplegia. Motor examination was notable for cogwheeling in the upper extremities bilaterally, especially on pronation-supination. The gait was remarkable for diminished bilateral arm swing. The posture and stance were remarkable for intermittent shocklike dipping of the head and shoulders with no apparent change in level of consciousness or postictal state.

Results of magnetic resonance imaging (MRI) (Figure 1) were focally abnormal with a single patch of increased T2 signal intensity and decreased T1 signal intensity in the subcortical white matter of the left frontal lobe. This focal lesion did not enhance with gadolinium. Results of an electroencephalogram (EEG) revealed high-amplitude bursts of periodic slow-wave complexes every 4–10 seconds, often accompanied by observable axial myoclonic spasms. The periodic slow-wave complexes arose from background activity that was essentially normal, except for some mild bifrontal dominant slowing (Figure 2). Cerebrospinal fluid (CSF) cytology, glucose, and total protein levels (15 mg/dL) were normal, but CSF immunoglobulin G (IgG) was elevated at 16.3 mg/dL (normal, 0.5–5.9 mg/dL). Measurement of specific antibodies by enzyme-linked immunosorbent assay revealed that rubeola (measles) IgG antibodies were markedly elevated in the CSF at 1:160 (normal, <1:5) and in the serum at 1:5120. Rubeola IgM antibody titers were undetectable in both CSF and serum. Both the EEG and CSF patterns were pathognomonic for SSPE and that diagnosis was made.

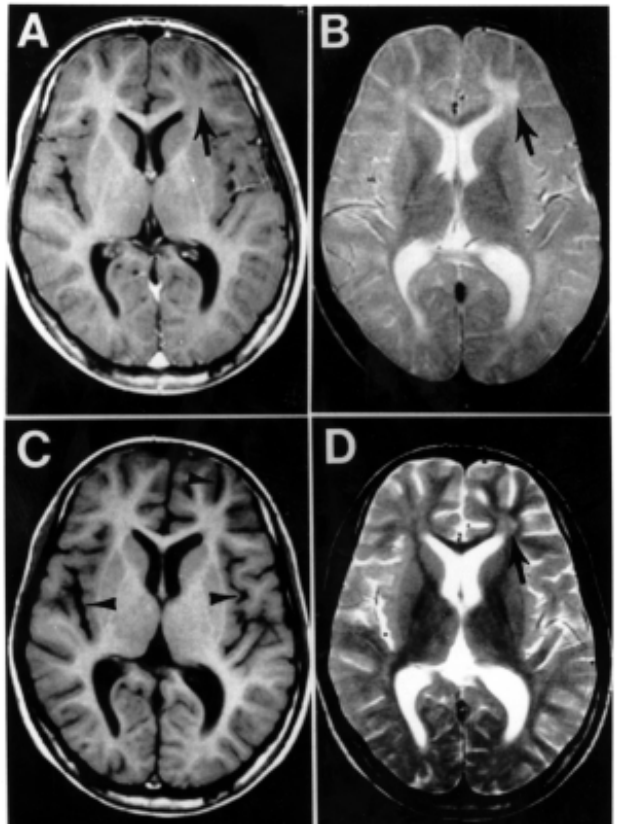

Figure 1. MRI scans of brain at time of presentation in the neurology clinic (A and B) and 3 months later (C and D). Panels A and C are T1-weighted images; B and D are T2-weighted images. The initial MRI scan (A and B) reveals a focal abnormality in the subcortical white matter of the left frontal lobe, consisting of a hypointense signal on the T1-weighted image (arrow in A) and a hyperintense signal on the T2-weighted image (arrow in B). In the follow-up scan, the focal abnormality in the left frontal lobe is less obvious than previously (arrow in D), but advanced and diffuse cortical atrophy is present, signified by the ventriculomegaly and markedly enlarged sulci (arrowheads in C).

The patient was placed on phenytoin, and the frequency of the drop attacks abated. Three months later, his neurologic status deteriorated rapidly, and he became obtunded. Repeat EEG again revealed high-amplitude, slow-wave complexes, but this time they arose from a diffusely and markedly slow background rhythm (Figure 2). Repeat MRI was most notable for advanced diffuse cortical atrophy that had not been present on the initial study. The focal abnormality in the subcortical white matter of the left frontal lobe was still detectable, but was less striking than initially (Figure 1). The patient died in June 1998, one week after the onset of acute deterioration. Permission for a postmortem study was denied.

Conclusions

SSPE is a neurodegenerative disease caused by persistent infection of the brain by an altered form of the measles virus. Neither the biology underlying the viral persistence nor the triggering mechanism for viral reactivation is well understood. In most cases, infected children remain symptom-free for 6-15 years after acute measles infection (3).

Several factors suggest that this patient contracted measles in Thailand. First, the most consistent risk factor for SSPE is acquiring measles before the second birthday (4); this child

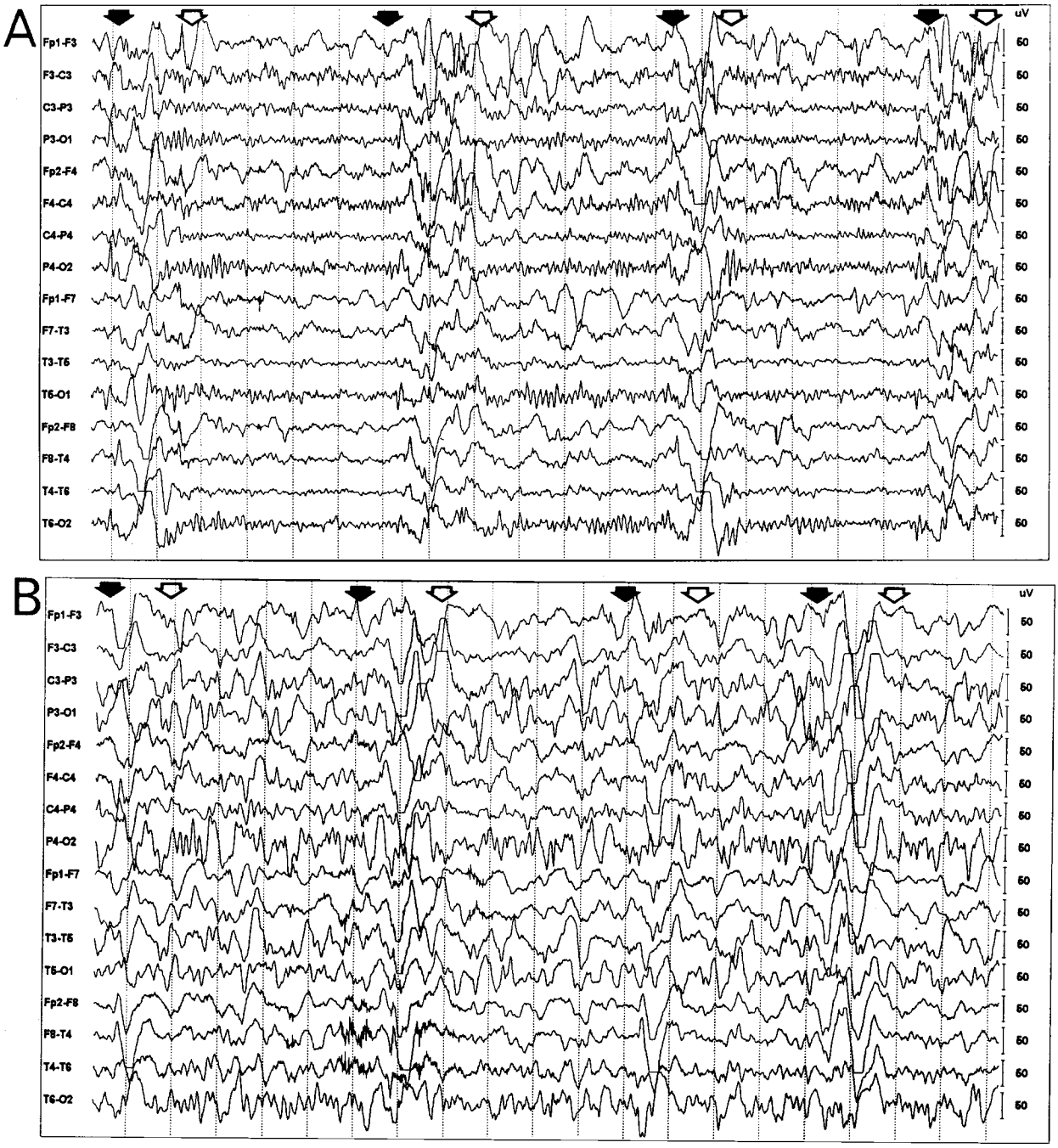

Figure 2. Electroencephalogram (EEG) at the time of presentation in the neurology clinic (A) and 3 months later (B). The initial EEG (A) reveals periodic bursts of high-amplitude, slow-wave complexes. (Onset of the complexes is indicated by solid arrows; offset, by open arrows.) The background rhythm is normal, except for bifrontal slowing. This "burst-suppression" pattern is highly characteristic of subacute sclerosing panencephalitis (4). EEG 3 months later, when the patient's clinical status has worsened (B), again shows periodic high-amplitude slow waves (again, between the solid and open arrows), but they now arise from a diffusely slowed background rhythm, which nearly obscures the periodic slow waves. In both A and B, the interval between each vertical dotted line is one second.

was still in Thailand at that age. Second, the incidence of SSPE in Asia is substantially higher than in North America; for example, the incidence is 2 per 100,000 population in India and 10 per 100,000 population in Pakistan, but only 1 per million population in the United States (4). Certainly, measles was endemic in Thailand when this patient lived there. Third, the number of cases of measles in Iowa during the nationwide outbreak in 1989–91 was relatively low; for example, there were no admissions for measles to the University of Iowa pediatrics ward during that period (unpublished data of authors). Finally, neither the parents nor the pediatrician noted any disease symptoms compatible with measles after the 4-year-old arrived in Iowa.

The initial symptoms of SSPE usually involve regressive changes in intellect and personality. Within several months, the psychological symptoms are compounded by neurologic ones, most often consisting of myoclonic jerks. A relentless mental and motor deterioration then ensues, culminating in extreme neurologic dysfunction and death within several years of the onset of symptoms (5). Our patient's clinical course reflected this typical natural history.

SSPE is accompanied by a unique set of laboratory abnormalities that facilitate its diagnosis. The persistent measles encephalitis induces a robust humoral immune response (6). Therefore, CSF in SSPE will typically have normal cellular components, glucose and total protein, but markedly elevated values of gammaglobulin (hyperglobulinorrachia greater than 20% of the total protein), and anti-measles antibodies (5,7). Typically, serum anti-measles antibody titers are also grossly elevated. In nearly all cases, EEG reveals a "burst-suppression" pattern at some point in the course of SSPE. This is one of two conditions in which a burst-suppression pattern is observed in a noncomatose patient. (The other condition is Creutzfeldt-Jakob disease.) The bursts of abnormal sharp and slow waves typically arise out of a normal background EEG activity early in the course of SSPE, but this background activity deteriorates to diffuse slow waves as the disease progresses (8). All of these characteristic immunologic and electrophysiologic abnormalities were observed in our patient. Because MRI came into clinical use after SSPE became rare in industrialized countries as a result of widespread measles vaccinations, few reports have been published on MRI findings in SSPE (9-11). The MRI profile of SSPE includes focal abnormalities in the subcortical white matter early in the course of disease and diffuse cerebral atrophy at later stages of the disease, as observed in our patient.

Further cases of SSPE are likely to occur among internationally adopted children. An increasing proportion of such children have spent their early childhood years institutionalized in crowded orphanages of Eastern Europe, Russia, and Asia, where conditions are fertile for outbreaks of measles and immunizations are often nonprotective (1-2). The incidence of SSPE among nonimmunized children is 100-200 times higher than among those who have been immunized effectively. (A recently reported case of SSPE in the United States involved an unimmunized child of Cambodian descent who contracted measles at the age of 1 year during the last outbreak in California in 1989 [12]. This child, who became ill with SSPE at age 9 years, had never traveled outside the United States and was not adopted.)

Because of the current widespread administration of measles vaccine in the United States, the incidence of acute measles infection has been dramatically reduced (13) and SSPE has been virtually eliminated and forgotten. However, measles, with the associated risk for SSPE in late childhood (14, 15), remains a recurrent public health hazard in developing nations.

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References

1. Hostetter MK. Infectious diseases in internationally adopted children: the past five years. Pediatr Infect Dis 1998; 17: 517-8.
2. Hostetter MK. Infectious diseases in internationally adopted children: Findings in children from China, Russia, and Eastern Europe. Adv Pediatr Infect Dis 1999; 14: 147-61.
3. Gascon GG. Subacute sclerosing panencephalitis. Semin Pediatr Neurol 1996; 3:260-9.
4. Britt WJ. Slow viruses. In: Feigin R, Cherry J, editors. Textbook of pediatric infectious diseases. 4th ed. Philadelphia: W.B. Saunders Company, 1998; p. 646-65.
5. Dyken PR. Subacute sclerosing panencephalitis: current status. Neurol Clin 1985; 3:179-96.
6. Norrby E, Kristensson K. Measles virus in the brain. Brain Res Bull 1997; 44:213-20.

7. PeBenito R, Naqvi SH, Arca MM, Schubert R. Fulminating subacute sclerosing panencephalitis: Case report and literature review. Clin Pediatr 1997; 36:149-54.
8. Crowell J. Clinical neurophysiology: Video EEG studies. Symposium to ICNA/CNS Subacute Sclerosing Panencephalitis meeting: Update. San Francisco, CA, Oct 1, 1994.
9. Winer JB, Pires M. Kernode A, Ginsberg L, Rosser M. Resolving MRI abnormalities with progression of SSPE. Neuroradiology 1991; 33:178-80.
10. Anlar B, Saatci I, Kose G, Yalaz K. MRI findings in subacute sclerosing panencephalitis. Neurology 1996; 47:1278-83.
11. Geller TJ, Vern BA, Sarwar M. Focal MRI findings in early SSPE. Pediatr Neurol 1987; 3:310-12
12. Park SY. Subacute sclerosing panencephalitis in an identical twin. Pediatrics 1999; 104:1390-4.
13. Notifiable diseases/deaths in selected cities. MMWR Morb Mortal Wkly Rep 2000; 48:1183-90.
14. Assaad F. Measles: Summary of worldwide impact. Rev Infect Dis 1983; 5:452-9.
15. Moodley M. Subacute sclerosing panencephalitis in the developing world. S Afr Med J 1992; 82:72-3.

Bacteremia and Endocarditis Caused by a *Gordonia* Species in a Patient with a Central Venous Catheter

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We report the first case of endocarditis caused by a *Gordonia* species genetically related to *G. sputi* but exhibiting some atypical biochemical features in a 31-year-old woman with a central venous catheter. This unusual pathogen may be a new cause of opportunistic infections in patients with severe underlying diseases.

Gordonia spp. are a gram-positive coryneform bacteria, recently identified in three patients as a cause of systemic infections (1,2), including two associated with indwelling implantable subcutaneous central venous catheters (2). We report *Gordonia* spp.'s capacity to infect a patient's undamaged cardiac valve through an implantable subcutaneous central catheter.

Case Report

The patient was a 31-year-old woman, who had undergone splenectomy at age 9 years, for severe double heterozygous hemoglobinopathy (beta-thalassemia and hemoglobin E disease). Multiple blood transfusions at that time were complicated by hepatitis C with cirrhosis and secondary hemochromatosis, treated at home with twice weekly deferoxamine by subcutaneous central venous catheter. Hemochromatosis was complicated by diabetes, adrenal insufficiency, and peripheral neuropathy. In September 1997, the patient became ill with *Staphylococcus aureus* bacteremia associated with localized renal and cutaneous abscesses; transesophageal echocardiography showed neither valvular vegetation nor a valvular defect suggestive of endocarditis. The bacteremia was successfully treated with intravenous fosfomycin, cefotaxime and netilmicin, followed by ciprofloxacin and oxacillin for 8 weeks. The subcutaneous central venous port was also changed after 6 weeks of treatment.

The patient remained afebrile until December 1998, when fever and chills developed. Physical examination 1 week after onset of symptoms revealed a temperature of 39°C and a new mitral systolic murmur. The site of the subcutaneous central venous port showed no signs of infection. Results of clinical laboratory tests showed a leukocyte count of 31.4×10^9/L, an erythrocyte sedimentation rate of 67 mm/h, and a C-reactive protein plasma level <5 mg/L. Transthoracic echocardiography revealed a mitral valvular vegetation (10x5 mm). Two blood cultures (one obtained from the central venous catheter) were performed at admission. One more peripheral blood sample was drawn for culture 12 hours later. All three cultures were positive for a gram-positive coryneform bacterium showing no extensive branching. This organism produced dry, raised, salmon-to-orange colonies without aerial hyphae. As the organism was weakly acid fast, according to the Kinyoun acid-fast stain modified for aerobic actinomycetes, we presumptively identified this organism as *Rhodococcus* sp. sensu lato. According to disk diffusion, the organism was susceptible to

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penicillin G, amoxicillin, cefotaxime, ceftriaxone, imipenem, gentamicin, netilmicin, ciprofloxacin, vancomycin, and erythromycin but was resistant to fosfomycin, ceftazidime, trimethoprim-sulfamethoxazole, and streptogramin. By E-test, the MICs of penicillin G, amoxicillin, and ceftriaxone were 0.047, 0.064 and 0.25 µg/mL, respectively. The patient was successfully treated with intravenous amoxicillin (3 g, 4/day) and intravenous netilmicin (150 mg, 2/day) for 1 week, then with intravenous amoxicillin alone for 3 weeks. This treatment was followed by home treatment with 2 g perfusion of ceftriaxone for 2 weeks. The central venous catheter was left in situ. At 1 year follow-up, the patient was not infected with *Gordonia* sp., and echocardiographic findings were consistent with mitral valve insufficiency without oscillating intracardiac mass on valve.

To accurately identify the organism, we analyzed the p-bromophenacyl esters of mycolic acids of the isolate by using high-performance liquid chromatography, obtaining a pattern consistent with that of *Gordonia* sp. The number of peaks and retention times were similar to those exhibited by the *G. sputi* type strain ATCC 29627. Biochemical test results were positive for the hydrolysis of urease and esculin but negative for the hydrolysis of xanthine, adenine, tyrosine, and hypoxanthine. When inoculated with aerobic, low-peptone carbohydrate slants, the strain produced acid from trehalose but not from L-rhamnose and D-mannitol. These biochemical characteristics fit those of *Gordonia* species, especially *G. bronchialis* (3). To determine partial 16S rRNA gene sequence, the two eubacterial universal primers P8-27 (5'-AGA GTT TGA TCC TGG CTC AG-3') and P1392-1372 (5'-AAG GCC CGG GAA CGT ATT CAC-3') were used for the amplification, then a direct sequencing method with an internal primer P535-514 (5'-GTA TTA CCG CGG CTG CTG GGC AC-3') 5' labeled with fluorescein isothiocyanate was performed (4). The sequence obtained coincides with the 450 5' base pairs of the 16S rRNA gene and matches totally that of *G. sputi* present in the GenBank-EMBL database. This sequence did not match other bacterial sequences, including those of other *Gordonia* species. The sequence of the isolate differed from the sequence of the *G. aichiensis* type strain by two nucleotides. DNA–DNA similarity experiments, according to the stringent nuclease S1 method, showed 55% DNA relatedness with *G. sputi* type strain and less than 28% with the type strain of other species including *G. aichiensis.* (The genetic definition of a species is more than 70% DNA similarity.) Thus, this isolate does not fit in any of the recognized *Gordonia* species, although it is taxonomically close to *G. sputi*.

Conclusions

The recent differentiation of *Gordonia* sp. as a distinct genus is the outcome of a taxonomic history complicated by several reclassifications (Table 1). Twelve organisms now belong to the genus *Gordonia*, including three species discovered in 1999 (5-9). To review the spectrum of clinical diseases in humans caused by *Gordonia* spp., we performed a Medline search for 1966 to 1999, using all the designations included in Table 1. Only *G. bronchialis* (10), *G. rubropertincta* (11),

Table 1. Classification of the genus *Gordonia*

Present designated species (date)	Former designated species (date)
Gordonia aichiensis[a] (1997)	*Gordona aichiensis* (1994) ← *Rhodococcus aichiensis* (1983)
Gordonia alkanivorans (1999)	--
Gordonia amarae[a] (1997)	*Gordona amarae* (1994) ← *Nocardia amarae* (1980)
Gordonia bronchialis[a] (1997)	*Gordona bronchialis* (1989) ← *Rhodococcus bronchialis* (1980)
Gordonia desulfuricans (1999)	--
Gordonia hirsuta[a] (1997)	*Gordona hirsuta* (1996)
Gordonia hydrophobica[a] (1997)	*Gordona hydrophobica* (1995)
Gordonia polyisoprenivorans (1999)	--
Gordonia rhizosphera (1998)	--
Gordonia rubropertincta[a] (1997)	*Gordona rubropertincta* (1989) ← *Rhodococcus rubropertinctus*[b] (1980)
Gordonia sputi[a] (1997)	*Gordona sputi* (1989) ← *Rhodococcus sputi*[c] (1975)
Gordonia terrae[a] (1997)	*Gordona terrae* (1989) ← *Rhodococcus terrae* (1980)

[a]The original spelling, *Gordona,* was changed to *Gordonia* in 1997 (5).
[b]Other former designations:*Bacillus rubropertinctus*, *Serratia rubropertincta*, *Mycobacterium rubropertinctum*, *Proactinomyces rubropertinctus*, *Nocardia rubropertincta.*
[c]Synonym: *Rhodococcus chubuensis.*

G. sputi (1,12), and *G. terrae* (2,13,14) have been shown to be pathogenic in humans (Table 2). They are derived from soil and may also be isolated in the sputa from patients with chest disorders (15). In an outbreak of sternal-wound infections (10), *G. bronchialis* was isolated from the hand, scalp, and vagina of a nurse as well as from her dog.

Systemic *Gordonia* spp. infections have been described in three patients. Buchman et al. (2) reported two cases of *Gordonia* spp. bloodstream infection associated with a Hickman catheter in two immunocompetent patients receiving long-term parenteral nutrition. Both strains were susceptible to vancomycin and gentamicin. In one case, the *Gordonia* sp. isolated from blood cultures and from a broth culture of the catheter tip was not clearly identified but was close to *G. rubropertincta*. The patient received intravenous vancomycin for 5 days and intravenous gentamicin for 19 days; the catheter was removed after 2 days. In the second case, the microorganism isolated from blood cultures was identified as *G. terrae,* and the patient was treated with intravenous vancomycin for 6 weeks with the catheter left in situ.

The only case of bacteremia caused by *G. sputi* was described in a 34-year-old immunocompromised patient (1) with metastatic melanoma treated with intravenous interleukin-2. The bacterium was thought to have reached the bloodstream through extensive desquamative skin rashes caused by the interleukin-2 treatment or by contamination of the catheter, although cultures from these specimens were negative. Because *G. sputi* was susceptible to ß-lactams, vancomycin, aminoglycosides, doxycycline, and rifampicin, the patient was first treated with amoxicillin-clavulanate (1,000 and 125 mg, respectively, every 3 hours). After 1 week of treatment, a second set of blood cultures yielded the same organism, and the treatment was changed to a combination of amikacin and piperacillin, which was successful (1). As with other *Gordonia* species, *G. sputi* may be isolated in the sputum of patients with pulmonary disease (15). Mediastinitis caused by *G. sputi* after coronary artery bypass grafting was recently described in an immunocompetent patient (12). The patient was treated with cefmetazole sodium (2 g per day for 3 weeks) and piperacillin sodium (2 g per day for 2 weeks) after surgical soft tissue debridement.

In our case, *Gordonia* sp. systemic infection associated with an implantable subcutaneous central venous catheter was complicated by endocarditis. The diagnosis was assessed as definitive on the basis of Duke criteria (one major and three minor, including echographic evidence of an oscillating intracardiac mass with a new regurgitant murmur, two persistently positive blood cultures yielding *Gordonia* sp., fever >38°C, and intravenous deferoxamin use). Our patient had neither neutropenia nor immunosuppressive medications, but underlying diseases may have impaired the immune system and facilitated infection. The bacteremia may be caused by manipulations of the implantable subcutaneous central venous catheter during routine home use. However, even if no other

Table 2. Types of infection caused by *Gordonia* species and patients' underlying conditions

Type of infection	Cases (No.)	Age (yrs)	*Gordonia* species	Underlying conditions	Authors
Sternal wound	7	51-68	*G. bronchialis*	Surgery	Richet et al. (10)
Mediastinitis	1	54	*G. sputi*	Surgery	Kuwabara et al. (12)
Brain abscess	1	40	*G. terrae*	None	Drancourt et al. (14)
	1	3	*G. terrae*	Cerebral tumor	Drancourt et al. (13)
Lung infection	1	29	*G. rubropertincta*	Tuberculosis	Hart et al.(11)
Bacteremia due to central venous catheter	2	43	*Gordonia* sp[a]	Breast and ovarian cancer	Buchman et al. (2)
		65	*G. terrae*	Chronic intestinal pseudo-obstruction syndrome	
Bacteremia due to cutaneous lesions	1	34	*G. sputi*	Metastatic melanoma IL2 treatment[b]	Riegel et al. (1)
Skin infection	1	7	*G. terrae*	None	Martin et al. (16)

[a]Not identified.
[b]IL2 = interleukin-2.

source for the bacteremia had been evident, no inflammation was observed at the port site, and semiquantitative culture was not available. An environmental investigation was not performed.

We conclude that *Gordonia* spp. may cause opportunistic infections, in particular bacteremia and endocarditis, in patients with severe underlying diseases and indwelling central catheters.

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The authors thank JP Euzéby (author of the folder "list of bacterial names with standing in nomenclature," http://www-sv.cict.fr/bacterio/) for providing helpful comments about *Gordonia*' s classification.

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References

1. Riegel P, Ruimy R, de Briel D, Eichler F, Bergerat JP, Christen R, et al. Bacteremia due to *Gordona sputi* in an immunocompromised patient. J Clin Microbiol 1996;34:2045-7.
2. Buchman AL, McNeil MM, Brown JM, Lasker BA, Ament ME. Central venous catheter sepsis caused by unusual *Gordona* (*Rhodococcus*) species: identification with a digoxigenin-labeled rDNA probe. Clin Infect Dis 1992;15:694-7.
3. McNeil MM, Brown JM. The medically important aerobic *actinomycetes*: epidemiology and microbiology. Clin Microbiol Rev 1994;7:357-417.
4. Heller R, Jaulhac B, Charles P, de Briel D, Vincent V, Bohner C, et al. Identification of *Mycobacterium shimoidei* in a tuberculosis-like cavity by 16S ribosomal DNA direct sequencing. Eur J Clin Microbiol Infect Dis 1996;15:172-7.
5. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchic classification system, *Actinobacteria classis* nov. Int J Syst Bacteriol 1997;47:479-91.
6. Takeuchi M, Hatano K. *Gordonia rhizosphera* sp. nov. isolated from the mangrove rhizosphere. Int J Syst Bacteriol 1998;48:907-12.
7. Kummer C, Schumann P, Stackebrandt E. *Gordonia alkanivorans* sp. nov., isolated from tar-contaminated soil. Int J Syst Bacteriol 1999;49: 1513-22.
8. Kim SB, Brown R, Olfield C, Gilbert SC, Goodfellow M. *Gordonia desulfuricans* sp. nov. a benzothiophenedesulphurizing actinomycete. Int J Syst Bacteriol 1999;49: 1845-51.
9. Linos A, Steinbuchel A, Sproer C, Kroppenstedt RM. *Gordonia polyisoprenivorans* sp. nov., a rubber-degrading actinomycete isolated from an automobile tyre. Int J Syst Bacteriol 1999;49: 1785-91.
10. Richet HM, Craven PC, Brown JM, Lasker BA, Cox CD, McNeil MM, et al. A cluster of *Rhodococcus (Gordona) bronchialis* sternal-wound infections after coronary-artery bypass surgery. N Engl J Med 1991;324:104-9.
11. Hart DHL, Peel MM, Andrew JH, Burdon JGW. Lung infection caused by *Rhodococcus*. Aust N Z J Med 1988;18:790-1.
12. Kuwabara M, Onitsuka T, Nakamura K, Shimada M, Ohtaki S, Mikami Y. Mediastinitis due to Gordonia sputi after CABG. J Cardiovasc Surg (Torino) 1999;40:675-7.
13. Drancourt M, McNeil MM, Brown JM, Lasker BA, Maurin M, Choux M, et al. Brain abscess due to *Gordona terrae* in an immunocompromised child: case report and review of infections caused by G. terrae. Clin Infect Dis 1994;19:258-62.
14. Drancourt M, Pelletier J, Cherif AA, Raoult D. *Gordona terrae* central nervous system infection in an immunocompetent patient. J Clin Microbiol 1997; 35:379-82.
15. Tsukamura M. Proposal of a new genus, *Gordona*, for slightly acid-fast organisms occurring in sputa of patients with pulmonary disease and in soil. J Gen Microbiol 1973;25:665-81.
16. Martin T, Hogan DJ, Murphy F, Natyshak I, Ewan EP. *Rhodococcus* infection of the skin with lymphadenitis in a nonimmunocompromised girl. J Am Acad Dermatol 1991;24:328-32.

Sin Nombre Virus in Deer Mice Captured Inside Homes, Southwestern Montana

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From 1996 through 1999, 35 deer mice (*Peromyscus maniculatus*) were captured in 25 urban and suburban homes in southwestern Montana. Mice were captured throughout the year except for January; seven mice (20%) from seven (28%) of the homes were seropositive for Sin Nombre virus. The infected mice were mostly adult males captured in the spring and fall.

Hantavirus pulmonary syndrome (HPS), first recognized in 1993, is caused by several related viruses in the genus *Hantavirus*. The primary reservoir of one of these, Sin Nombre virus (SNV), is the deer mouse, *Peromyscus maniculatus*. Deer mice adapt to a variety of habitats and are found across most of the United States; their presence in and around homes has been implicated as a risk factor for HPS. Case-control studies of the original outbreak in the Four Corners region of the southwestern United States found that hantavirus infection prevalence (as indicated by the presence of antibody) in deer mice in and around urban and rural homes was 27.5% to 32.5%, with no significant difference in prevalence between homes of case and control patients. The prevalence of hantavirus infection in deer mice that invade homes in other parts of the United States has not been determined because of the difficulty in obtaining samples from occupied homes. Because the rates of deer mouse infestation in urban and suburban homes are low, extensive random sampling is both nonproductive and disruptive to home owners.

We describe the temporal patterns and age and sex characteristics of deer mice in urban (residential subdivisions) and suburban (>2-ha lots near the edge of town) homes in a nonoutbreak area and determine the prevalence of infection in deer mice captured inside these homes. This study was conducted in and near Butte (population 30,000), Silver Bow County, Montana, USA. Although 12 cases of HPS, with three deaths, have been documented in Montana, none occurred in this area of the state.

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The Study

From November 1996 through September 1999, we provided Sherman live traps to persons who contacted us or local exterminators with complaints of rodent infestation in their homes. Traps containing animals were placed in plastic bags and brought to us for processing according to the protocols of Mills et al.(1). Sex and age were recorded for each captured mouse. Age was based on the following weight categories: <14 g juvenile, 14 to 17 g subadult, and >17 g adult (2). Mice were examined for injuries (scarred tails or torn or nicked ears) possibly indicating fights. Blood samples were collected from the retroorbital sinus of each animal with a heparinized capillary tube and were stored on dry ice until transferred to –70°C freezers for storage. Serologic testing was conducted at Montana State University, Bozeman, Montana. Samples of whole blood were tested for antibody reactive with SNV recombinant nucleocapsid protein by enzyme-linked immunosorbent assay (3).

We captured 35 *P. maniculatus* from 25 homes (Table). Seven mice from seven of the homes were seropositive for antibodies to SNV, an overall prevalence of 20%. Sex and age ratios were similar between seropositive and negative deer mice. More adult than subadult and juvenile and more male than female mice were captured inside homes. Most seropositive mice (71.4%) were male, and >50% of these were adult. One

seropositive mouse (14.3%) had evidence of injuries. *P. maniculatus* were captured inside homes throughout the year with the exception of January (Figure). However, seropositive mice were captured only in the spring and fall. Juvenile deer mice were captured only in September and October.

Table. Distribution of antibody-positive and -negative deer mice, by sex, age,[1] and evidence of injuries

Characteristic	Antibody positive no. (%)	Antibody negative no. (%)	Total (%)
Sex			
Male	5 (71.4)	18 (64.3)	23 (65.7)
Female	2 (28.6)	10 (35.7)	12 (34.3)
Total	7	28	35
Age			
Juvenile	1 (14.3)	1 (3.7)	2 (5.9)
Subadult	2 (28.6)	11 (40.7)	13 (38.2)
Adult	4 (57.1)	15 (55.6)	19 (55.9)
Total	7	27	34
Injuries			
Yes	1 (14.3)	3 (10.7)	4 (11.4)
No	6 (85.7)	25 (89.3)	31 (88.6)
Total	7	28	35

[1]Age information was not collected for one mouse.

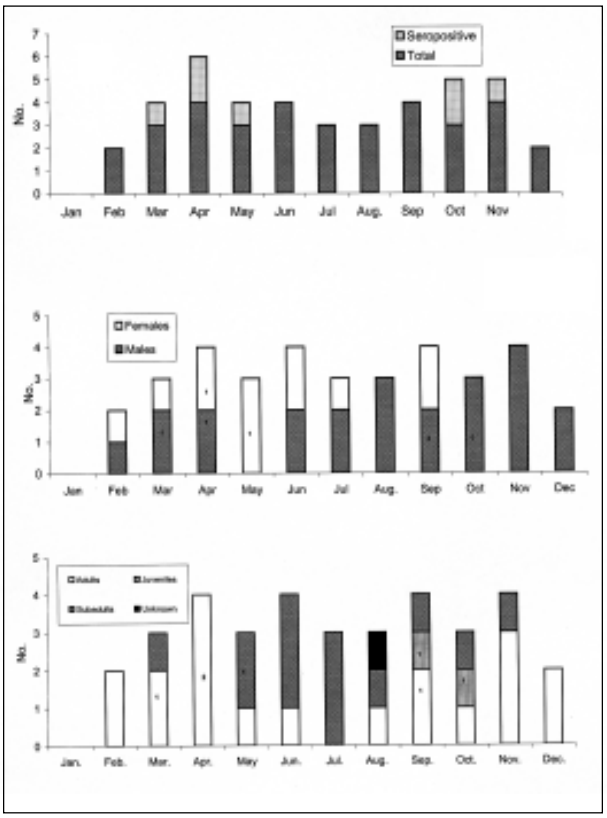

Figure. Temporal patterns of deer mice trapped in urban and suburban homes, November 1996 to September 1999. Numbers inside bars indicate seropositive mice.

Conclusions

The overall prevalence of antibodies reactive with SNV antigen in deer mice invading homes was 20%. This prevalence is lower than that of deer mice captured in and around homes during the 1993 Four Corners outbreak but higher than the overall prevalences of sylvatic deer mice populations in Montana, Colorado, Kansas, national parks in eastern and central United States, and major biotic communities in the southwestern United States.

Most of the mice invading homes were adult males. Prevalence of infection (as indicated by antibody) was higher in male and sexually mature rodents, as in other studies (4-7). Although we detected few direct signs of aggressive encounters in individual mice, others have reported a higher incidence of injuries in infected mice (4,8), which implicates fighting as a mode of transmission.

Deer mice were trapped inside homes throughout the year except for January, which indicates that mice do not necessarily invade homes more readily during cold weather. Seropositive mice, however, were captured only during spring and fall, which indicates a higher prevalence of infection in deer mice populations during those times. Sylvatic populations of *P. boylii* in northern Arizona show a bimodal pattern of hantavirus transmission, with peaks in the spring and fall (6). Cases of human infection in the Four Corners region have a spring-summer seasonal pattern (9,10). However, peridomestic populations of deer mice from two study sites near Butte, Montana, had no distinct seasonal patterns of transmission of hantavirus infection (unpub. data, Douglass and Kuenzi). The seasonal patterns we found in deer mice captured in homes may be an artifact of sample size.

Infestation of homes by deer mice is not restricted to rural environments. SNV infection may be as prevalent in deer mice captured in homes in urban and suburban environments as in populations in sylvatic habitats. Urban and suburban homeowners are not exempt from the risk for hantavirus infection and should follow recommendations for risk reduction (11).

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References

1. Mills JN, Yates TL, Childs JE, Parmenter RR, Ksiazek TG, Rollin PE, et al. Guidelines for working with rodents potentially infected with hantavirus. J Mammal 1995;76:716-22.
2. Fairbairn DJ. The spring decline in deer mice: death or dispersal? Can J Zool 1977;55:84-92.
3. Feldmann H, Sanchez A, Morzunov S, Spiropoulou CF, Rollin PE, Ksiazek TG, et al. Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. Virus Res 1993;30:351-67.
4. Calisher CH, Sweeney W, Mills JN, Beaty BJ. Natural history of Sin Nombre virus in western Colorado. Emerg Infect Dis 1999;5:126-34.
5. Mills JN, Ksiazek TG, Ellis BA, Rollin PE, Nichol ST, Yates TL, et al. Patterns of association with host and habitat: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the southwestern United States. Am J Trop Med Hyg 1997;56:273-84.
6. Abbott KD, Ksiazek TG, Mills JN. Long-term hantavirus persistence in rodent populations in central Arizona. Emerg Infect Dis 1999;5:102-12.
7. Kuenzi AJ, Morrison ML, Swann DE, Hardy PC, Downard GT. A longitudinal study of Sin Nombre virus prevalence in rodents, southeastern Arizona. Emerg Infect Dis 1999;5:113-7.
8. Childs JE, Glass GE, Korch GW, LeDuc JW. Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, Maryland. Am J Trop Med Hyg 1987;37:648-62.
9. Khan AS, Khabbaz RF, Armstrong LR, Holman RC, Bauer SP, Graber J, et al. Hantavirus pulmonary syndrome: the first 100 U.S. cases. J Infect Dis 1996;173:1297-303.
10. Engelthaler DM, Mosley DG, Cheek JE, Levy CE, Komatsu KK, Ettestad P, et al. Climatic and environmental patterns associated with hantavirus pulmonary syndrome, Four Corners region, United States. Emerg Infect Dis 1999;5:87-94.
11. Centers for Disease Control and Prevention. Hantavirus infection—southwestern United States: interim recommendations for risk reduction. MMWR Morb Mortal Wkly Rep 1993;42(RR-11):1-13.

Evidence of *Rickettsia helvetica* Infection in Humans, Eastern France

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A 37-year-old man living in eastern France seroconverted to *Rickettsia helvetica* in August 1997, 4 weeks after the onset of an unexplained febrile illness. Results of a serosurvey of forest workers from the area where the patient lived showed a 9.2% seroprevalence against *R. helvetica*. This organism may pose a threat for populations exposed to *Ixodes ricinus* ticks.

Spotted fever group rickettsiae are gram-negative intracellular bacilli associated with arthropods and transmitted by ticks. The most common clinical features of rickettsioses in humans are fever, headache, rash, and inoculation eschar. Five well-characterized and three recently proposed rickettsioses of humans have been identified in the past 13 years. Extensively studied rickettsioses include Japanese spotted fever (caused by *Rickettsia japonica*), Astrakhan fever (Astrakhan fever rickettsia), Flinder's Island spotted fever (*R. honei)*, California flea typhus (*R. felis)*, and African tick-bite fever (*R. africae)*. In France, *R. conorii*, the agent of Mediterranean spotted fever, which is transmitted through the bite of the brown dog tick *(Rhipicephalus sanguineus*), is the main pathogenic rickettsia and is encountered in the southern part of the country. Recently, "*Rickettsia mongolotimonae*," *R. slovaca*, and *R. helvetica* have also been identified as agents of human rickettsioses in Europe. Other species of unknown pathogenicity—including *R. rhipicephali*, *R. massiliae*, and Bar 29—have been isolated from ticks in Europe.

R. helvetica has been isolated from *Ixodes ricinus* collected in Switzerland, France, Slovenia, and Sweden. These ticks, which readily bite humans, are also the vectors in Europe of *Borrelia burgdorferi* and *Ehrlichia phagocytophila*, the agent of human granulocytic ehrlichiosis. *I. ricinus* is widely prevalent in parts of Europe (1), including eastern France, and frequently bites humans (Figure 1). Consequently, the potential transmission of *R. helvetica* by *I. ricinus* to humans seemed likely, but its pathogenic capacity in this regard remained uncertain until Nilsson et al. demonstrated its role in the development of perimyocarditis and sudden death in two young patients (2).

In this report, we describe a human infectious syndrome in which specific seroconversion against *R. helvetica* occurred. We also present the results of a serosurvey on rickettsioses conducted in Alsace (eastern France) among forest workers,

Figure 1. Map of Europe showing the distribution of *Ixodes ricinus* (1). The areas where *I. ricinus* is prevalent are shaded in black and the prevalence of *R. helvetica* in *I. ricinus*, where estimated, is indicated.

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which showed high prevalence of antibody reactive with this bacterium.

Case Report

A 37-year-old, immunocompetent man was admitted to a hospital in Strasbourg, Alsace, on August 18, 1997, with prolonged fever, fatigue, myalgias, and headache of unknown etiology. The fever had begun on August l, 18 days after the man walked in a forest near Strasbourg, where he observed numerous ticks but not any tick bites. On physical examination, the physician observed low-grade fever (38.1°C) but no rash, lymphadenopathy or inoculation eschar.

Results of laboratory tests included an elevated C-reactive protein (48 mg/L); increased fibrinogen level (6.0 g/L); accelerated erythrocyte sedimentation rate (34 mm, first hour); leukocyte count of 6,700/mm^3; platelet count of 165,000/mm^3; and increased rates of alanine-aminotransferase (60 IU/L) and aspartate aminotransferase (52 IU/L). Blood cultures taken on August 18 were negative. The patient received no antibiotic treatment and recovered spontaneously within 2 weeks. Rickettsial serologic tests were performed on serum samples (taken on days 17, 27, and 86 after onset) at the Unité des Rickettsies. Antibody levels against *B. burgdorferi*, *Francisella tularensis*, *E. phagocytophila*, and hepatitis A, B and C viruses were measured in Strasbourg but were negative.

Serosurvey

A convenience collection of serum samples from 379 forest workers from Alsace, all of whom were state employees, was obtained. These samples had been initially collected as part of a systematic serosurvey organized by the state department of occupational medicine to evaluate for the presence of antibodies to *B. burgdorferi*. This population was composed of 377 men and 2 women, 20 to 59 years of age; 360 (95.5%) reported frequent tick bites but were clinically asymptomatic; the remaining 19 reported no tick bites. No questionnaire was administered. After informed consent from the patients, antibodies to *R. helvetica*, *R. conorii*, *R. slovaca,* and *"R. mongolotimonae"* were determined.

Serologic Tests

To determine the specificity of cross-reactions, serum samples, including those from the survey, were adsorbed with *R. conorii*, *R. helvetica*, *R. slovaca* or *R. mongolotimonae* antigens. Cross-adsorption procedures were performed (3). *R. helvetica*, *R. conorii*, *R. slovaca*, and "*R. mongolotimonae*" Western blot analyses were performed on the resultant supernatants. Purified *R. helvetica*, *R. conorii*, *R. slovaca*, and *"R. mongolotimonae"* were suspended in sterile water and adjusted to 2 mg/mL with a UV spectrophotometer. Western blotting procedures using unheated antigens were performed (4). Antibodies reacting against antigens of 20 to 50 kDa were assumed to be directed primarily against the lipopolysaccharide in *R. helvetica*, *R. conorii*, *R. slovaca*, and *"R. mongolotimonae."* Serologic specificity toward the different spotted fever group rickettsia species was determined by the relative immunoglobulin G (IgG) reactivity to species-specific antigens in the 110- to 145-kDa region. Intensity measures were compared by video image acquisition (The Imager, Appligene, Illkirch, France).

Case Findings

The first serum sample, taken from the patient on August 18, was negative for all tested antigens. Samples taken on August 27 and October 27 yielded IgG titers of 1:128, 1:64, 1:128 and 1:256, and IgM titers of 1:64, 1:16, 1:32 and 1:128 against *R. conorii*, *R. slovaca*, *"R. mongolotimonae,"* and *R. helvetica*, respectively, but were negative for *Coxiella burnetii* and *E. phagocytophila*. Western immunoblotting of the patient's serum samples demonstrated cross-reactivity between *R. helvetica*, *R. slovaca*, *"R. mongolotimonae,"* and *R. conorii* antigens, but adsorption with *R. helvetica* antigen eliminated all antibodies. After adsorption with *R. conorii*, *R. slovaca*, and "*R. mongolotimonae"* antigens, antibodies to *R. helvetica* were still present, but antibodies to the other three antigens were not observed, indicating that *R. helvetica* was the etiologic agent.

Serosurvey Findings

Of the 379 patients included in our serosurvey, 35 (9.2%) demonstrated anti-*R. helvetica* IgG titers 1:64 (16 had an IgG titer of 1:64; 13 a titer of 1:128; 5 a titer of 1:256; and one a titer of 1:512). IgM was not detected in any patient. In all positive serum samples, titers were greater against *R. helvetica* than against any of the other three tested antigens by two or more dilutions (Figure 1). Following adsorption with

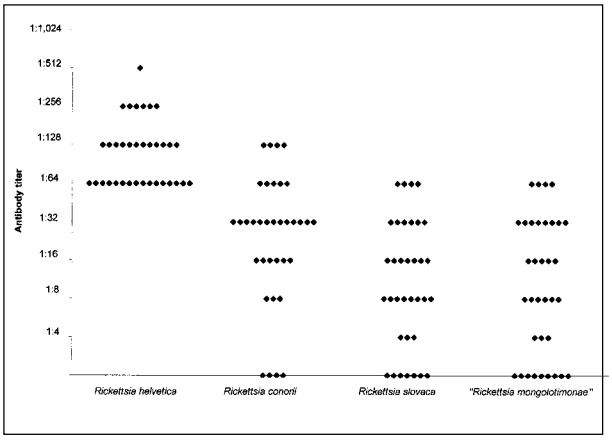

Figure 2. Antibody titers against *Rickettsia helvetica*, *R. conorii*, *R. slovaca*, and "*R. mongolotimonae*" in 35 forest workers, Alsace.*

*Each diamond represents the IgG titer in serum samples from the patients. *R. conorii* Moroccan strain (ATCC VR 141), *R. helvetica* C9P9 strain, *R. slovaca* 13-B strain, and "*R.* mongolotimonae" HA91 strain were grown on confluent layers of Vero cells in a 150-cm^2 flask. Infection was monitored by Gimenez staining. When 90% of the cells were infected, cell layers and supernatants were harvested, pelleted by centrifugation (10,000 x *g* for 10 min), and resuspended in 15 mL of phosphate-buffered saline (PBS, pH 7.3). Antigen purification was performed (5). The final suspension was resuspended in sterile water, and the protein content of the purified organisms was determined by UV spectrophotometry and adjusted to 1 mg/mL. All four rickettsial antigens were applied by pen point to each well of 30-well microscope slides (Dynatech Laboratories Ltd., Billingshurt, United Kingdom), air dried, and fixed with acetone for 10 min. This allowed a direct comparison of fluorescence intensity between antigens. All serum samples, including positive and negative controls, were diluted 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128 in PBS with 3% nonfat dry milk. Microimmunofluorescence procedures were performed (3). All serum samples found to be positive for total immunoglobulins were diluted serially (twofold dilutions ranging from 1/32 to 1/2,048 or more), and the titers of IgG and IgM were determined. Before detection of IgM, serum was adsorbed with a rheumatoid factor absorbent (RF-absorbent, Behring-werke AG, Marburg, Germany). Titers at or above 1:64 for IgG or 1:32 for IgM were considered positive.

R. helvetica antigen, all antibodies were eliminated.

Conclusions

Epidemiologic evidence suggests that our patient was exposed to *I. ricinus* during his forest walk. He did not report any tick bites, but larvae or nymphs are often not detected by patients. Clinically, he presented with a prolonged low-grade fever, headache and myalgias. Unfortunately, no blood specimen was available for culture. Though neither cardiac symptoms nor a rash developed, the latter is sometimes lacking in other spotted fever group rickettsioses, such as African tick-bite fever or *R. slovaca* infection.

The usual method for the diagnosis of rickettsioses is serologic testing; the easiest method is microimmunofluorescence. Although serological cross-reactions are common among spotted fever group rickettsiae (6), *R. helvetica* is phylogenetically distant from other spotted fever group rickettsiae as well as from typhus group rickettsiae. Thus, one could expect the antibody response to this bacterium to be higher than against other spotted fever group rickettsiae. In our patient and 35 (9.2%) of 379 tested forest workers, antibody titers against *R. helvetica* were higher than those detected against *R. conorii*, *R. slovaca*, and *"R. mongolotimonae."* The specificity of the antibody response was confirmed by cross-adsorption and Western blotting. The forest workers lived in Alsace, an area where *R. conorii* and *Rhipicephalus* sp. are absent but where *I. ricinus* is highly prevalent, and were frequently exposed to ticks. To date, *I. ricinus* has only been demonstrated to harbor one rickettsial species, *R. helvetica*. Therefore, the observed seroprevalence was mainly or fully caused by *R. helvetica*. Although none of the forestry workers in our study reported any tick-bite-related disease, such results suggest that this occupational group may be exposed to the bites of *I. ricinus* and to *R. helvetica* infection. Prospective evaluation of ill patients and molecular identification of the causative agents need to be conducted.

Our data support the notions that this rickettsia, found in ticks (*I. ricinus)* that bite humans and are widely distributed in forest areas in Central Europe, could be frequent and that *I. ricinus* should be considered in the diagnosis of tick-transmitted infection in Europe.

Dr. Fournier is a physician in the French reference center for the diagnosis and study of rickettsial diseases. His research interests include the clinical and epidemiologic features of rickettsioses.

References

1. Gilot B. Bases biologiques, écologiques et cartographiques pour l'étude des maladies transmises par les tiques (Ixodidae et Argasidae) dans les alpes françaises et leur avant pays) [thesis]. Grenoble, France: Universite de Grenoble]; 1985.

2. Nilsson K, Lindquist O, Pahlson C. Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. Lancet 1999;354:1169-73.
3. Brouqui P, Harle JR, Delmont J, Frances C, Weiller PJ, Raoult D. African tick bite fever : an imported spotless rickettsiosis. Arch Intern Med 1997;157:119-24.
4. Teysseire N, Raoult D. Comparison of Western immunoblotting and microimmunofluoresence for diagnosis of Mediterranean spotted fever. J Clin Microbiol 1992;30 :455-60.
5. Raoult D, Dasch GA. Line blot and Western blot immunoassays for diagnosis of Mediterranean spotted fever. J Clin Microbiol 1989;27:2073-9..
6. Hechemy KE, Raoult D, Fox J, Han Y, Elliott LB, Rawlings J. Cross-reaction of immune sera from patients with rickettsial diseases. J Med Microbiol 1989;29:199-202.

Antibiotic Resistance in *Escherichia coli* from Nigerian Students, 1986–1998

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We tested 758 fecal *Escherichia coli* isolates, recovered from Nigerian students in 1986, 1988, 1990, 1994, and 1998, for susceptibility to seven antimicrobial drugs. The prevalences of strains resistant to tetracycline, ampicillin, chloramphenicol, and streptomycin were 9% to 35% in 1986 and 56% to 100% in 1998. These findings demonstrate that resistance gene reservoirs are increasing in healthy persons.

Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries (1-4). Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control (5). Normal intestinal flora are a reservoir for resistance genes; the prevalence of resistance in commensal *Escherichia coli* is a useful indicator of antibiotic resistance in bacteria in the community (6,7). Studies with *E. coli* are of particular relevance because this species can occupy multiple niches, including human and animal hosts (8). In addition, *E. coli* strains efficiently exchange genetic material with pathogens such as *Salmonella, Shigella, Yersinia*, and *Vibrio* species, as well as pathogenic *E. coli* (8,9).

Few studies have evaluated antimicrobial resistance in sub-Saharan Africa. Most available data are specific to pathogenic organisms, and trends over time in this region are rarely followed. We monitored trends in antibiotic resistance prevalence in *E. coli* isolates from apparently healthy Nigerian students by measuring resistance to seven antimicrobial drugs in *E. coli* isolated from 758 stool specimens collected over a 13-year period.

The Study

Stool specimens were collected from apparently healthy student volunteers at the Obafemi Awolowo University in 1986, 1988, 1994, 1996, and 1998. The students, who provided informed consent, were 16 to 32 years of age; 347 (45.8%) were female. All volunteers who had taken antimicrobial drugs or been ill in the previous month were excluded from participation.

The specimens were collected into Stuart's transport medium and subcultured onto MacConkey agar plates. Colonies with morphologic characteristics of *E. coli* were subcultured onto fresh plates. Identity was confirmed by conventional biochemical tests. The standard disk diffusion method was used for susceptibility testing (10). The antibiotic disks used were ampicillin (10 µg), chloramphenicol (30 µg), streptomycin (30 µg), sulfisomidine (250 µg), nalidixic acid (30 µg), trimethoprim (5 µg), and tetracycline (30 µg) (AB Biodisk, Sweden). *E. coli* NCTC 10418 and K-12 C600 were used as controls. All isolates recovered at the same time from the same person with identical biochemical and antibiotic susceptibility profiles were considered identical. The prevalence of resistance to each drug for each year was computed. Trends were analyzed by Pearson's regression and the chi-square test for trend (Mantel-Haenszel test).

No sampling was conducted in 1990 or 1992, and streptomycin resistance was not tested in 1994. Despite these gaps in the study, trends toward increasing resistance in *E. coli* were observed with tetracycline, sulfonamides, ampicillin, chloramphenicol, and streptomycin (Figure). The proportion of isolates resistant to chloramphenicol increased from 13.5% in 1986 to 59.8% in 1998, while isolates resistant to tetracycline increased from 34.9% to 100%. The trends for tetracycline and streptomycin were statistically significant ($p < 0.05$, Pearson's

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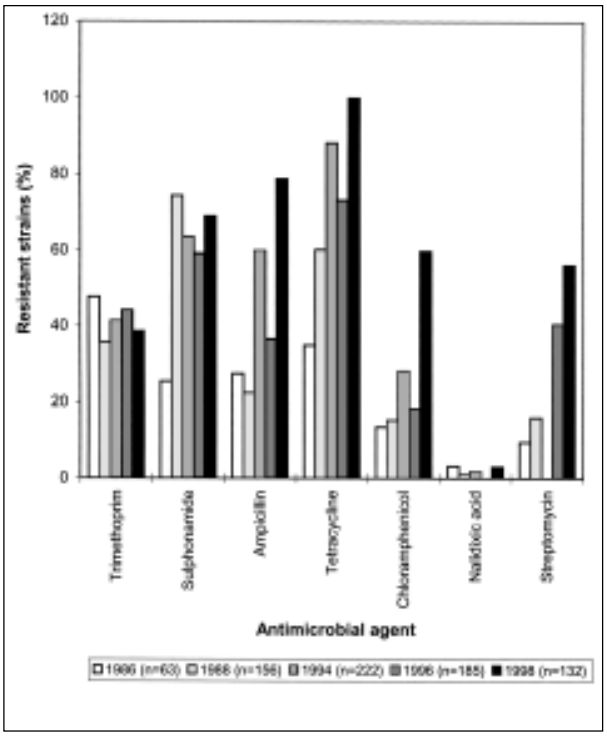

Figure. Percentage of isolates resistant to seven antimicrobial drugs, 1986-1998.
*No data are available for streptomycin in 1994.

regression; p <0.10, chi-square test for trend). The prevalence of sulfonamide resistance increased sharply from 1986 to 1988 (from 25.4% to 74.3%), then remained fairly constant. The moderate levels of resistance to trimethoprim (35.7% and 47.6%) and low levels to nalidixic acid (0% to 3.2%) did not change appreciably.

As strains susceptible to all drugs became less common, the proportion of isolates resistant to multiple antibiotics increased (Table). In 1986, 19 (30.2%) isolates were susceptible to all the drugs tested, but by 1998 all the isolates were resistant to at least one drug. The proportion of isolates resistant to three or more drugs increased steadily, from 30.2% in 1986 to 70.5% in 1998 (p <0.05, Pearson's regression; p <0.10, chi-square test for trend). Only one isolate from 1986 was resistant to at least six drugs, but 15.9% of the isolates obtained in 1998 demonstrated this phenotype. Three isolates were resistant to all drugs tested. Two of these had only low levels of resistance to nalidixic acid and chloramphenicol (in both cases) and ampicillin in one case. One isolate recovered in 1998 had high-level resistance

Table. Proportions of universally susceptible and multiply resistant *Escherchia coli*, Nigeria, 1986–1998

	No. (%) isolates		
Yr/(No. isolates)	Sensitive to all drugs tested	Resistant to 3 drugs	Resistant to 6 drugs
1986/(63)	19 (30.2)	19 (30.2)	1 (1.6)
1994/(222)[a]	24 (15.4)	37 (23.7)	0 (0)
1996/(185)	34 (18.4)	105 (56.8)	6 (3.2)
1998/(132)	0 (0)	93 (70.5)	21 (15.9)
p value[b]	-0.768 (0.232)	0.960 (0.04)	0.768 (0.232)

[a]Streptomycin was not tested in 1994.
[b]p value based on Pearson's regression coefficient for trend.

to all the drugs tested, as well as to cefalotin and fluoroquinolones (ofloxacin, ciprofloxacin, and norfloxacin); however, it was susceptible to spectinomycin, tobramycin, and gentamicin.

Conclusions

Residents in and visitors to developing countries acquire antibiotic-resistant *E. coli* as part of their normal flora (1,2,11). Our data show that the prevalence of resistance to most drugs tested in *E. coli* isolates from apparently healthy students is within the high range reported previously (2) and has increased from 1986 to 1998. The increases in prevalence of resistance to streptomycin and tetracycline were statistically significant. In most drugs tested, the proportion of resistance isolates has increased rapidly, so that the usefulness of drugs moderately effective in 1986 has been severely compromised. The prevalence of resistance in these commensal *E. coli* during the latter sampling points reached >50% in 1998 for all drugs except trimethoprim and nalidixic acid. For tetracycline, the proportion of resistant strains increased from <40% to 100% in the 13-year period. As in other studies (2), the general trend toward increasing prevalence of resistance was marked by the recovery of an increasing proportion of strains that were simultaneously resistant to several drugs. These data sound a warning because the indiscriminate use of antibiotics, along with poor hygiene and infection control (risk factors for antibiotic resistance in bacteria) are highly prevalent in Nigeria and other developing countries (4,12).

The five drugs for which a considerable rise in resistance was seen from 1986 to 1998

(ampicillin, sulfonamides, streptomycin, chloramphenicol, and tetracycline) are extensively used in Nigeria and other developing countries (4,12). These five inexpensive drugs are widely available without prescription from authorized health institutions and pharmacies, as well as from unauthorized patent medicine shops and other distributors (4,12). Streptomycin, which must be injected, and chloramphenicol, because of its toxicity, are less popular than the other three drugs—and had lower resistance prevalence—but are nonetheless used more often than indicated. The prevalence of resistance to a sixth agent, nalidixic acid, remained low, corresponding to the low consumption of this and related drugs in health institutions and the community. The recent introduction of fluoroquinolones in clinical practice may alter this profile, since resistance to the fluoroquinolones and nalidixic acid has been shown to spread rapidly (3,13).

Resistance to trimethoprim was higher than to other drugs in 1986 but did not increase substantially in subsequent years. This drug is heavily used in health institutions and in the community, generally in combination with sulfamethoxazole. The selective pressure generated by overuse explains the relatively high prevalence of resistance in *E. coli* isolates in 1986. However, it is not clear why the trend observed with other widely used drugs was not seen in this case. Any increases in prevalence of strains resistant to trimethoprim may have occurred before 1986. A similar plateau with sulfonamide resistance followed a rapid rise from 1986 to 1988. Why this plateau was seen with two drugs and not others is not known, but the observation has been reported in other locations (14).

Ingestion of antibiotics is known to provide selective pressure ultimately leading to a higher prevalence of resistant bacteria, even among persons who have not taken antibiotics (7,8). As recent antibiotic use was a criterion for exclusion from the study, selection of the resistance strains isolated in the study may have occurred before the volunteer hosts were colonized. The source of resistant organisms in our study population is not known, but possible sources are food, water, and person-to-person transfer. Suboptimal sanitary conditions and overcrowding in student hostels may facilitate the spread of these organisms.

We observed rapid increases in the prevalence of resistance in commensal *E. coli* to most of the older, less expensive antimicrobial drugs used in the management of infections in Nigeria. Not only are these strains potential causes of infection, but they are also potential reservoirs of resistance genes that could be transferred to pathogens. For this reason, the trends seen with commensal *E. coli* may also occur with pathogenic organisms. Studies in other developing countries have shown that the trend in enteric pathogens is toward increasing antibiotic resistance (3). Our study emphasizes the need to monitor commensal organisms as well as pathogens by susceptibility testing to guide treatment. Control of antibiotic resistance is needed to conserve the usefulness of the remaining drugs. The data suggest that nalidixic acid and possibly trimethoprim may be useful in treating infections caused by pathogenic *E. coli* and other related bacteria in Nigeria. The future usefulness of these drugs will, however, depend on effective interventions to halt the selection and spread of resistance among enteric organisms.

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References

1. Calva JJ, Sifuentes-Osornio J, Ceron C. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. Antimicrob Agents Chemother 1996;40:1699-702.
2. Lamikanra A, Okeke IN. A study of the effect of the urban/rural divide on the incidence of antibiotic resistance in *Escherichia coli*. Biomed Lett 1997;55:91-7.
3. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin Infect Dis 1998;26:341-5.
4. Hart CA, Kariuki S. Antimicrobial resistance in developing countries. Br Med J 1998;317:647-50.

5. O'Brien T. The global epidemic nature of antimicrobial resistance and the need to monitor and manage it locally. Clin Infect Dis 1997;24(Suppl 1):S2-8.
6. Levy S, Marshall B, Schleuderberg S, Rowe B, Davis J. High frequency of antibiotic resistance in human fecal flora. Antimicrob Agents Chemother 1988;32:1801-6.
7. Levin B, Lipsitch M, Perrot V, Schrag S, Antia R, Simonsen L, et al. The population genetics of antibiotic resistance. Clin Infect Dis 1997;24:S9-16.
8. Levy SB. Antibiotic resistance: an ecological imbalance. Ciba Found Symp 1997;207:1-9; discussion 9-14.
9. Tauxe RV, Cavanagh TR, Cohen ML. Interspecies transfer in vivo producing an outbreak of multiply resistant Shigellosis. J Infect Dis 1989;160:1067-70.
10. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 2nd ed. Approved standard. Villanova (PA): The Committee; 1990.
11. Murray BE, Mathewson JJ, DuPont HL, Ericsson CD, Reves RR. Emergence of resistant fecal *Escherichia coli* in travelers not taking prophylactic antimicrobial agents. Antimicrob Agents Chemother 1990;34:515-8.
12. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavorial factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis 1999;5:18-27.
13. Green S, Tillotson G. Use of ciprofloxacin in developing countries. Pediatr Infect Dis J 1997;16:150-9; discussion 160-2.
14. O'Brien T. Resistance of bacteria to antibacterial agents: report of Task Force 2. Reviews of Infectious Diseases 1987;9:S244-60.

Ehrlichia chaffeensis Antibodies in White-Tailed Deer, Iowa, 1994 and 1996

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Surveillance of 2,277 white-tailed deer for antibodies against *Ehrlichia chaffeensis* in Iowa showed seropositivity rates of 12.5% in 1994 and 13.9% in 1996. From 1994 to 1996, the estimated number of seropositive deer increased to 54,701 (28%). The increasing deer population and expanding tick distribution may increase risk for human monocytic ehrlichiosis.

Human monocytic ehrlichiosis (HME) is a newly recognized disease caused by *Ehrlichia chaffeensis*, a rickettsialike, gram-negative, pleomorphic bacterium (1). HME causes clinical illness, from mild and flulike to life threatening. A prolonged incubation period and ambiguous symptoms complicate the diagnosis. Early recognition and treatment of the disease are key to preventing death. Identifying the ecologic niches for the etiologic agent and vector is important for characterizing ehrlichiosis and the occupational and environmental risk for illness. The southern counties of Iowa border the region of endemic HME, and an important reservoir host, the white-tailed deer (*Odocoileus virginianus*), is common in Iowa, as is the vector, the Lone Star tick (*Amblyomma americanum)* (2,3).

Although humans may be hosts for nymph and adult ticks, they are not the preferred mammal for the tick and therefore are accidental dead-end hosts of HME (4). Person-to-person transmission is not known to occur. Most likely to be infected with *Ehrlichia* are populations with an increased risk for tick exposure, including hunters, farmers, hikers, campers, foresters, and park rangers. To define the prevalence and geographic distribution of *E. chaffeensis* in Iowa, we tested blood collected from white-tailed deer from every county in the state in 1994 and 1996.

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White-tailed deer were used as sentinel animals for HME because 1) nonspecific symptoms complicate the diagnosis of mild and subclinical cases in humans; 2) deer are primary hosts for *A. americanum*; 3) the limited home range of deer (4 km^2 [5]) allows accurate determination of geographic distribution; and 4) hunters in Iowa are willing to provide deer blood samples. In addition, tick collection, speciation, and analysis by polymerase chain reaction would be prohibitive for evaluation of *E. chaffeensis* in all 99 counties in Iowa. Testing ticks would likely require analysis of huge numbers of specimens to detect significant numbers of positives. However, each deer during its lifetime may be bitten by thousands of ticks, and any one of the bites may lead to infection and seroconversion.

The Study

Randomly selected licensed deer hunters (2,500 in the 1994 and 2,000 in the 1996 hunting seasons) were sent packets containing an explanation of the study, two Nobuto blood filter strips (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and a postage-paid return envelope. Hunters were instructed to collect a deer blood sample on the Nobuto strip, allow the sample to dry, place it in a resealable bag, and mail it, along with a map showing the county where the deer was killed. The dried whole blood samples (0.1 mL) were eluted from the Nobuto strips by soaking in phosphate-buffered saline (PBS), pH 7.4, for 3 hours at 4°C. The eluted samples were stored at -70°C until tested. Twelve-well, Teflon-coated, glass microscope slides were coated with *E. chaffeensis*-infected DH82 canine malignant

macrophages. All blood specimens were analyzed by immunofluorescent antibody (IFA) assay at a dilution of 1:64 in PBS (6). Specimens with at least 1+ fluorescence were considered positive.

To calculate the statistical significance of the difference in statewide and regional prevalence rates for 1994 and 1996, chi-square tests of homogeneity of binomial proportions were used. A chi-square test for trend in binomial proportions was used to determine significance of the north-to-south trend. All tests were performed by using EpiInfo 6.04b, and an alpha <0.05 was considered significant.

Of the 4,500 blood-sampling packets mailed to hunters, 2,881 were returned, of which 2,877 were usable (64% overall: 71% in 1994 and 55% in 1996) (Table 1). All 99 counties of Iowa were represented in the 1994 samples (mean 17.9 specimens per county [2 to 82]). The 1996 samples were collected in 97 of the 99 counties (mean 11.2 specimens per county [0 to 82]). To facilitate analysis of geographic trends in seroprevalence, we divided the Iowa map horizontally into three rows of counties (Figure).

The deer population has been increasing in Iowa (Table 1). To establish estimates of statewide prevalence, population estimates of deer per county were obtained from the Iowa Department of Natural Resources (DNR) (W.J. Suchy, pers. comm.). From 1994 to 1996 the deer population in Iowa increased by 14.0%, or more than 45,000 deer. The deer population was distributed with 23.3% and 27.0% in the north in 1994 and 1996, respectively; 43.5% and 40.3% were located in the southern region in 1994 and 1996, respectively. Although the population density was higher in the south, most increases in deer population occurred in the north. This increase accounts for the difference in the regional distribution of blood samples across years (Table 1). The estimated proportions of deer in Iowa included in this study (Table 2) are 0.55% sampled in 1994 and 0.30% in 1996. The ratio of the 1994 proportion to the 1996 shows no difference in the regional distribution of population-based sampling across years (North, 1.84; Central, 1.84; South, 1.82). The statewide seroprevalence rate was 12.5% in 1994 but increased to 13.9% in 1996 (Table 2). The maps demonstrate that seropositivity followed a north-to-south gradient, with the highest prevalence rates in the southernmost counties (Figure). Six

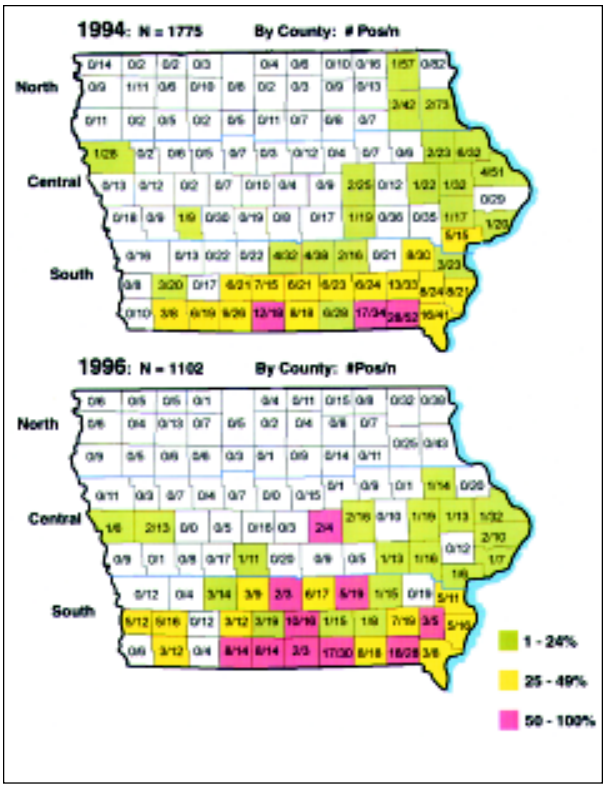

Figure. Iowa maps showing *Ehrlichia chaffeensis* seropositive specimens by county. The total number of positive specimens was 102 of 1,775 in 1994 (top map) and 91 of 1,102 in 1996 (bottom map). Within each of the 99 counties is listed the number of positive specimens over the total submitted for the county. Colors indicate the percentage of positive specimens as listed in the key.

Table 1. Geographic distribution of deer blood specimens collected and estimated deer population, Iowa, 1994 and 1996

	1994			1996			Pop. increase
Region	Est. pop.	No. sampled	% sampled	Est. pop.	No. sampled	% sampled	%
North	75,252	444	25.0	99,531	318	28.9	32.3
Central	107,344	617	34.8	120,134	374	33.9	11.9
South	140,470	714	40.2	148,569	410	37.2	5.8
Iowa	323,067	1,775	100.0	368,234	1,102	100.0	14.0

Table 2. Deer seropositivity for antibodies to *Ehrlichia chaffeensis*, Iowa, 1994 and 1996.

Year	Result	North	Central	South	Statewide
1994	No. of samples	444	617	714	1,775
	% of population sampled	0.59	0.57	0.51	0.55
	No. positive	6	26	189	221
	% positive	1.35	4.21	26.5	12.5
	Est. no. of positive deer	1,017	4,523	37,183	42,724
	Odds ratio	1.0	3.2	26.3[a]	
1996	No. of samples	318	374	410	1,102
	% of population sampled	0.32	0.31	0.28	0.30
	No. positive	0[b]	18	135	153
	% positive	0	4.81	32.9	13.9
	Est. no. of positive deer	0	5,782	48,919	54,701
	Odds ratio	1.0	16.1	156.1[c]	

[a]Chi square for trend = 183, p<0.00001
[b]One positive case was assumed for this cell to allow calculation of the odds ratio
[c]Chi square for trend = 173, p<0.00001

seropositive deer in the 1994 survey and none in 1996 were found in the northern counties. Five of the six positive specimens in the north were near the Mississippi River, the eastern border of Iowa. In the central region, only one county yielded >24% positive specimens, on the basis of four samples. These findings contrast with the results from the southern region, where in 1996 seropositive deer were found in 25 of the 31 counties and 20 counties had >25% positive samples.

Seroprevalence data, odds ratios and estimated numbers of seropositive deer were calculated to determine the strength of the trend of increasing seroprevalence with location (Table 2). In both survey years, a highly significant trend was observed (1994: chi square = 183, p <0.00001; and 1996: chi square = 173, p <0.00001). Analysis of seroprevalence data in each region and for the whole state across survey years demonstrated a significant increase only in the south (chi square = 5.3, p = 0.021) (Table 2). These figures indicate a 28.0% increase in statewide seroprevalence from 1994 to 1996, attributable mostly to increases in the southern region.

Conclusions

When prevalence rates were assessed for three regions of Iowa, a gradient of increasing seroprevalence was shown from north to south. This information can help direct occupational and public health efforts to regions of greatest risk for HME. In the southern region, one-fourth to one-third of deer were seropositive for antibodies against *E. chaffeensis*. Our data show that potential for HME is present in southern Iowa because of proximity to a disease-endemic area and the presence of the vector tick. This heavily wooded area provides ideal conditions for deer and ticks to come into contact. *E. chaffeensis*-seropositive deer were also found in the Mississippi River valley on the eastern border and near several interior rivers. These uninterrupted wet, wooded regions facilitate the spread of *E. chaffeensis* northward and westward in Iowa. The north-central area, which is relatively flat, has an abundance of deer but fewer forested areas, and the Lone Star tick is uncommon. Therefore, deer in this region are unlikely to have as much contact with the Lone Star tick as deer in southern Iowa or along wooded river valleys. Our data support the conjecture that the north-central area should be a region of sustained low prevalence of HME.

This study has several limitations. First, information on the deer population by county may have the same selection bias as our study, since much of the data are drawn from numbers of deer killed by hunters. According to Iowa DNR wildlife biologists, the actual deer population may differ from estimates by as much as 10%. Second, many counties had a limited number of specimens submitted. However, deer hunters tend to hunt where deer are most plentiful. Third, IFA has high sensitivity but unknown specificity;

therefore, cross-reactions with nonpathogenic *Ehrlichia* species could occur. However, cross-reactions to species such as *E. canis* and *E. ewingii* were unlikely, and no reports indicate that deer become infected with these organisms. As an emerging pathogen, *E. chaffeensis* has only recently been studied extensively. Although human cases of disease have occurred in Illinois, Missouri, and other states south of Iowa, disease transmission in Iowa has not been recognized. Most physicians are familiar with Lyme disease and Rocky Mountain spotted fever, but few are aware of ehrlichiosis and may not include it in differential diagnoses.

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References

1. Dumler JS, Bakken JS. Human ehrlichiosis: newly recognized infections transmitted by ticks. Annual Rev Med 1998;49:201-13.
2. Lockhart JM, Davidson WR, Stallknecht DE, Dawson JE. Site-specific geographic association between *Amblyomma americanum* (Acari: Ixodidae) infestations and *Ehrlichia chaffeensis* reactive (Rickettsiales: *Ehrlichieae)* antibodies in white-tailed deer. J Med Entomol 1996;33:153-8.
3. Ewing SA, Dawson JE, Kocan AA, Barker RW, Warner CK, Panciera RJ, et al. Experimental transmission of *Ehrlichia chaffeensis* (Rickettsiales: *Ehrlichieae)* among white-tailed deer by *Amblyomma americanum* (Acari: Ixodidae). J Med Entomol 1995;32:368-74.
4. Fishbein DB, Dawson JE, Robinson LE. Human ehrlichiosis in the United States, 1985 to 1990. Ann Intern Med 1994;120:736-43.
5. Hiller ILO. Habitat and food in the white-tailed deer. 1st ed. College Station (TX): Texas A&M University Press; 1997. p 17-8.
6. Dawson JE, Fishbein DB, Eng TR, Redus MA, Greene NR. Diagnosis of human ehrlichiosis with the indirect fluorescent antibody test: kinetics and specificity. J Infect Dis 1990;162:91-5.

Antimicrobial Resistance in *Salmonella* Enteritidis, Southern Italy, 1990-1998

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During 1990 to 1998, we identified multidrug-resistant isolates of *Salmonella* Enteritidis in southern Italy. Plasmids containing class I integrons and codifying for synthesis of extended-spectrum ß-lactamases were detected. Active surveillance for resistance to antimicrobial agents is needed to guard against the possible spread of resistant clones.

In the last decade, the incidence of *Salmonella* Enteritidis infections has increased in many countries. In Europe, this serotype now predominates among Salmonella isolates from humans (1). In southern Italy, identification of *S.* Enteritidis has increased steadily since 1990, in parallel with increases throughout Europe. After a temporary decline in 1995 and 1996, isolation rates from both sporadic cases and foodborne outbreaks increased. During 1998, records from the Center for Enteric Pathogens in southern Italy show identification rates of approximately 45% in all human Salmonella isolates and 61% in isolates from patients hospitalized for enteritis. In the Enteritidis serotype, resistance to antimicrobial drugs is rare, but resistance to antibacterial agents has been increasing in some Mediterranean countries (2).

We conducted a retrospective study of antimicrobial drug resistance patterns of *S.* Enteritidis isolates identified from human, animal, and environmental sources in southern Italy from 1990 to 1998. We also investigated mechanisms of resistance at the molecular level.

The Study

From 1990 to 1998, 1,889 strains of *S.* Enteritidis were referred to the Center for Enteric Pathogens, Palermo, southern Italy: 86% were of human origin, 2.9% from infected animals (mainly poultry), 6.7% from sewage plant effluents and surface water, and 4.4% from foods (mainly eggs and egg-based dishes). All strains were biochemically identified by standard tests and were serotyped for somatic and flagellar antigen identification. Phage types were determined with 10 typing phages (3).

Forty-four (2.2%) of the 1,889 strains tested were resistant to at least one antibiotic; we examined patterns of antibiotic resistance, phage types, and plasmid profiles of these 44 strains (Table). Resistance to ampicillin, alone or associated with other ß-lactams, and tetracycline, alone orassociated with aminoglycosides, sulfonamides, and trimethoprim, were the most commonly encountered phenotypes among the *S.* Enteritidis isolates studied. Of the 17 tetracycline-resistant strains, nine and eight, respectively, had transferrable plasmids of 80 and 30 MDal.

Six strains isolated from pediatric patients with enteritis (three in 1992, 1994, and 1996 in Sicily and three in 1997 in Calabria) were resistant to ampicillin, aztreonam, cephalotin, third-generation cephalosporins, and sulfonamides by the Kirby-Bauer method (7). Two of the 1997 isolates were also resistant to chloramphenicol. The double-disk synergy test was positive for all six isolates, suggesting the production of ESBL. In five cases, plasmids of 38, 70, and 80 MDa were shown by conjugation to mediate the complete pattern of resistance. In one strain identified in 1992, a 30-MDal plasmid was detected, but the resistance traits could not be transferred to recipient cells.

Six isolates of *S.* Enteritidis carried integrons with inserted regions of DNA of 0.8 to 2.5 kb (Table). Transconjugant *Escherichia coli* from these strains was also positive, indicating that

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Table. Resistance patterns of *Salmonella* Enteriditis strains, southern Italy, 1990–1998

Year	Source	Region	Phage types	Resistance pattern[a]	Plasmid pattern (mol. wt., MDa)	Resistance pattern of recipient *Escherichia coli*	Integrons (size of inserted regions, kb)
1990	human	Sicily	RDNC	Ap	36, 25		
1991	cake[b]	Sicily	4	Su, Tp, Tc	**80**,[c] 36	Tp, Tc	2.5
1992	seafood	Apulia	4	Ap	**30**	Ap	
1992	seafood	Apulia	4	Ap	36, **30**	Ap	
1992	human	Sicily	4	Ap, Kf, Atm, Caz, Cro, Ctx, Su	30		
1992	dog	Sicily	RDNC	Ap, Kf, Sm, Su, Tc	**30**	Ap, Sm, Su, Tc	
1992	human	Calabria	4	Gm, Sm, Su	80, 70		0.8
1992	human	Sicily	RDNC	Sm, Su, Tp	**80**, 36	Sm, Su, Tp	
1992	human	Calabria	1	Su, Tp, Tc	**80**, 36	Tp, Tc	1.5
1993	human	Calabria	7	Gm, Sm, Tc	**80**	Tc	
1993	human	Sicily	4	Sm, Tc	**80**, 36	Tc	
1993	human	Sicily	4	Sm, Su, Tp, Tc	**80**, 36	Sm, Su, Tp, Tc	
1993	human	Sicily	7	Sm, Su, Tp, Tc	**80**, 36	Sm, Su, Tp, Tc	
1994	human	Sicily	4	Ap, Kf, Atm, Caz, Cro, Ctx, Su	**80**, 36	Ap, Kf, Atm, Caz, Cro, Ctx	2.0
1994	human	Sicily	RDNC	Tc	36, **30**	Tc	
1994	human	Sicily	4	Tc	36, **30**	Tc	
1995	human	Calabria	4	Tc	36, 30	Tc	
1995	human	Apulia	4	Tc	**80**, 36	Tc	
1995	human	Apulia	7	Tc	36, **30**	Tc	
1996	human	Sicily	4	Ap, Kf, Atm, Caz, Cro, Ctx, Su	**80**, 36	Ap, Kf, Atm, Caz, Cro, Ctx	2.0
1996	human[b]	Sicily	RDNC	Tc	36, **30**	Tc	
1996	human	Apulia	RDNC	Tc	**30**	Tc	
1997	human	Sicily	4	Ap	36, 30		
1997	human	Sicily	4	Ap	36		
1997	human	Sicily	1	Ap	36, 30		
1997	human	Calabria	4	Ap, Kf, Atm, Caz, Cro, Ctx, Cm, Su	**70**, 36	Ap, Kf, Atm, Caz, Cro, Ctx, Cm	
1997	human	Calabria	4	Ap, Kf, Atm, Caz, Cro, Ctx, Cm, Su	**38**, 36	Ap, Kf, Atm, Caz, Cro, Ctx, Cm	
1997	human	Calabria	RDNC	Ap, Kf, Atm, Caz, Cro, Ctx, Cm, Su	**80**, 36	Ap, Kf, Atm, Caz, Cro, Ctx	2.0
1997	human	Apulia	1	Ap, Sm, Tc	36, **30**	Ap, Sm, Tc	
1997	human	Calabria	1	Ap, Sm, Tc	36, **32**	Ap, Sm, Tc	
1997	human	Sicily	4	Cm, Su, Tp	36, **32**	Cm, Su, Tp	
1997	human	Sicily	4	Su, Tp	36		
1997	poultry	Sicily	14b	Tc	**80**, 36	Tc	
1997	human	Sicily	14b	Tc	**80**	Tc	
1997	human	Sicily	NT	Tc	**80**	Tc	
1997	human	Sicily	13	Tc	**80**, 36	Tc	
1997	human	Sicily	RDNC	Tc, Nal	**80**, 36	Tc	
1998	human	Sicily	4	Ap	**70**, 36	Ap	
1998	sewage	Sicily	RDNC	Ap, Kf	36		
1998	human	Sicily	RDNC	Tc	36, **30**	Tc	
1998	human	Sicily	7	Tc	**30**	Tc	
1998	human	Sicily	6a	Tc	**80**, 36	Tc	
1998	human	Sicily	RDNC	Tc	36, **30**	Tc	
1998	poultry	Sicily	RDNC	Tc	**30**	Tc	

Ap, ampicillin; Kf, cephalotin; Atm, aztreonam; Caz, ceftazidime; Cro, ceftriaxone; Ctx, cefotaxime; Cm, chloramphenicol; Gm, gentamicin; Sm, streptomycin; Su, sulfonamides; Tp, trimethoprim; Tc, tetracycline; Nal, nalidixic acid; RDNC, reaction did not conform; NT, not typable.

[a]The strains were screened for resistance to ampicillin (10 µg), cephalotin (30 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), sulfonamides (300 µg), tetracycline (30 µg), and trimethoprim (5 µg). Strains resistant to cefotaxime were subsequently tested for susceptibility to aztreonam (30 µg), ceftazidime (30 µg), and ceftriaxone (30 µg). Resistance was determined by disk diffusion (4). The double-disk synergy test was performed (4) on strains presumed to produce extended-spectrum ß-lactamase (ESBL). Plasmid DNA was extracted by an alkaline lysis method (5). Electrophoresis on 0.7% agarose gels was performed on samples of plasmid DNA. The approximate molecular weight of plasmids was estimated by comparison with plasmids of known molecular size extracted from *Escherichia coli*. Conjugation experiments were carried out in Luria-Bertani broth. Transconjugant colonies of *E. coli* were selected after growth on MacConkey agar containing rifampin (300 µg/ml) and ampicillin (50 µg/ml), streptomycin (30 µg/ml), chloramphenicol (30 µg/ml), or tetracycline (30 µg/ml). All resistant isolates were screened for class I integrons by a strict protocol with oligonucleotide primers specific for the sequence of the 5'-CS and 3'-CS regions adjacent to the site-specific recombinational insertion sequence (6). Primer sequences were 5'-CS, GGCATCCAAGCAGCAAG and 3'-CS,A AGCAGACTTGACCTGA (5).

[b]Source in outbreak.

[c]Numbers in bold indicate the approximate molecular size of resistance plasmids.

the integrons were carried on plasmids. DNA fragments of approximately 2.0 kb were obtained from ESBL-producing strains.

Conclusions

During the 9-year study, a small proportion of resistant strains was found within Enteritidis, 2.3% showing resistance to at least one antimicrobial drug and 0.9% to three or more. Prevalence in southern Italy was similar to that in other European countries, such as England and Wales (8) and the Czech Republic (9); however, it was lower than prevalence detected from 1987 to 1993 in Greece, where up to 67.4% of strains of *S.* Enteritidis from human and nonhuman sources were resistant to antibiotics and the resistance rate increased steadily until 1991 (2). No temporal trend or possible association with source was investigated in resistance patterns identified in southern Italy because resistant strains are rare and usually from human sources.

The unusual characteristics of antimicrobial resistance of some *S.* Enteritidis isolates highlight the problem of emergence of drug resistance in a common serotype of Salmonella, transmitted in popular food items and often implicated in foodborne outbreaks. We identified six ESBL-producing isolates from epidemiologically unrelated cases, a rare finding (10-12). All six strains were isolated from community-acquired enteritis cases in otherwise healthy children, who had no recent history of hospitalization or antimicrobial therapy. This observation is not consistent with the hypothesis that multidrug-resistant clones are selected or resistance determinants are acquired as a consequence of antibiotic treatment. Moreover, the presence of integrons in strains isolated as long ago as 1991 is of particular concern because of the ability of these elements to disseminate resistance traits by intra- and inter-specific gene transfer (13,14).

Although most isolates identified in southern Italy were susceptible, some aspects of the epidemiology of *S.* Enteritidis are cause for concern. Active monitoring of *S.* Enteritidis strains for resistance to antibacterial drugs seems crucial because of the public health implications of a potential spread of resistant clones.

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References

1. Fisher J. Salm/Enter-Net records a resurgence in *Salmonella* enteritidis infection through the European Union. Eurosurveillance Weekly 1997;1:June 26.
2. Tassios PT, Markogiannakis A, Vatopoulos AC, Katsanikou E, Velonakis EN, Kourea-Kremastinou J, et al. Molecular epidemiology of antibiotic resistance of *Salmonella* enteritidis during a 7-year period in Greece. J Clin Microbiol 1997;35:1316-21.
3. Ward LR, De Sa JDH, Rowe B. A phage-typing scheme for *Salmonella* enteritidis. Epidemiol Infect 1987;99:291-4.
4. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. Villanova (PA): The Committee; 1997.
5. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 1979;7:1513-23.
6. Lévesque C, Piché L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother 1995;39:185-1.
7. Bauer AW, Kirby MMW, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
8. Threlfall EJ, Ward LR, Skinner JA, Rowe B. Increase in multiple antibiotic resistance in nontyphoidal Salmonellas from human in England and Wales: a comparison of data for 1994 and 1996. Microb Drug Resist 1997;3:263-6.
9. Sramova H, Karpiskova R, Dedicova D, Sisak F, Rychlik I. Properties of Salmonella isolates in the Czech Republic. Epidem Mikrobiol Immunol 1999;48:111-6.
10. Cherian BP, Singh N, Charles W, Prabhakar P. Extended-spectrum beta-lactamase-producing *Salmonella* enteritidis in Trinidad and Tobago. Emerg Infect Dis 1999;5:181-2.
11. Blahova J, Lesicka-Hupkova M, Kralikova K, Krcmerry V, Krcmeryova T, Kubonova K. Further occurrence of extended-spectrum-beta-lactamase-producing *Salmonella* enteritidis. J Chemother 1998;10:291-4.
12. Gaillot O, Clement C, Simonet M, Philippon A. Novel transferable beta-lactam resistance with cephalosporinase characteristics in *Salmonella* enteritidis. J Antimicrob Chemother1997;39:85-7.
13. Stokes H, Hall RA. A novel family of potential mobile DNA elements encoding site-specific gene integration functions: integrons. Mol Microbiol 1989;3:1669-83.
14. Rankin SC, Coyne MJ. Multiple antibiotic resistance in *Salmonella* enterica serotype enteritidis. Lancet 1998;351:1740.

Lyme Disease Surveillance in England and Wales, 1986–1998

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Improved surveillance indicates that Lyme borreliosis, an emerging zoonosis in the United Kingdom, has increased from 0.06/100,000 during 1986-1992 to 0.32/100,000 since 1996. Case reports peaked in the third quarter of each year. Several high-incidence localities were identified. Erythema migrans was reported in 41% of patients; arthritis in 4%; musculoskeletal symptoms in 18%; and neuroborreliosis in 15%.

Lyme borreliosis, a zoonosis caused by the spirochete *Borrelia burdorferi* sensu lato and transmitted by *Ixodes* ticks, is the most prevalent and widespread vector-borne human infection in the Northern Hemisphere, with enzootic cycles that can be maintained in a wide range of ecologic conditions (1). Reported annual incidence rates throughout Europe range from 16 cases/100,000 voluntarily reported in France, to 80/100,000 in seven counties in southern Sweden where the disease became temporarily reportable in 1992-93, and 120/100,000 in Slovenia (2), where it is also reportable. Lyme borreliosis appears to be rare in the United Kingdom, although *B. burgdorferi* s.l. has been detected by polymerase chain reaction (PCR) in many tick populations and in archived specimens collected over the last 100 years (3).

The Study

Surveillance for Lyme borreliosis has been ongoing in England and Wales since September 1986, based mainly on voluntary reporting of serologically confirmed cases by laboratories to the Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre (CDSC)(4). In 1992, the case definition of the Centers for Disease Control and Prevention became available (5), and in 1996 a European case definition came into use (6). Serologic diagnosis in the two reference laboratories in England and Wales follows an internationally recommended two-step approach (7) of antibody screening tests followed by immunoblots of reactive or equivocal samples. Reference laboratory serologic tests have improved, with continuing refinement of immunoblot techniques and interpretation, as well as introduction of immunoglobulin (Ig)M tests. Borrelial cultures, PCR, and genotyping have also been introduced, mainly for research. Recently, commercial antibody test kits have become more widely available, and many diagnostic laboratories now use them for preliminary screening and send only reactive samples to reference laboratories for immunoblot testing. Some kits have IgM detection components (either singly or in combination with IgG) and may detect antibody production at an earlier stage of infection than previously possible.

Serologically confirmed cases of Lyme borreliosis should be voluntarily reported to CDSC by the primary laboratory that referred the patient's specimen for confirmation, but reporting has been incomplete. An enhanced surveillance scheme was introduced in 1996, with reference laboratories also reporting to CDSC. Since 1997 the PHLS reference laboratory has routinely sent a questionnaire to primary laboratories seeking information on patient demographics, clinical features, and tick exposure. This strategy has greatly improved Lyme borreliosis surveillance, with an 85% return rate

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of questionnaires in the first full year of implementation. From 1986 to 1992, CDSC received 227 reports, a mean annual rate of 0.06/100,000. This rate increased to an average annual rate of 0.11/100,000 during 1992-1996 (n=235) and to 0.32/100,000 in the first 2 years of enhanced surveillance (n=334).

The age, sex, and seasonal distribution of cases were similar in each period. Of the 796 patients, 85 (10.6%) were children < 15 years of age, 45 (5.7%) were 15-24 years of age, 220 (27.6%) 25-44, 300 (37.7%) 45-64, and 129 (16.2%) were ≥65 years of age; in 17 cases, the patient's age was not known. The male:female ratio was approximately equal in all age groups.

A clear, consistent seasonal pattern was seen, with 48% of cases reported in the third quarter of each year. The peak in laboratory reports was in September, representing peak onset of symptoms in early summer. Cases were reported by laboratories in 68 counties (Figure), but only 14 counties had more than 10 cases each. Forty-five percent of reports were of cases in three contiguous counties in southern England: 219 from Hampshire, 72 from Wiltshire, and 61 from Dorset. This area includes well-known foci of Lyme borreliosis in and near the New Forest and Salisbury Plain. Other counties with a relatively high number of case reports were Devon (47 cases) and Somerset (32 cases) in southwestern England and Norfolk in East Anglia (29 cases). One hundred eighteen (14.8%) of the 796 cases were reported to have been acquired abroad, mainly in the United States, France, Germany, Austria, and Scandinavia. Most of these cases occurred in vacationers. Forty-six (5.8%) cases were apparently acquired occupationally: nine through work with or hunting of deer, seven through forestry work, three through farm work, one through exposure during tick ecology studies, and another through work as a veterinary surgeon. For the others, occupational details were not reported. The proportion of Lyme borreliosis patients with a reported history of tick bites has risen steadily, from 24% in 1986-1992 and 32% in 1993-1996 to 39% in 1997-98. Other patients (30%, 34% and 42% in the corresponding periods) gave histories of insect bites and may not have distinguished between insects and ticks.

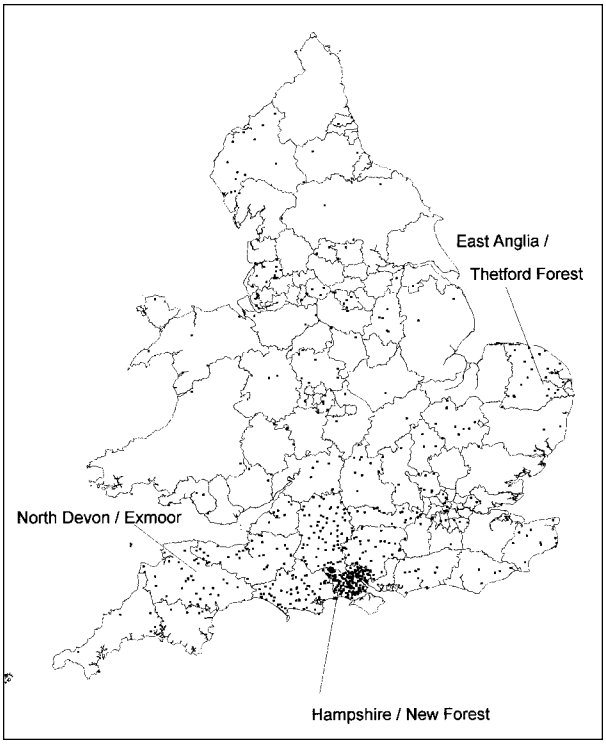

Figure. Density plot of distribution of indigenously acquired Lyme borreliosis cases, by county of primary testing laboratory, England and Wales, 1986-1998.

Clinical features (Table) reported over the 13-year period include neuroborreliosis (facial palsy, radiculopathy, meningitis, and "neuroborreliosis") and nonspecific neurologic symptoms (e.g., fatigue, malaise, and headache). Neuroborrelosis was reported in 23% (21/91) of patients ≤ 14 years of age, compared with 14% (96/701) in other age groups ($x^2 = 5.6$, $p = 0.02$), a difference explained by a higher reported incidence of isolated facial palsy in children.

Musculoskeletal symptoms have been divided into "arthritis" and "other," which includes arthralgia and myalgia. Arthritis was reported in 9 (20%) of 45 patients 15-24 years of age, compared with 22 (3%) of 746 patients in other age groups ($x^2 = 28.5$; $p < 0.001$). The proportions of cases associated with both a tick bite and erythema migrans increased from 12% in 1986-1992 to 22% in 1997-98. Only three cases of acrodermatitis chronica atrophicans were reported, and cardiac involvement was reported in only five cases.

Arthritis was reported in 6 (14%) of 42 cases acquired in the United States, compared with none of 8 Scandinavian cases, 1 of 55 other European cases, and none of 47 New Forest cases (U.S. vs. other odds ratio [OR] = 18; 95% confidence interval [CI] 2-835; Fisher's exact p =

Table. Clinical features reported in 796 patients with Lyme borreliosis, England and Wales, 1986–1998

	1986-1992 No. (%)	1993-1996 No. (%)	1997-98 No. (%)	Total No. (%)
Total reports[a]	227 (100)	235 (100)	334 (100)	796 (100)
Any insect bite	67 (30)	79 (34)	140 (42)	286 (36)
Tick bite	53 (23)	74 (32)	128 (38)	255 (32)
Arthritis	16 (7)	5 (2)	11 (3)	32 (4)
Other musculo- skeletal symptoms	58 (26)	31 (13)	49 (15)	138 (17)
Skin involvement	80 (35)	125 (53)	204 (61)	409 (51)
Erythema migrans	64 (28)	97 (41)	164 (49)	325 (41)
Erythema migrans & tick bite	27 (12)	41 (17)	72 (22)	140 (18)
Neuroborreliosis	47 (21)	28 (12)	43 (13)	118 (15)
Other neurologic symptoms	46 (20)	16 (7)	20 (6)	82 (10)
Cardiac involvement	2 (0.9)	1 (0.4)	2 (0.6)	5 (0.6)

[a]Patients may have multiple exposures/symptoms.

0.001). Neuroborreliosis was reported in 6 (14%) of 42 U.S.-acquired cases, none of 8 Scandinavian cases, 6 (11%) of 55 other European cases, and 12 (26%) of 47 New Forest cases. Erythema migrans was reported in 13 (31%) of 42 U.S.-acquired cases, 7 (88%) of the 8 Scandinavian cases, 21 (38%) of 55 other European cases, and 28 (60%) of 47 New Forest cases (U.S. v other OR = 0.26; CI = 0.11-0.58; $x^2 = 11.8$; $p < 0.001$).

Conclusions

The large increase in Lyme disease incidence in 1997 and 1998 may represent a surveillance artefact resulting from increased awareness of the disease, greater access to diagnostic facilities, more sensitive diagnostic methods, and more complete reporting to CDSC. Awareness of the infection has increased rapidly as a result of media interest and ready access to medical information from Internet sources. However, Lyme borreliosis is probably not as widely recognized in the United Kingdom as in some other European countries (8). Enhanced long-term surveillance and additional cross-checking between the PHLS reference laboratory and CDSC are needed to allow detection of the time trends in disease incidence. The apparent widening of the geographic areas reporting cases is probably due to improved reporting, but current surveillance data indicate areas with high disease incidence from recreational and occupational exposures.

Population surveillance has clarified the clinical manifestations of Lyme borreliosis in England and Wales. However, the proportion of patients with erythema migrans may be relatively low, either because patients with erythema migrans are easily identified without serologic tests or because serologic tests have relatively low sensitivity at this early stage. This underreporting could bias reporting towards a higher proportion of the more complicated later-stage infections (2) or those with atypical acute symptoms.

Differences between clinical symptoms in the United States and Europe have been attributed to differences in prevalence of *B. burgdorferi* genospecies. In the United States, *B. burgdorferi* sensu stricto predominates, with an associated pattern of musculoskeletal complications. Thus, patients with U.S.-acquired infections had a higher rate of arthritis than those whose cases were acquired in mainland Europe. In Europe, at least two other pathogenic genospecies are found in addition to *B. burgdorferi* s.s.: *B. afzelii*, which is associated with skin manifestations, and *B. garinii*, which is associated with neurologic complications (9). Another strain, *B. valaisiana*, does not appear to be associated with manifestations of disseminated borreliosis. These genospecies have been identified in British tick populations, consistent with the broad range of clinical presentations seen in the United Kingdom. The relatively high prevalence of the apparently less pathogenic *B. valaisiana* in some British tick populations may be a factor in the low incidence of Lyme borreliosis observed in the United Kingdom.

Both the prevalence of *B. burgdorferi*-infected ticks and the incidence of Lyme borreliosis are highest in eastern Europe and decrease westward across the continent, including the

British Isles (2). Throughout Europe, heterogenous deciduous woodlands provide favorable ecologic conditions for host species that maintain both ticks and spirochetes (10), and the high-incidence areas in the United Kingdom conform to this description. However, the distribution of infected ticks is far wider than that of reported human cases, and Lyme borreliosis is an important potential emerging zoonosis in England and Wales. Factors that could increase incidence include changing patterns of land use, especially for recreation, and changes in the density of animals that act as reservoir hosts. Climatic factors such as drought or prolonged cold weather can substantially affect tick populations and their level of activity, which may be reflected in fluctuations in the incidence of Lyme borreliosis (11).

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References

1. Gern L, Estrada-Peña A, Frandsen F, Gray JS, Jaenson TGT, Jongejan F, et al. European reservoir hosts of *B.burgdorferi* sensu lato. Zentralbl Bakteriol 1998; 287:196-204.
2. O'Connell S, Granstrom M, Gray JS, Stanek G. Epidemiology of European Lyme borreliosis. Zentralbl Bakteriol 1998; 287:229-40.
3. Hubband MJ, Baker AS, Carr KJ. Distribution of *Borrelia burgdorferi* s.l. spirochaete DNA in British ticks (*Argasidae* & *Ixodidae*) since 19th century assessed by PCR. Med Vet Entomol 1998; 12:89-97.
4. Grant AD, Eke B. Application of information technology to the laboratory reporting of communicable disease in England and Wales. Commun Dis Rep 1993;3:R75-8.
5. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance: Lyme disease (revised 9/96). MMWR Morbid Mortal Wkly Rep 1997; 6(suppl RR-10):20-1.
6. Stanek G, O'Connell S, Cimmino M, Aberer E, Kristoferisch K, Granstrom M, et al. European Union concerted action on Lyme Borreliosis: clinical case definitions for Lyme borreliosis. Wien Klin Wochenschr 1996;106:742-7.
7. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the second national conference on serological diagnosis for Lyme disease. MMWR Morbid Mortal Wkly Rep 1995;4:590-1.
8. Gray JS, Granstrom M, Cimmino M, Daniel M, Gettinby G, Kahl O, et al. Lyme borreliosis awareness. Zentralbl Bakteriol 1998; 287:253-65.
9. Saint Girons I, Gern L, Gray JS, Guy EC, Korenburg E, Nuttall PA, et al. Identification of *Borrelia burgdorferi* sensu lato species in Europe. Zentralbl Bakteriol 1998;287:190-5.
10. Gray JS, Kahl O, Robertson JN, Daniel M, Estrada-Peña A, Gettinby G, et al. Lyme borreliosis habitat assessment. Zent bl Bakteriol 1998;287:211-28.
11. Gray JS. The development and seasonal activity of the tick *Ixodes ricinus*: a vector of Lyme borreliosis. Rev Med Entomol 1991;79:323-33.

Borrelia burgdorferi and the Causative Agent of Human Granulocytic Ehrlichiosis in Deer Ticks, Delaware

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During the 1998 hunting season in Delaware, 1,480 ticks were collected from 252 white-tailed deer; 98% were *Ixodes scapularis*, a significant increase from the 85% reported in 1988. Ticks were tested for *Borrelia burgdorferi* and the causative agent of human granulocytic ehrlichiosis. Infection rates remained stable in New Castle and Kent counties, but increased from <1% to 8% in Sussex County.

Delaware has one of the highest incidence rates of Lyme disease in the United States, with an average annual rate over the past 10 years of 14.43 cases/100,000 (1). *Ixodes scapularis*, the primary vector of *Borrelia burgdorferi*, the causative agent of Lyme disease, has also been implicated in transmission of the causative agent of human granulocytic ehrlichiosis (HGE) (2). From 1986 to 1997, 449 cases of HGE were reported in the United States (3), none of them in Delaware, where HGE is not a reportable illness.

To determine if there has been an increase in *I. scapularis* or *B. burgdorferi* infection rate and if the causative agent of HGE is present in Delaware, we tested ticks by polymerase chain reaction (PCR) for both *B. burgdorferi* and the causative agent of HGE (4,5). The only available data on *B. burgdorferi* infection rates and tick density in Delaware are from a 1988 study of ticks parasitizing hunter-harvested white-tailed deer, *Odocoileus virginianus* (6).

The Study

During the 1998 hunting season, hunters brought deer to six check stations operated in three counties by the Delaware Division of Natural Resources. Deer were inspected for ticks during November 13–21, 1998. Information collected for each deer included the county of origin, sex, and approximate age and weight. As in the 1988 study, one side of the head, neck, and shoulder of each deer was combed for ticks. All ticks were placed in 70% ethanol and held for identification. The percentage of *I. scapularis* among the ticks collected in each county and statewide was compared with the percentage reported in the 1988 study (Z-test for comparisons among proportions) (7).

Because heme can interfere with PCR accuracy (8), engorged ticks were excluded from testing. Fifty *I. scapularis* ticks were randomly selected from each of the three Delaware counties from the remaining males and unengorged females. Bacterial DNA was recovered from the ticks by methods modified from those described by Persing et al. (9). Briefly, ticks were placed in a sterile microfuge tube along with 20 µl of sterile 0.5-mm glass beads treated with 1% bovine albumin. Sterile 0.1 M Tris buffer (25 µl, pH 8.3) was added, and the ticks were crushed into the beads for one min to release the midgut contents. Samples were boiled for 5 min, immediately placed in ice water for 2 min, and held at 4°C until used for PCR.

For *B. burgdorferi*, oligonucleotide primers OSP-A1 and OSP-A2 (5) were obtained from Only DNA (Midland, TX). PCR was performed in a Hybaid thermal cycler by using 0.5 µM of each primer, 5 µL of tick extract (template), and 25 µL REDTAQ PCR mixture (Sigma). Components were denatured at 94°C for 2 min and then subjected to 30 cycles of denaturing (94°C for 30 sec), annealing (54°C for 30 sec), and extension (72°C for 2 min). Samples were analyzed by

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electrophoresis on 0.8% agarose, stained with ethidium bromide, and viewed on a UV transilluminator. Samples were considered positive if the expected 158-bp fragment was seen.

For the HGE agent, we used a nested PCR as described by Massung et al. (10). Primers ge3a and ge10r were obtained from Only DNA. The first round of PCR was carried out in a Hybaid thermal cycler with 0.5 µM of each primer, 5 µL of tick extract (template), and 25 µL of REDTAQ PCR mixture (Sigma). Components were denatured at 95°C for 2 min and then subjected to 40 cycles of denaturing (94°C for 30 sec), annealing (55°C for 30 sec), and extension (72°C for 2 min). Samples were analyzed by electrophoresis on 0.8% agarose, stained with ethidium bromide, and viewed on a UV transilluminator. Samples were considered positive if the expected 932-bp fragment was seen.

Positive specimens were analyzed in a second round of PCR with primers ge9f and ge2 (10) from Only DNA and 5 µL of the positive primary PCR solution. Amplification and analysis conditions were identical to those of the first round, except 30 cycles were used. Samples were considered positive if the expected 546-bp product was seen in the second round of PCR. Quality control measures included positive controls from infected *I. scapularis* nymphs maintained in colonies at Yale University, as well as from field-collected adults. Negative samples were reagent blanks with buffer instead of tick extract.

During November 13-21, 1998, 252 deer were examined, and 1,480 ticks were collected (Table). The percentage of *I. scapularis* collected in New Castle County remained stable from 1988 (99%) to 1998 (98%) (Figure 1). In Kent County, Sussex County, and statewide, the proportion of *I. scapularis* increased significantly, from 93% in 1988 to 100% in 1998 ($z = 5.0$, $\alpha = 0.05$). In Sussex County in 1988, 62% of the ticks collected were *I. scapularis*; by 1998 the proportion was 96% ($z = 17.0$, $\alpha = 0.05$). Statewide, the proportion of *I. scapularis* also rose significantly, from 85% in 1988 to 98% in 1998 ($z = 13.3$, $\alpha = 0.05$).

Table. Ticks obtained from hunter-harvested deer in Delaware, November 1998

County	No. deer inspected	% deer infested with ticks	*Dermacentor albipictus*	*Ixodes scapularis*	Total
New Castle	102	85	9	564	573
Kent	59	83	0	301	301
Sussex	91	84	15	591	616
Statewide	252	84	24	1,456	1,480

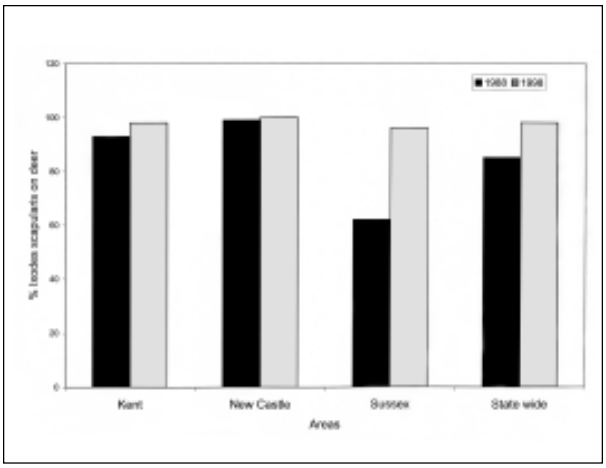

Figure 1. The proportion of *Ixodes scapularis* among ticks parasitizing white-tailed deer in Delaware, 1988 (2) and 1998.

Of the 50 ticks tested from New Castle County, six were positive for *B. burgdorferi* and two for the HGE agent. In both Kent and Sussex counties, 4 of the 50 ticks tested positive for *B. burgdorferi*, while none tested positive for the HGE agent. None were positive for both organisms.

Conclusions

This study confirms the presence of the causative agent of HGE in New Castle County, Delaware. Although the infection rate in ticks is low (4%), physicians should be aware of the risk for this disease. In Alabama, where an infection rate of 3% was reported for *B. burgdorferi* in *I. scapularis* (11), 54 cases of Lyme disease were reported by 1996 (12). Most confirmed cases of HGE occur in states that, like Delaware, have high incidence rates of Lyme disease (13). Although neither of the HGE-positive ticks from Delaware tested positive for *B. burgdorferi*, such simultaneous infection has been reported elsewhere in ticks (3,14,15), as well as humans (16).

In Delaware, Lyme disease was reported more frequently in 1998 than in 1988 (1). The largest increase in the *B. burgdorferi* infection rate corresponds with the greatest increase in *I. scapularis*. Sussex County had the largest increase in the proportion of *I. scapularis* among

ticks found on deer. In 1988, 62% of the ticks parasitizing deer were *I. scapularis*; this proportion increased to 96% by 1998 (Figure 1). Sussex County also had the largest increase in *B. burgdorferi* infection rates (Figure 2). Our data indicate that the high Lyme disease incidence may be attributed to an increase in the *B. burgdorferi* infection rate in *I. scapularis* in some areas. Previous studies have demonstrated a correlation between the number of cases of Lyme disease and the numbers of *I. scapularis* (17,18).

To test ticks concurrently for both *B. burgdorferi* and the causative agent of HGE, we used PCR, while the 1988 study used immunofluorescent assay (2). Comparing infection rates obtained by different procedures may be problematic; for example, the elevated infection rate of *B. burgdorferi* in Sussex County could result from the greater sensitivity of PCR (15). However, we saw a large increase in infection rate only in Sussex County, where there is a corresponding increase in the proportion of *I. scapularis*. Our results show that at least in Sussex County *I. scapularis* is more common, the infection rate for *B. burgdorferi* is higher, and thus the risk for Lyme disease is higher now than it was 10 years ago. To allow comparison of infection rates between studies, we examined ticks parasitizing deer, even though a better assessment of human risk would have been to examine infection rates in questing ticks.

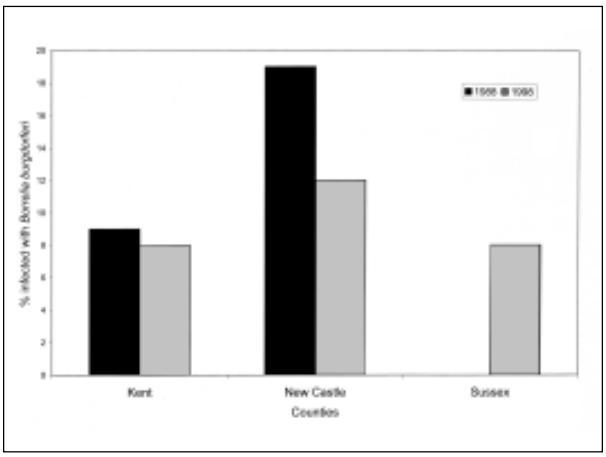

Figure 2. *Borrelia burgdorferi* infection rates in *Ixodes scapularis* parasitizing white-tailed deer in Delaware, 1988 (2) and 1998.

The distribution of Lyme disease and HGE in Delaware remains poorly defined. Examining ticks collected from hunter-harvested animals provides only a rough indication of where the ticks and bacteria occur. Deer can travel great distances; hunters may not reveal the exact location of the hunt; and infection rates can vary within the same region (15). Therefore, relying on ticks collected from deer does not provide sufficient information about specific locations where risk for exposure to Lyme or HGE may be elevated.

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References

1. Centers For Disease Control and Prevention. Lyme disease: epidemiology. 1999. [cited 1999 Dec 1] Available from URL: http://www.cdc.gov/ncidod/dvbid/lymeepi.htm
2. Pancholi P, Kolbert CP, Mitchell PD, Reed KD, Dumler JS, Bakken JS, et al. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J Infect Dis 1995;172:1007-12.
3. McQuiston JH, Paddock CD, Holman RC, Childs JE. The human ehrlichioses in the United States. Emerg Infect Dis 1999;5:635-42.
4. Persing DH, Telford SR, Spielman A, Barthold SW. Detection of *Borrelia burgdorferi* infection in *Ixodes dammini* ticks with the polymerase chain reaction. J Clin Microbiol 1990;28:566.
5. Magnarelli LA, Stafford KC, Mather TN, Yeh MT, Horn KD, Dumler JS, et al. Hemocytic Rickettsia-like organisms in ticks: serologic reactivity with antisera to ehrlichiae and detection of DNA of agent of human granulocytic ehrlichiosis by PCR. J Clin Microbiol 1995;33:2710-4.
6. Wolfe D, Fries C, Reynolds K, Hathcock L. The epidemiology of Lyme disease in Delaware 1989-1992. Del Med J 1994; 66:603-13.
7. Walpole RE, Myers RH. Probability and statistics for engineers and scientists. 2nd ed. New York: Macmillan Publishing Co., Inc.; 1978.
8. Schwartz IS, Varde S, Nadelman RB, Wormser GP, Fish D. Inhibition of efficient polymerase chain reaction amplification of *Borrelia burgdorferi* DNA in blood fed ticks. Am J Trop Med Hyg 1997;56:339-42.

9. Persing DH, Telford SR, Rys PN, Dodge DE, White TJ, Malawista SE, et al. Detection of *Borrelia burgdorferi* DNA in museum specimens of *Ixodes dammini* ticks. Science 1990;249:1420.
10. Massung RF, Slater K, Owens JH, Nicholson WI, Mather TN, Solberg VB, et al. Nested PCR assay for detection of granulocytic Ehrlichia. J Clin Microbiol 1998;36:1090.
11. Luckhart S, Mullen GR, Wright JC. Etiological agent of Lyme disease, *Borrelia burgdorferi*, detected in ticks (Acari:Ixodidae) collected at a focus in Alabama. J Med Entomol 1991;28:652-7.
12. Centers for Disease Control and Prevention. Lyme disease—United States, 1996. MMWR Morb Mortal Wkly Rep 1997;46:531-5.
13. Centers for Disease Control and Prevention. Statewide surveillance for ehrlichiosis—Connecticut and New York, 1994-1997. MMWR Morb Mortal Wkly Rep 1998;47:476-80.
14. Varde S, Beckley J, Schwartz I. Prevalence of tick-borne pathogens in *Ixodes scapularis* in a rural New Jersey county. Emerg Infect Dis 1998;4:97-9.
15. Daniels TJ, Boccia TM, Varde S, Marcus J, Le J, Bucher DJ, et al. Geographic risk for Lyme disease and human granulocytic ehrlichiosis in southern New York state. Appl Environ Microbiol 1998;64:4663-9.
16. Nadelman RB, Horowitz HW, Hsieh TC, Wu JM, Aguero-Rosenfeld ME, Schwartz I, et al. Simultaneous human granulocytic ehrlichiosis and Lyme borreliosis. N Engl J Med 1997;337:27-30.
17. Centers for Disease Control and Prevention. Lyme disease—United States, 1987-1988. MMWR Morb Mortal Wkly Rep 1989;38:668-72.
18. Falco RC, McKenna DF, Daniels TJ, Nadelman RB, Nowakowski J, Fish D, et al. Temporal relation between *Ixodes scapularis* abundance and risk for Lyme disease associated with Erythema migrans. Am J Epidemiol 1999;149:771-6.

Primary Care Surveillance for Acute Bloody Diarrhea, Wales

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A sentinel group of primary-care physicians in Wales actively reported cases of acute bloody diarrhea from February 1997 through December 1998. The estimated annual rate was 18 cases per 100,000 population. Most (80%) cases were due to *Campylobacter* or *Salmonella;* however, 18% were undiagnosed.

The number of cases of acute bloody diarrhea in the United Kingdom and elsewhere is unknown. In addition, while some established etiologic agents of acute bloody diarrhea (*Salmonella*, *Campylobacter*, *Shigella,* and Shiga toxin-producing *Escherichia coli* [STEC] O157) are routinely detected in local diagnostic laboratories, others, such as non-O157 STEC, are not and therefore would not be ascertained by current surveillance. In the United Kingdom, all residents are registered with a general practitioner (a primary-care physician). Since blood in the stool is disturbing to patients, they would be likely to report it to their physicians. We therefore used surveillance by a sentinel group of volunteer general practitioners to estimate cases of acute bloody diarrhea in Wales, detect any clusters, and identify the etiologic agents.

An established network of 34 volunteer general practitioners' offices in Wales (combined registered practice population 223,465 in 1998, representing 8% of the 2,933,324 population) routinely reports cases of measles, mumps, rubella, shingles, chickenpox, influenza, whooping cough, and infective gastroenteritis to the Public Health Laboratory Service Communicable Disease Surveillance Centre (CDSC) (Wales) (1,2). Acute bloody diarrhea was introduced as a reporting category in February 1997, with a clinical case definition of "diarrhea and visible blood of acute onset (first episode only)." Reporting was weekly by paper; data items recorded were age, sex, practice name, and report date. From February 1997 to December 31, 1998, stool samples, requested from the patient by the general practitioner, were submitted to the local clinical diagnostic laboratory. Bacteriologic tests were performed for *Salmonella, Campylobacter, Shigella*, and STEC O157 by culture; ova and parasites were examined by microscopy. Laboratories were contacted for results.

Data were analyzed by person, time, and place. Clusters were defined as three or more cases of acute bloody diarrhea reported in any 2 consecutive weeks by the same practice. (A practice is required, contractually, to specify to the health authority a defined geographic area where all its registered patients should reside.) We used the Poisson distribution to calculate 95% confidence intervals (CIs) for the number of cases; rates were calculated by using the combined practice population of 223,465 as the denominator.

A total of 81 cases of acute bloody diarrhea were reported during the 23-month study period (mean annual rate, 18 per 100,000); 31 cases (95% CI = 21 to 44) were reported from February through December 1997, and 50 cases (95% CI = 37 to 65) during 1998. The age-sex distribution was similar each year, with the highest incidence in young children ages ≤4 years, particularly girls, and the lowest in adults over 65 years. The age distribution differed by sex: rates did not vary in male patients >5 years of age but were high in 25- to 44-year-old women (Table 1). No seasonal distribution pattern was noted.

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Table 1. Age and distribution of cases (and annual rates per 100,000 practice population with 95% confidence intervals) of acute bloody diarrhea reported by sentinel general practitioners in Wales, 1997–98

Age	Total	Males	Females
≤4	15 (63:35-104)	6 (50:18-109)	9 (76:35-144)
5-14	9 (16:7-30)	4 (14:14-36)	5 (18:6-42)
15-24	6 (12:4-26)	4 (15:4-38)	2 (8:1-29)
25-34	15 (25:14-41)	5 (16:5-37)	10 (33:16-61)
35-44	14 (24:13-40)	4 (14:4-36)	10 (34:16-63)
45-64	15 (13:7-21)	6 (11:4-24)	9 (16:7-30)
65+	7 (9:4-19)	3 (9:2-26)	4 (8:2-20)
Total	81 (18:14-22)	32 (15:10-21)	49 (22:16-29)

In 1997, three clusters were identified, one with four cases, and two with three cases each. Within the first cluster, *Campylobacter* was isolated in two cases, but in the other two clusters both *Salmonella* and *Campylobacter* were isolated. General practitioners' records did not show links between cases within these clusters. In 1998, two clusters were identified. In the first, five persons had *Salmonella;* three patients belonged to the same family, but the other two were not linked to the family or to each other. The second cluster was composed of two sisters with *Salmonella* and two unlinked cases, one *Salmonella* and one *Campylobacter*.

Stool specimens were obtained from 74 (91%) of 81 patients with acute bloody diarrhea. *Salmonella* organisms were isolated from 30 (41%) patients, 11 male and 19 female, ages 8 months to 74 years. *Campylobacter* was isolated from 29 (39%) patients, 13 male and 16 female, ages 1 month to 65 years. All five cases of acute bloody diarrhea in men ages 25-34 years were *Campylobacter*. *Shigella* was isolated in one case, a woman in the 35- to 44-year age group. STEC O157 was not detected (upper 95% CI = 3.7 cases, equivalent to 1.6 per 100,000 total population in Wales). *Cryptosporidium* was detected in one case. A total of 13 (18%) cases (7 in male, 6 in female patients) were undiagnosed by the local laboratory. Stool specimens in 3 of these cases were sent to the Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale, London, for testing for other STEC serogroups; none was detected.

This is the first measurement of cases of acute bloody diarrhea in Wales. Since the study sample is broadly representative of the Welsh population (2), the mean annual rate of 18 per 100,000 practice population would equate to 530 cases in Wales as a whole. While a sentinel practice-based scheme cannot measure the true prevalence, it is an economical and efficient way to estimate the order of magnitude of the disease. The low frequency of acute bloody diarrhea and the rarity with which it is encountered by any individual practitioner limit greater precision. The range calculated from the 95% CIs in the study extrapolates to 275 to 850 cases per year in Wales. The difference between the number of cases reported during 11 months in 1997 and the whole of 1998 is not statistically significant since the 95% CIs overlap, but some increase may have occurred as reporting became more efficient.

A high proportion of patients (91%) supplied stool specimens, compared with 67% in a general practitioner-based survey of patients with nonbloody diarrhea (3). *Campylobacter* and *Salmonella*, known causes of bloody diarrhea, were isolated from 80% of specimens, and the epidemiologic characteristics of cases of acute bloody diarrhea frequently reflected those of these organisms (4). For example, the higher annual incidence of acute bloody diarrhea in children <5 years of age (63 per 100,000, compared with 18 per 100,000 for the entire study population) is reflected in laboratory reports to the CDSC (Wales) during the study period (i.e., for *Campylobacter*, an annual incidence of 130 per 100,000, with an incidence in children <5 years of age of 234 per 100,000; for *Salmonella,* an incidence of 68 per 100,000, with an incidence in children <5 years of age of 156 per 100,000 (CDSC [Wales], unpub. data).

One anomaly, however, is the higher rates of acute bloody diarrhea in 25- to 44-year-old women. This is largely accounted for by higher rates of salmonellae (Table 2), something not

Table 2. Cases of acute bloody diarrhea[a] reported by sentinel general practitioners, Wales, 1997–98, by etiologic agents and age

	Salmonella		*Campylobacter*		No organism found	
Age	M[b]	F	M	F	M	F
≤4	3	4	2	3	1	1
5-14	2	1	1	3	1	0
15-24	1	2	2	0	1	0
25-34	0	4	5	4	0	1
35-44	1	2	1	2	1	3
45-64	2	5	2	3	1	1
65+	2	1	0	1	1	0
Total	11	19	13	16	6	6

[a]One isolate of *Shigella* was reported in a woman in the 35- to 44-year age group.
[b]M = males; F = females.

seen in routine surveillance of *Salmonella* isolates in this period (CDSC [Wales], unpub. data). If this finding is true, women in this age group may be more likely than men to exhibit acute bloody diarrhea when infected with salmonellae. No cases of STEC O157 were detected through this surveillance system; however, this organism is rare in Wales. The mean annual rate between 1990 and 1998 was 1.6 cases per 100,000; in 46.3% of cases, blood was detected in the stool (5).

Thirteen (18%) cases for which stool specimens were submitted were of unknown etiology. Although noninfectious causes such as inflammatory bowel disease may also be involved, more diagnoses could almost certainly be made if specimens were sent to a reference laboratory. This could provide useful information about the emergence of, for example, non-O157 STECs. Although difficult to diagnose, such organisms are emerging causes of acute bloody diarrhea and the serious sequelae of hemolytic uremic syndrome worldwide (6).

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References

1. Thomas DR, Salmon RL, Westmoreland D, Palmer SR. Surveillance of influenza: interpreting sentinel general practice rates using contemporaneous laboratory data: opportunities and limitations. J Epidemiol Community Health 1998;52(Suppl 1):28S-31.
2. Palmer SR, Smith RMM. GP surveillance of infections in Wales. Commun Dis Rep 1991;1:R25-8.
3. Palmer S, Houston H, Lervy B, Ribeiro D, Thomas P. Problems in the diagnosis of foodborne infection in general practice. Epidemiol Infect 1996;117:479-84.
4. Skirrow MB. A demographic survey of campylobacter, salmonella and shigella infections in England. Epidemiol Infect 1987;99:647-57.
5. Chalmers RM, Parry SM, Salmon RL, Smith RMM, Willshaw GA, Cheasty T. The surveillance of Vero cytotoxin-producing *Escherichia coli* O157 in Wales, 1990 to 1998. Emerg Infect Dis 1999;5:566-9.
6. Nataro JP, Kaper JB. Diarrheogenic *Escherichia coli*. Clin Microbiol Rev 1998;11:142-201.

Epidemic Spread of Adenovirus Type 4-Associated Acute Respiratory Disease between U.S. Army Installations

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A large outbreak of adenovirus type 4-associated acute respiratory disease (ARD) occurred at Fort Jackson, South Carolina, in 1997. A laboratory-based ARD surveillance program was initiated at Fort Gordon, Georgia, where advanced individual training was heavily populated with Fort Jackson soldiers. Adenovirus type 4 was isolated from 50% of 147 trainees hospitalized with ARD. Most (88%) introduced cases were in trainees from Fort Jackson.

A large outbreak of adenovirus type 4-associated acute respiratory disease (ARD) occurred at Fort Jackson, South Carolina, the U.S. Army's largest basic training center (1) from May through December 1997. During the latter half of 1997, ARD hospitalizations were reported in basic trainee populations from all army training centers. However, the highest rates (≥1% of all trainees per week) were at Fort Jackson and at Fort Leonard Wood, Missouri (2).

After completing an 8-week basic training, soldiers generally enter the second phase of military specialty training, advanced individual training, which is offered at various U.S. Army posts. Fort Gordon, Georgia, receives soldiers directly from Fort Jackson (approximately 150 km away) and other basic training centers. From August to December 1997, the average advanced training population at Fort Gordon was 3,600 soldiers; 80% came from Fort Jackson, approximately 10% from Fort Leonard Wood, and 10% from other sites. Most soldiers proceed directly to advanced training upon graduation from basic training at Fort Jackson. Concern that adenovirus-associated ARD might spread to advanced training students at Fort Gordon led to an intensive, laboratory-based ARD surveillance program at this site on April 1, 1997.

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The Study

Female and male soldiers in advanced training live in barracks at Fort Gordon and receive medical care at the army hospital. Soldiers report to outpatient clinics during the day or to the emergency department during evenings and weekends. Local medical policy requires hospitalization of advanced training soldiers with ARD symptoms (one or more signs or symptoms of acute respiratory infection and oral temperature of ≥38.06°C). Approximately 80% of advanced training soldiers admitted to the Fort Gordon hospital who met the case definition were enrolled in the surveillance program.

A questionnaire was used to record basic training site, date of reporting to Fort Gordon, and date of onset of symptoms for each trainee. A pharyngeal swab was taken from each trainee, placed directly into virus transport medium (Viromed Laboratories, Inc., Minneapolis, MN), and immediately transported to the laboratory at the Dwight David Eisenhower Army Medical Center, Fort Gordon. All procedures performed on human subjects met ethical standards

established by the Institutional Review Committee of the medical center.

Viruses were isolated and identified in the Virus Isolation Laboratory, Dwight David Eisenhower Army Medical Center. Adenoviruses were isolated on human lung carcinoma (A-549) cells. Serotypes were determined by virus neutralization (3) with type-specific antisera (Centers for Disease Control and Prevention, Atlanta, GA) by the Reed-Muench method for calculation of the 50% lethal dose titer (4). Cultures were also examined by standard virus isolation and identification for influenza A and B; parainfluenza 1, 2, and 3; herpes simplex; and enteroviruses. In addition to human lung carcinoma, primary rhesus monkey kidney and human foreskin cell lines were used to screen for viral agents. A pharyngeal swab was obtained from most study participants for isolation of *Streptococcus pyogenes*, Group A, by using standard methods.

The incubation period for adenovirus ARD is generally ≤10 days (5,6). Patients who had been at Fort Gordon for ≤10 days before the onset of ARD symptoms were classified as having been infected elsewhere (introduced cases). Patients who had been at Fort Gordon for >10 days before onset were classified as having acquired adenovirus infection at Fort Gordon (secondary cases).

From April 17 to May 14, 1997, a survey was undertaken to determine if adenoviruses were circulating in trainees who did not exhibit classic ARD. Throat swabs for virus isolation were performed on 180 randomly selected Fort Gordon advanced training soldiers who reported to clinic for treatment of minor illnesses, including afebrile upper respiratory infections.

The first isolate of adenovirus type 4 was from a specimen collected on August 19, 1997, from an advanced training soldier whose illness met the case definition of ARD. None of the specimens obtained from advanced training soldiers without ARD yielded adenovirus. From August 1, 1997, through December 31, 1997, 147 advanced training cases from four basic training sites that met the ARD case definition were studied; 73 (49.7%) patients had positive test results for adenovirus type 4. Each began basic training after adenovirus vaccination had ceased. Of the 73, 7 (9.6%) completed basic training at Fort Leonard Wood; 6 (8.2%) trained at Fort Sill, Oklahoma; 3 (4.1%) trained at Fort Knox, Kentucky; and 57 (78.1%) trained at Fort Jackson. The 57 adenovirus type 4-positive soldiers included 47 (82.5%) men and 10 (17.5%) women with a median age of 19 years (range 17 to 33 years). This gender distribution closely mirrored that of the total advanced training population at Fort Gordon (approximately 80% male and 20% female).

Of 119 cases in soldiers who had performed basic training at Fort Jackson, 57 (47.9%) were adenovirus type-4 positive; of these cases, 22 (38.6%) were introduced and the remaining 35 (61.4%) were attributed to local transmission at Fort Gordon (Figure 1). Thirty-three other ARD

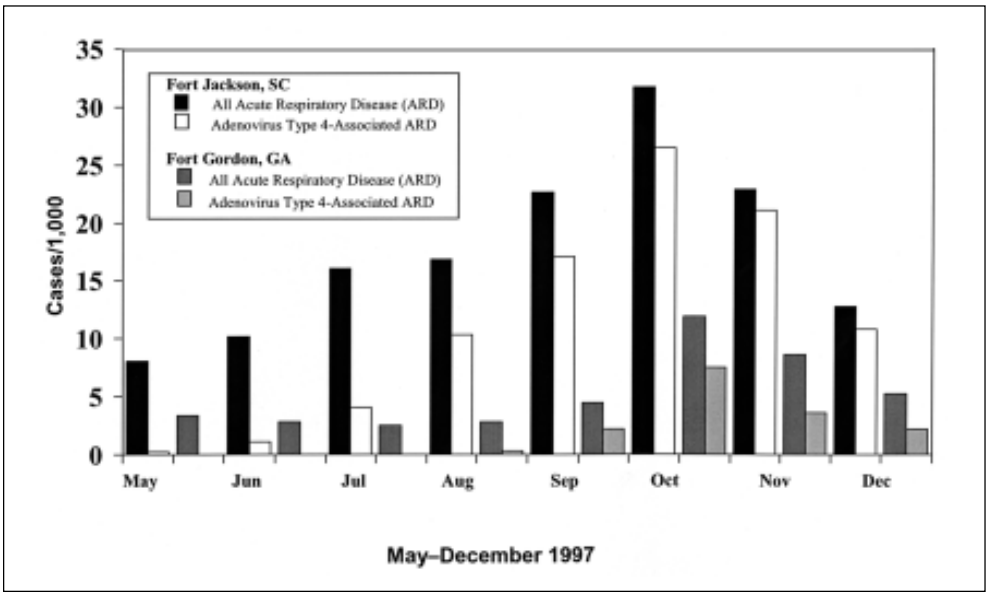

Figure 1. Numbers of introduced and secondary cases of adenovirus type 4-associated acute respiratory disease (ARD) at Fort Gordon, Georgia, USA, in soldiers who initially trained at Fort Jackson, South Carolina, August through December 1997.

causative agents were isolated from the 62 persons who were adenovirus type-4 negative. This resulted in an identifiable cause of ARD in 90 (75.6%) soldiers. Twenty-seven of the other agents were viruses. These included eight isolates of herpes simplex; eight of adenovirus type 21; four of parainfluenza type 3; five of adenovirus type 2; one of influenza B; and one mixed infection of adenovirus types 2 and 3. Six other trainees were positive for *Streptococcus pyogenes*, Group A. Two trainees with mixed infections of *S. pyogenes*, Group A, and adenovirus type 2 and adenovirus type 4, respectively, were identified.

The patient with the initial adenovirus type 4 isolate on August 19, 1997, had arrived from Fort Jackson on June 20, 1997. Twenty-five (34.2%) of the 73 cases of adenovirus type 4-associated ARD were classified as introduced; 48 (65.8%) were classifed as secondary (Figure 2). Of the introduced cases, 22 (88.0%) were in trainees from Fort Jackson. The remaining three were in trainees from three basic training sites, who arrived at Fort Gordon on October 15 or later. Twelve of the trainees from Fort Jackson who had introduced cases had onset of symptoms 1 day before arriving at Fort Gordon (day –1) to 4 days after arrival (day+4). The earliest identified, introduced cases were in trainees who arrived at Fort Gordon on August 29. One became ill the day of arrival, and the second, 3 days after arrival.

The outbreak persisted until December 12, 1997, when the last adenovirus type 4 isolate was identified as a secondarily acquired infection in an advanced training soldier who had not trained at Fort Jackson (Figures 1, 2).

Conclusions

Adenovirus type 4 was not isolated in 180 non-ARD patients cultured during April and May, indicating that the virus was not then circulating widely, if at all, at Fort Gordon. We stopped culturing non-ARD patients because of the increased workload in the medical center's virus laboratory caused by the ARD outbreak at Fort Jackson. Adenovirus type 4 was not isolated from ARD patients at Fort Gordon until August 19. The index patient had reported to Fort Gordon 58 days before onset of illness, a period much longer than the usual incubation period for adenovirus type 4; we classified that case as secondary, although it is possible that the patient was infected at Fort Jackson and excreted adenovirus type 4 at Fort Gordon without symptoms of clinical illness until August. Virus watch programs recognize infections with little or no illness and brief excretion of virus as well as persistent infections often characterized by illness and periods of intermittent virus excretion that can exceed 100 days (7,8). We were unable to identify the trainee who introduced adenovirus type 4 to Fort Gordon from a basic training center.

Our data are consistent with the introduction of adenovirus type 4 (with subsequent virus circulation) to Fort Gordon during June to

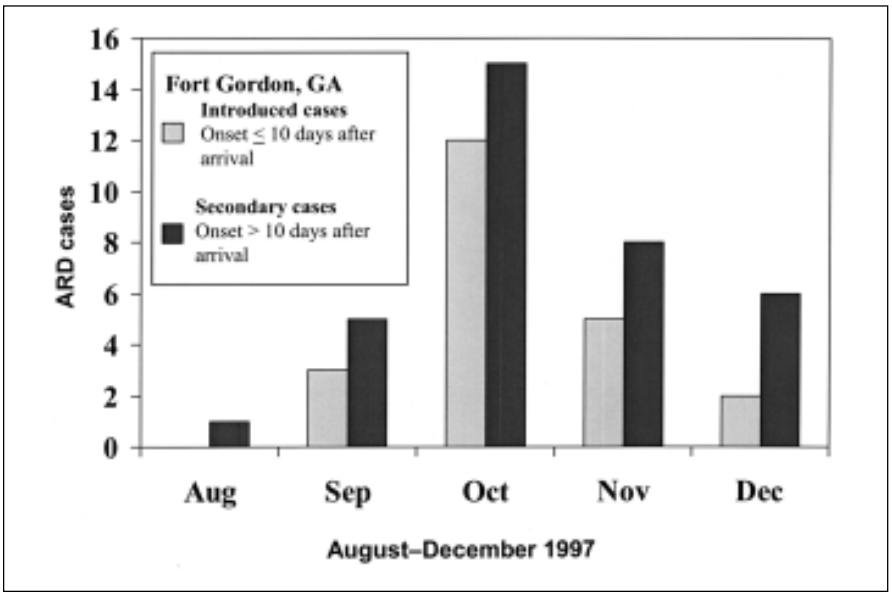

Figure 2. All acute respiratory disease (ARD) and adenovirus type 4-associated ARD incidence rates (cases/1,000 trainees/month) at Fort Jackson, South Carolina, and Fort Gordon, Georgia, May through December 1997.

August, probably by a trainee from Fort Jackson. The likely spread of adenovirus type 4 from Fort Jackson to Fort Gordon is supported by the temporal relationship between the outbreaks at the two sites and the laboratory-proven adenovirus type 4 infections in soldiers arriving at Fort Gordon from Fort Jackson. Immunizations for adenovirus type 4 ceased at Fort Jackson and elsewhere in the army on April 1, 1997, and the adenovirus type 4 ARD outbreak in Fort Jackson basic trainees began in May (1). Since basic training was 8 weeks, the first cohort of nonimmunized soldiers arrived at Fort Gordon at the beginning of June. Strain variations have been described in isolates of adenovirus type 4 (9). Molecular identification procedures were not available in military laboratories at the time of this outbreak, and the cost of molecular characterization or other tests to compare isolates from basic training centers and Fort Gordon were beyond the study budget.

The adenovirus type 4 outbreak in the advanced training population at Fort Gordon differed from the much larger outbreak in Fort Jackson basic trainees. At Fort Jackson, adenovirus type 4 accounted for more than 90% of ARD hospitalizations toward the end of the 1997 epidemic (3). At Fort Gordon, isolation of adenovirus type 4 occurred less often: <50% of soldiers hospitalized with ARD at Fort Gordon had an adenovirus isolated. Among the remaining 50%, a potential respiratory pathogen was identified for 53.2%. Laboratory tests to identify *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* as causes of ARD were not available for this study (10).

ARD rates were much lower at Fort Gordon than at Fort Jackson (Figure 2). At its peak, the Fort Jackson outbreak produced an attack rate of 26.45 cases per 1,000 soldiers per month. The highest attack rate at Fort Gordon in advanced training soldiers was 7.50 cases per 1,000 soldiers per month. Various factors including naturally acquired immunity from exposure to outbreaks during basic training, less crowded housing in advanced training (two-person rooms in advanced training versus open barracks in basic training), and less physical stress in the highly technical Fort Gordon training program may have limited the magnitude of that outbreak.

The outbreak among advanced training soldiers at Fort Gordon supports the hypothesis that the spread of adenovirus type 4 from one training site to another can occur. This outbreak also demonstrates that adenovirus type 4-associated ARD occurs in advanced training soldiers and results in hospitalizations. Since a new adenovirus vaccine manufacturer has not yet been identified, the U.S. military may not have adenovirus type 4 or 7 vaccines until 2004 or beyond. Surveillance must be conducted at basic and advanced training centers with special consideration given to studying adenovirus transmission. Understanding the variables associated with virus spread could lead to effective nonvaccine interventions.

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References

1. McNeill KM, Hendrix RM, Lindner JL, Benton FR, Monteith SC, Tuchscherer MA, et al. Reemergence of adenovirus type 4-associated acute respiratory disease in U.S. army trainees: report of a large, persistent epidemic. Emerg Infect Dis 1999;5;798-801.
2. ARD Surveillance Update. Medical Surveillance Monthly Report, Army Medical Surveillance Activity 1998;4:13.
3. Lennette DA. General principles for laboratory diagnosis of viral, rickettsial, and chlamydial infections. In: Lennette EH, Lennette DA, Lennette ET, editors. Diagnostic procedures for viral, rickettsial, and chlamydial infections. 7th ed. Washington: American Public Health Association; 1995. p. 3-25.
4. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. American Journal of Hygiene 1938;27:493-7.
5. Foy HM. Adenoviruses. In: Evans AS, Kaslow RA, editors. Viral infections in humans. 4th ed. New York: Plenum Press; 1997; p. 119-38.
6. Gaydos CA, Gaydos JC. Adenovirus vaccines, 1999. In: Plotkin SA, Orenstein WA, editors. Vaccines. 3rd ed. Philadelphia: WB Saunders; 1999. p. 609-28.
7. Fox JP, Brandt CD, Wassermann FE, Hall CE, Spigland I, Kagan A, et al. The virus watch program: a continuing surveillance of viral infections in metropolitan New York families. VI. Observations of adenovirus infections: virus excretion patterns, antibody response, efficiency of surveillance, patterns of infection, and relation to illness. Am J Epidemiol 1969;89:25-50.

8. Fox JP, Hall CE, Cooney MK. The Seattle virus watch. VII. Observations of adenovirus infections. Am J Epidemiol 1977;105:362-86.
9. Crawford-Mikoza LK, Nang RN, Schnurr DP. Strain variation in adenovirus serotypes 7 and 7a causing acute respiratory disease. J Clin Microbiol 1999;37:1107-12.
10. Gray GC, Callahan JD, Hawksworth AW, Fisher CA, Gaydos JC. Respiratory diseases among U.S. military personnel: countering emerging threats. Emerg Infect Dis 1999;5:379-87.

Drug-Resistant *Salmonella enterica* Serotype Paratyphi A in India

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The incidence of enteric fever caused by *Salmonella enterica* serotype Paratyphi A has been increasing in India since 1996. In 1998, the incidence of enteric fever caused by drug-resistant *S.* Paratyphi A abruptly increased in the New Delhi region. In the first 6 months of 1999, 32% of isolates were resistant to both chloramphenicol and cotrimoxazole and another 13% were resistant to more than two antibiotics.

Enteric fever (typhoid) is classically caused by *Salmonella enterica* serotype Typhi, but a similar syndrome may be observed with *S.* Paratyphi A and other serotypes. Outbreaks of enteric fever associated with *S.* Paratyphi A have rarely been reported in India (1–3). Although multidrug-resistant outbreaks of *S.* Typhi with an increase in numbers of strains with decreased susceptibility to ciprofloxacin have occurred, cases of drug-resistant *S.* Paratyphi A have been relatively uncommon (2). We report a sudden increase in enteric cases caused by drug-resistant *S.* Paratyphi A unresponsive to ciprofloxacin therapy.

The Study

We screened all recent isolates of *S.* Paratyphi A from hospitals in Delhi and adjoining areas for susceptibility (MICs) to various drugs. A total of 105 sporadic isolates of *S.* Paratyphi A from All India Institute of Medical Sciences (67 isolates), Safdarjang Hospital (31 isolates), New Delhi and Rohtak Medical College, Haryana (7 isolates) (an Indian state near New Delhi) were collected from April 1996 to July 1999 and tested for susceptibility to chloramphenicol, cotrimoxazole, amoxicillin, and ciprofloxacin by comparative disc diffusion (4). MICs to ciprofloxacin were estimated by E-test (AB-Biodisc, Sweden) according to guidelines from the National Committee for Clinical Laboratory Standards (NCCLS).

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In the study period, *S.* Paratyphi A isolations in enteric fever cases were 10, 16, 57, and 22, in 1996 (April), 1997, 1998, and 1999 (through July), respectively. During 1996-97, isolates were uniformly susceptible to all antibiotics, including ciprofloxacin and ceftriaxone, commonly used in the treatment of enteric fever. However, in 1998, the incidence of enteric fever caused by drug-resistant *S.* Paratyphi A abruptly increased (up to 24% of isolates), and the number of drug-resistant isolates susceptible to ciprofloxacin markedly decreased. MICs of 0.25 to 1.5 mg/L were recorded (Table). In the first 6 months of 1999, 7 (32%) of 22 isolates were resistant to both chloramphenicol and cotrimoxazole and another 3 (13%) were resistant to more than two drugs. Compared with isolates from 1996 to 1998, most drug-resistant isolates in 1999 showed higher MICs to ciprofloxacin.

Conclusions

S. Paratyphi A, which causes 1%-15% of enteric fever cases in India, has been increasing since 1996 (3). Our study found that 32% of isolates from the New Delhi region had decreased susceptibility to ciprofloxacin (MIC >2.0 mg/L), the drug of choice for enteric fever in India. One sequela of this increased resistance was delay in the resolution of symptoms. Although strains may appear sensitive at this level, when subjected to ciprofloxacin-susceptibility testing by disc diffusion, treatment failure may still occur.

The mechanisms proposed for quinolone resistance involve alteration in the permeability of the drug (outer membrane protein gene

Table. Resistance pattern and ciprofloxacin MIC of *Salmonella* Paratyphi A isolates, New Delhi, India, 1996–1999

Year	Strains (no.)	Drug-resistance pattern							Ciprofloxacin MIC	
		Cl	Cz	Ax	Cp	Cl+ Cz	Cl+ Cz+ Ax	Total (%)	Range (mg/L)	Total (%)
1996	10	-	-	-	-	1	-	1 (10)	<0.0025	-
1997	16	2	-	-	-	1	-	3 (18)	<0.045	-
1998	57	-	11	-	-	-	3	14 (24)	0.25-1.5	12 (21)
1999[a]	22	-	-	-	-	7	3	10 (45)	2.0	7 (32)

[a]until July 1999.
- = sensitive range; Cl=chloramphenicol; Cz=cotrimoxazole; Ax=amoxicillin; Cp=ciprofloxacin.

mutation) or alteration of the target enzyme DNA gyrase (5) within the treated bacterium as its adaptive reflex. Since resistance to quinolones is independent of resistance to other drugs that are mainly plasmid mediated, it may occur in otherwise sensitive strains. Similar R-plasmids of the IncHi Group have been documented: four strains of drug-resistant *S*. Paratyphi A were shown to harbor such plasmids encoding transferrable resistance to many drugs (ampicillin, chloramphenicol, sulfamethoxazole, and tetracycline) other than ciprofloxacin (6). The incidence of plasmids conferring multidrug resistance is increasing in *Salmonella* serotypes, including Enterobacteriaceae, where transfer of these R-plasmids to *S*. Paratyphi A strains may have occurred. Continuous surveillance for the susceptibility patterns of current isolates is needed.

However, development of resistance to ciprofloxacin has been suggested as partly related to exposures of these organisms to concentrations near their MICs. With increases in MICs, clinicians may be tempted to administer higher doses of ciprofloxacin to achieve serum levels required for effective therapy; however, higher doses could have unwanted clinical and public health consequences. Rather, this increased resistance may warrant a restructuring of the chemotherapeutic regimen for enteric diseases, as well as restricting use of ciprofloxacin to atypical cases in which lack of clinical response to other therapeutic drugs is noted.

Chloramphenicol and amoxicillin may need to be reconsidered as the drugs of choice in cases of enteric fever because of the increased susceptibilities of such cases to these drugs (>90% for reemerging isolates of *S*. Typhi [3]). However, these recommendations might not be appropriate in view of the substantial increase in drug-resistant *S*. Paratyphi A infections, which often obfuscate the clinical diagnosis and management of enteric fever. The increase in incidence of enteric fever caused by *S*. Paratyphi A could possibly be related to widespread use of vaccines and quinolones against *S*. Typhi in the past decade. Regardless, the frequency and geographic diversity of these cases increase the potential for large outbreaks of drug-resistant *S*. Paratyphi A in India.

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References

1. Thong KL, Nair S, Chaudhry R, Seth P, Kapil A, Chandel DS, et al. Molecular analysis of *Salmonella* Paratyphi A from an outbreak in New Delhi, India, by ribotyping and pulsed-field gel electrophoresis. Emerg Infect Dis 1998;4:507-8.
2. Mahanta J. Drug sensitivity of Salmonella paratyphi A isolated from a suspected outbreak of enteric fever in Duliajan. J Indian Med Assoc 1994;92:49-50.
3. Sood S, Kapil A, Dash N, Das BK, Goel V, Seth P, et al. Paratyphoid fever in India: An emerging problem. Emerg Infect Dis 1999;5:483-4.
4. Stokes EJ, Ridgway GL. Clinical bacteriology: antibacterial drugs. 5th ed. London: Edward Arnold; 1980. p.205-19.
5. Cullman W, Steieglitz M, Bams B, Opferkuch W. Comparative evaluation of newly developed quinolone compounds, with a note on the frequency of resistant mutants. Chemotherapy 1985;31:19-28.
6. Rangnekar VM, Banker DD, Jhala HI. Antimicrobial resistance and R-plasmids of Salmonella paratyphi A isolated in Bombay. Indian J Med Res 1983;77;5-9.

Dengue Epidemic in Southern Vietnam, 1998

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A widespread epidemic of dengue hemorrhagic fever (DHF) occurred in southern Vietnam in 1998, with 438.98 cases/100,000 population and 342 deaths. The number of DHF cases and deaths per 100,000 population increased 152.4% and 151.8% , respectively, over a 1997 epidemic. Dengue viruses were isolated from 143 patient blood samples; DEN-3 virus was identified as the predominant serotype, although a resurgence of DEN-4 was noted.

Since 1963, the incidence of dengue hemorrhagic fever (DHF), a leading cause of hospitalization and death in children, has steadily increased in Vietnam. In 1998, a widespread DHF epidemic affected 19 provinces in southern Vietnam (Figure 1); 119,429 cases of DHF and 342 deaths were reported (Figure 2); and the rates per 100,000 population were 438.98 and 1.26, respectively, for a case-fatality rate of 0.29%, an increase of 152.4% and 151.8%, respectively, over those of a 1997 epidemic (288.02 and 0.83)(1). The epidemic curve was similar to those of previous years: cases increased substantially from June to November (1-4). Peak transmission occurred from July to September, closely associated with the rainy season, a breeding period for the mosquito vector. DHF cases were reported in the first quarter in Ben Tre (1,387.2/2.4/100,000), Binh Phuoc (635.1/0), and Kien Giang provinces (568.4/2.9).

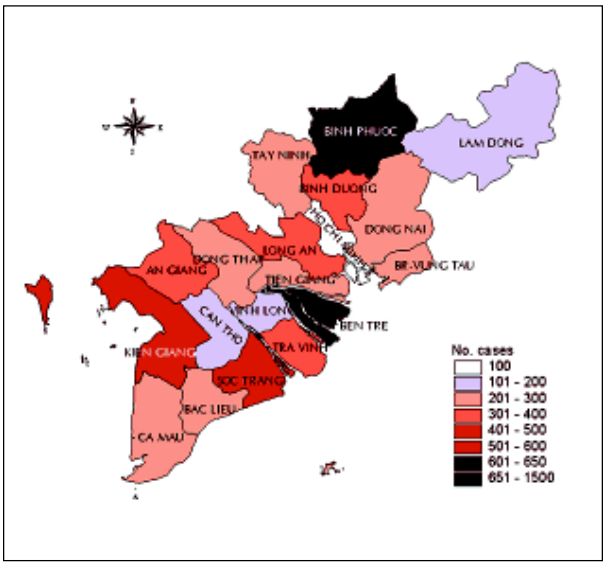

Figure 1. Nineteen provinces in southern Vietnam with mortality rates per 100,000 population, 1998.

We describe epidemiologic, virologic, and serologic studies carried out during the epidemic.

The Study

Reports of DHF cases and deaths were gathered by hospitals and Departments of

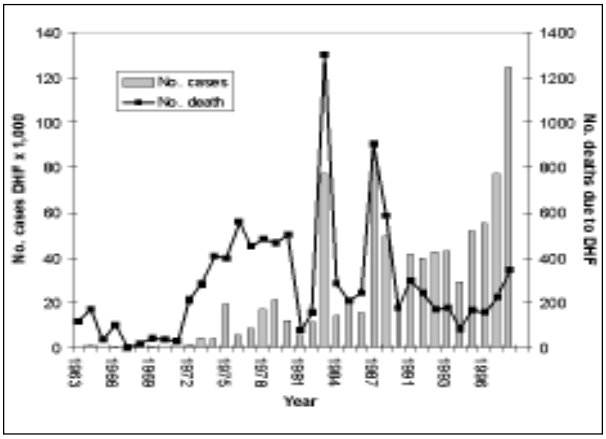

Figure 2. Reported cases of dengue hemorrhagic fever in southern Vietnam, 1963-1998.

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Hygiene and Preventive Medicine at the district level, then sent to the Provincial Centers of Preventive Medicine. These data were reported weekly to the Pasteur Institute in Ho Chi Minh City. Seventeen of the 19 provinces submitted blood samples to the Institute for virus isolation. One hundred forty-three dengue viruses were isolated from 1,236 blood samples, for a positivity rate of 11.6% (Table 1). Although DEN-1 and DEN-2 had been the most common serotypes (1-4), DEN-3 was isolated in 15 provinces.

The blood samples were obtained on days 1 to 4 after the onset of illness and were stored at -20°C or -70°C before being injected into C6/36 (*Aedes albopictus*) cell cultures seeded at 3 x 10^5 cells per mL in 1-mL glass tubes. Undiluted blood was injected into duplicate tubes (0.05 mL per tube) and incubated at 28°C for 7 days. Infected cell cultures were harvested and assayed for dengue virus by the direct and indirect fluorescent antibody techniques, with the monoclonal antibody SLE 6B6C-1/FITC conjugate and four serotype-specific monoclonal antibodies: DEN-1 (Hawaii 15F3-1-15 and D2-1F1-3), DEN-2 (NGC 3H5-1-21), DEN-3 (H87 5D4-11-24), DEN-4 (H241 1H10-6-7), and Japanese encephalitis (Nakayama 14H5) (5). To detect dengue-specific IgM antibody, samples were tested by IgM-capture enzyme-linked immunosorbent assay (Mac-ELISA) by using the monoclonal antibody SLE 6B6C-1/HRP conjugate (6).

Sixteen of 19 provinces in southern Vietnam submitted patient sera for dengue serodiagnosis. Seropositive results were seen in all provinces throughout the year, and the confirmation rates increased during the DHF season (Table 2). Despite the high sensitivity and specificity of Mac-ELISA for dengue diagnosis, the seropositivity rates in eight provinces were low (< 50%). Clinical diagnoses of DHF during the epidemic in these provinces may have been overestimated, especially in cases of suspected DHF or fever of

Table 1. Dengue viruses isolated, by province, 1998

No.	Prov.	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1	Lam Dong				2D3		2D3	1D3	5D1					5D1
									5D3					10D3
2	Dong Nai						1D2	1D2			1D2			3D2
								1D3			3D3	1D3		5D3
3	Binh Phuoc						1D3							1D3
4	Binh Duong											2D3		2D3
5	BR-V Tau						1D1							1D1
6	HCMC	2D3	1D3				2D3	2D3	2D3	2D3	7D3			18D3
7	Tien Giang						1D1	1D1	4D3		3D2			2D1
							5D3	1D2			1D3			4D2
														21D3
8	Dong Thap	1D4												1D4
9	Vinh Long		2D1				1D2							2D1
			6D2											7D2
			3D3											3D3
10	Tra Vinh							1D1			4D3	2D4		1D1
								4D2			1D4			4D2
								1D3						5D3
11	Can Tho					1D3			1D3					2D3
12	Soc Trang								1D1					1D1
									5D3					5D3
13	Ben Tre	1D1	1D1			2D3	3D1	1D3						5D1
		1D3	2D3				8D3							14D3
14	An Giang	1D2			1D3				1D3			1D3		1D2
		1D3												4D3
15	Bac Lieu					1D3			1D3					2D3
16	Ca Mau					1D3								1D3
17	Kien Giang	2D2												2D2
		8D3												8D3
Total		1D1	3D1		3D3	5D3	5D1	2D1	6D1	2D3	4D2	4D3		17D1
		3D2	6D2				2D2	6D2	19D3		15D3	2D4		21D2
		12D3	6D3				18D3	17D3			1D4			101D3
		1D4												4D4
No./No. specimens		17/90	15/142	0/10	3/57	5/103	25/244	25/179	25/104	2/78	20/161	6/53	0/15	143/1,236

Dengue virus serotypes: D1 = DEN1; D2 = DEN2; D3 = DEN3; D4 = DEN4

Table 2. Specimens positive for dengue virus, by province, 1998

Prov	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total & rate (%)
Lam Dong				0*/10	3/10								3/20 (15)
Dong Nai						18/22	4/4			12/16			34/42 (80.95)
Br-V Tau						2/2	4/4						6/6 (100)
HCMC	9/38	35/82	6/23	20/48	14/21	38/54	78/117	71/79	40/83	63/162	24/54	16/66	414/827 (50.06)
Long An		0/38				16/24		6/7	2/3				24/72 (33.33)
Tien Giang					1/6	23/67			6/8	4/4	10/10	3/3	47/98 (47.96)
Ben Tre					9/14		25/32	16/21	12/26	3/3			65/96 (67.71)
Vinh Long		17/98				41/57							58/155 (37.42)
Tra Vinh							19/27	5/6	10/17	5/6			39/56 (69.64)
Dong Thap	3/5		1/3	1/5					3/4				8/17 (47.06)
Can Tho			2/3	1/2	11/17								14/22 (63.64)
Soc Trang							1/2	8/28					9/30 (30)
An Giang	28/118	25/101	45/138	51/117	72/116	55/68	62/86	88/114	50/88	37/60	26/38	3/4	542/1,048 (51.72)
Ca Mau					6/7	8/12	1/6			9/12	4/4		28/41 (68.29)
Bac Lieu				1/2	3/11								4/13 (30.77)
Kien Giang	1/17	0/5					0/7			11/13	0/1	0/1	12/44 (27.27)
Total	41/178	77/324	54/167	74/184	119/202	201/306	194/285	194/255	123/229	144/276	64/107	22/74	1,307/2,587 (50.52)

*Number of positive specimens/total number of sera tested by IgM capture enzyme-linked immunosorbent assay (Mac-ELISA)

unknown origin. As a result, hospitals in these provinces were overwhelmed by patients, to the extent that the quality of treatment has been affected.

Conclusions

During 1990-1998, dengue viruses were most often recovered in children 5 to 14 years of age (3). In the 1998 outbreak, more dengue viruses were isolated from adults (18.2%) than in the previous 4 years. Adults are not likely to have been exposed to the emerging DEN-3 virus.

From 1987 to 1998, the dengue virus serotypes in circulation changed (3). DEN-2 was responsible for the 1987 epidemic. From 1990 to 1995, DEN-1 predominated, but had decreased to 11.9% by 1998. DEN-2 accounted for 42.2% of the serotypes identified in 1997, but had decreased to 14.7% by 1998. The circulation of DEN-3 was the lowest during 1987-1994; increased to 29.5% by 1996, 42.2% by 1997, and 70.6% in 1998; and was the predominant serotype of the 1998 epidemic.

DEN-3 virus was first detected in 1987 only in Ho Chi Minh City, but by 1991 it was also identified in Tien Giang Province (7). In 1994 it appeared in Tien Giang and Soc Trang, in 1997 in four additional provinces, and by 1998 in 15 provinces. After a 5-year absence, DEN-4 virus was also detected in Dong Thap and Tra Vinh provinces in the Mekong Delta.

During a 1998 DHF epidemic affecting 19 provinces in southern Vietnam, 119,429 cases and 342 deaths were reported, for an increase of 152.4% and 151.8%, respectively, over 1997. It was the largest DHF epidemic in Vietnam since 1963. DEN-3, which began to emerge in southern Vietnam in 1994, was the serotype associated with the 1998 epidemic. The simultaneous

emergence of DEN-4 should alert public health officials to the potential for outbreaks associated with that serotype. Virologic and serologic surveillance indicate that dengue is endemic in southern Vietnam and that the Dengue Control Program should be implemented in the interepidemic phase—in the first quarter of every year.

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References

1. Do QH, Vu TQH, Huynh TKL, Cao MT. Dengue activity in southern Vietnam, 1995-1997. Journal of Medicine and Pharmacy Activity III 1998;special issue:259-63 (in Vietnamese).
2. Do QH, Nguyen KT, Dinh TH, Vu TQH, Nguyen TL, Vo DT, et al. Epidemic DHF in South Vietnam, 1987: epidemiological and virological studies. Dengue Newsletter (WHO) 1989;14:46-57.
3. Do QH, Vu TQH, Huynh TKL, Dinh QT, Deubel V. Dengue haemorrhagic fever in the south of Vietnam during 1975-1992 and its control strategy. Jap Trop Med 1994;36:187-201.
4. Do QH, Vu TQH, Huynh TKL, Pham KS. Situation of DHF in South Vietnam, 1991-1994. Dengue Bulletin WHO 1996;20:55-61.
5. Gubler DJ. Application of serotype-specific monoclonal antibodies for identification of dengue viruses. In: Yunker C, editor. Arboviruses in arthropod cells in vitro. Boca Raton: CRC Press; 1986. p. 3-14.
6. Kuno G, Gomez I, Gubler DJ. Detecting artificial anti-dengue IgM immune complexes using an enzyme-linked immunosorbent assay. Am J Trop Med Hyg 1987;36:153-9.
7. Do QH, Vu TQH, Huynh TKL, Cao MT. Virological surveillance of dengue haemorrhagic fever in southern Vietnam during 1987-1998. Vietnamese Journal of Preventive Medicine IX 1999;3:17-27 (in Vietnamese).

Getting It Right in Prime Time: Tools and Strategies for Media Interaction

> "I only know what I read in the papers."
> Mark Twain

Increasingly scientists are faced with the challenge of communicating with a public that may well have little understanding (or considerable misunderstanding) of their work. Bold headlines all over the world scream out urgent new health emergencies, from necrotizing fasciitis (Killer Bug Ate My Face) to avian influenza (Chicken Flu). When the popular media seek answers and information for the public, a communications strategy that uses the concept of message development and delivers timely and accurate information is very effective.

Both reporters and the public have begun to ask probing questions: Why should physicians in the United States be concerned about an outbreak of Ebola in Zaire? Why is the risk for *Escherichia coli* O157:H7 infection higher when eating undercooked hamburger than when eating undercooked steak? Should we lose sleep over West Nile virus infection? It is incumbent on the public health community to provide readily understood answers and make the communications leap from medical science to public concern. Popular access to science provided by the media has understandably created more questions.

Flow of information to the media can be facilitated by the "single overriding communication objectives" (SOCOs) approach. Use of this strategy early in the development of communication objectives streamlines data and focuses on the primary audiences. All concerned know what the message is, who the audience is, and who is going to deliver the message. This harmony is achieved by having investigators, collaborators, administrators, communications personnel, and key agency officials answer the following questions: 1) What is the key point of this interview?—Your statement should reflect what you would like to see as the lead paragraph in a newspaper story or broadcast news report about this subject. 2) What are the three facts or statistics you would like the public to remember after reading or hearing about this story? 3) Who is the main audience or population segment you would like this story to reach? Is there a secondary audience? 4) What is the single message your audience needs to take away from this report? 5) Who in your department will serve as the primary point of contact with the media and when will this person be available?

These questions are at the core of translating scientific data into useful and direct messages for the public. The process requires that the investigator scan the entire empirical structure of available data for what needs to be at the top of the data pyramid for use by the consumer. The limited time that the media will devote to this single issue must be used to deliver the most powerful message. This process ensures a uniform and effective message. For example, *E. coli* O157:H7 is a complex pathogen whose proliferation is tied to issues as far-reaching as meat production and processing, day-care centers, cooking times, handwashing, and pasteurization. But the message for the public may be as simple as "Cook hamburger until well done, drink pasteurized beverages, and wash your hands well and frequently."

If we liken the experience of being interviewed by a television or newspaper reporter to diving into a pool of water, we can see the challenge. Think of the pool of water as the data pool, and the leap into that body of water (or data) as the response to a question. The persons interviewed must decide how deep into the data they must dive. So much of scientific training dictates meticulous description of methods, a discussion of findings, assessment of validity, and statement of conclusions. But when the message is delivered to the public, communication must address the public's concerns not the scientist's.

The challenge in developing a communications strategy to deal with evolving and complex issues of public and media interest is to create a mind-set where the communicator and the institution understand the value of information exchange and can develop single overriding communication objectives for both the short-term and long-term communication goals. As an issue evolves so may the communication objectives. The initial message may be one of a warning or an advisory alerting the public to a threat. Subsequent communications may direct the public on what actions to take regarding prevention and control. Communication objectives evolve quickly and require frequent and careful development that tracks the progression of the scientific findings. The process has proven valuable in both short- and long-term communi-

cation programs. In the short term, it allows focusing on clear useful messages for the public, as was the case during the hantavirus outbreak in the southwestern United States, where residents were given simple timely health advice: "Avoid contact with rodents; don't provide havens for rodents; and report all hanta-like symptoms to your doctor immediately." In the long term, the communication process places diseases in proper perspective. Even though human cases of Ebola virus infection had not reached the shores of the United States, a global village message stressed that whether it is Ebola or West Nile virus, what happens in Zaire or the Sudan today may well be a problem in the United States tomorrow. "We live in a global village" and "diseases are only a plane flight away" are messages that everyone can understand.

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Neisseria meningitidis Serogroup W135 Isolates Associated with the ET-37 Complex

To the Editor: As of April 20, 2000, >300 laboratory-confirmed cases of meningococcal disease caused by *N. meningitidis* serogroup W135 (NMW135) have been reported in Saudi Arabia and nine other countries among Hajj pilgrims or their close contacts (1). This is the first reported multinational outbreak of W135 meningococcal disease. NMW135 accounts for <5% of meningococcal disease in the United States and worldwide. The main cause of meningococcal disease outbreaks, especially in the African "meningitis belt," which extends from Ethiopia in the east to Senegal in the west, has long been *N. meningitidis* serogroup A. An epidemic, caused by a particular clonal group of serogroup A, identified by multilocus enzyme electrophoresis (MEE) as subgroup III-1, occurred in Nepal (1983 and 1984), Pakistan, and India (1985) (2) and may have caused earlier epidemics in China. In 1987, an outbreak of group A meningococcal disease caused by the same clonal group occurred in association with the annual Moslem pilgrimage to Mecca (Hajj) (3). At that time, Saudi Arabia implemented vaccination requirements for all entering pilgrims. The vaccine formulations vary; bivalent A/C vaccine or quadrivalent A/C/Y/W135 vaccine (as licensed in the United States) is used.

We present molecular characterization results of NMW135 isolates from four U.S. patients with meningococcal disease. These cases were epidemiologically linked to this year's Hajj in Saudi Arabia, which concluded on March 17, 2000 (4). We examine the origin and potential relatedness of the NMW135 isolates to some of the major *N. meningitidis* virulent clones. Two patients (both vaccinated with quadrivalent A/C/Y/W135 vaccine) were returning pilgrims; one was a close household contact of another pilgrim, the other had other possible contacts with the U.S. pilgrims. Three isolates were identified as NMW135 at the New York City Health Department and the fourth at the California Department of Health. Serogroup identification was confirmed at the Centers for Disease Control and Prevention (CDC) by standard microbiologic methods (5).

MEE has long been the standard method for molecular subtyping of *N. meningitidis* and has allowed identification of several major epidemic-prone clones. When the MEE system established by Caugant et al. (6) and that used at CDC were recently compared, they demonstrated excellent correlation segregating individual electrophoretic types (ETs) into similar clusters. A virulent clone, designated ET-37 complex, which contains >50 different ETs, is mainly composed of *N. meningitidis* serogroup C. In the United States, strains of ET-24 (within the ET-37 complex) are the main cause of meningococcal disease outbreaks and among the most frequent causes of sporadic meningococcal disease (7). The four Hajj-associated NMW135 isolates were of ET-927, which is located in a small cluster more closely related to the ET-37 complex (at a genetic distance <0.20) than to any other NMW135 isolate or any other major virulent clonal group among >2,000 *N. meningitidis* isolates in our collection (8). This cluster contains two other NMW135 isolates: one from Indonesia from a pilgrim returning from the 1996 Hajj and another from Canada (1997) for which additional epidemiologic information is not available.

Serotyping and serosubtyping showed that all four W135 isolates were 2a:P1.5,2, most frequently seen in *N. meningitidis* serogroup B and C isolates (9). Sequencing of the variable regions (VR) of the *porA* gene showed that these four isolates had VR1 and VR2 sequences identical to those of the prototype P1.5,2 strain. Strains of the same serogroup/serosubtype have been already identified in France and the Netherlands isolated from four patients with Hajj 2000 association (1), and earlier in Gambia (1990-1995) and Mali (1995) (10). A single NMW135 isolate from Gambia and one from Mali are listed in the multilocus sequence typing (MLST) database (Oxford database) as being of sequence type 11, typically seen in isolates of the ET-37 complex (11). MLST provides results comparable to those of MEE for classification and taxonomic purposes. We are evaluating the usefulness of MLST for outbreak studies. The DNA sequences of the 16S rRNA gene of the four Hajj-associated NMW135 isolates were identical to each other and to those of the California (1995) outbreak-related serogroup C ET-24 strains and an Ohio (1997)

sporadic serogroup C ET-24 strain. The 16S rRNA gene sequences of 66 *N. meningitidis* isolates representing serogroups A, B, C, W135, Z, and Y were diverse, with nine different sequences among the NMW135 isolates. Finally, all four recent NMW135 isolates had identical *Nhe*I pulsed-field gel electrophoresis (PFGE) patterns distinct from patterns seen in other NMW135 isolates. All these molecular markers were clearly unique in NMW135 isolates previously identified in the United States or isolated at the same time as the Hajj-associated isolates but with no epidemiologic link to the current outbreak. These unique markers allowed easy differentiation of the imported, Hajj-associated isolates from other sporadic NMW135 isolates circulating in the United States.

It has been shown previously that NMW135 strains can exist in widely divergent clonal groups. Our data suggest that strains like those associated with this year's Hajj have been in circulation in human populations for at least several years in different parts of the world. Given that the Hajj is a large, yearly event, high-level exposure of pilgrims to respiratory secretions and subsequent spread of infection to many countries by returning pilgrims may turn W135 meningococcal disease into a global health threat. Continued surveillance, as well as increased awareness of meningococcal disease caused by *N. meningitidis* of this serogroup by physicians and the public, is needed. Efforts to measure the efficacy of the quadrivalent meningococcal vaccine for prevention of W135 meningococcal disease should be considered. To get a better global understanding of W135 meningococcal disease, we are conducting a large multicenter study on molecular characterization of >50 Hajj-associated NMW135 isolates from Saudi Arabia, France, Singapore, and Finland, and 50 other W135 isolates from throughout the world.

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References

1. Samuelsson S, Handysides S, Ramsay M, Lyytikainen O, Nygard K, Perrocheau A, et al. Meningococcal infection in pilgrims returning from the Haj: update from Europe and beyond. Eurosurveillance Weekly 2000:17:1-5.
2. Achtman M. Molecular epidemiology of epidemic bacterial meningitis. Rev Med Microbiol 1990:1:29-38.
3. Novelli VM, Lewis RG, Dawood ST. Epidemic group A meningococcal disease in Haj pilgrims. Lancet 1987:2:863.
4. Centers for Disease Control and Prevention. Serogroup W135 meningococcal disease among travelers returning from Saudi Arabia—United States, 2000. MMWR Morb Mortal Wkly Rep 2000:49:345-6.
5. Popovic T, Ajello G, Facklam R. Laboratory manual for the diagnosis of meningitis caused by *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae.* Geneva: World Health Organization; 1999.
6. Caugant D. Population genetics and molecular epidemiology of *Neisseria meningitidis.* APMIS 1998:106:505-25.
7. Rosenstein NE, Perkins BA, Stephens D, Lefkowitz L, Cartter ML, Danila R, et al. The changing epidemiology of meningococcal disease in the United States, 1992-1996. J Infect Dis 1999:180:1894-901.
8. Reeves MW, Perkins BA, Diermayer M, Wenger JD. Epidemic-associated *Neisseria meningitidis* detected by multilocus enzyme electrophoresis. Emerg Infect Dis 1995:1:53-4.
9. Sacchi CT, Lemos APS, Brandt ME, Whitney AM, Melles CAE, Solari CA, et al. Proposed standardization of *Neisseria meningitidis* PorA variable-region typing nomenclature. Clin Diagn Lab Immunol 1998:5:845-55.
10. Kwara A, Adegbola RA, Corrah PT, Weber M, Achtman M, Morelli G, et al. Meningitis caused by a serogroup W135 clone of the ET-37 complex of *Neisseria meningitidis* in West Africa. Trop Med Int Health 1998:3:742-6.
11. Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci 1998:95:3140-5.

Gnathostomosis in Fish from Tres Palos Lagoon, Guerrero, Mexico

To the Editor: Since the first two cases of human gnathostomosis in Mexico were reported in 1970 (1), >1,500 cases have been reported in Nayarit, Sinaloa, Oaxaca, Guerrero, Veracruz, and Tamaulipas states (2). In Acapulco, Guerrero, 98 cases have been described; the intermediate or definitive hosts in this region are unknown (3,4).

During a survey of parasitic helminths of wild vertebrates from Tres Palos Lagoon, in Guerrero, Mexico, we found *Gnathostoma* sp. advanced third-stage larvae ($AdvL_3$) in the skeletal muscle of several fish species. Fish were caught from March to August 1999 in Tres Palos Lagoon (16° 41' to 16° 50'N and 99° 37' to 99° 47'W), Acapulco Municipality, 25 km south of Acapulco Bay (5). Fish muscle was ground individually, compressed between glass plates, and examined with a magnifying glass and a lamp. The infection was characterized as by Margolis et al. (6).

Of nine fish species examined, five were positive for *Gnathostoma* $AdvL_3$: Eleotridae: *Dormitator latifrons* ("popoyote," n = 83), *Gobiomorus maculatus* ("guavina," n = 66), *Eleotris pictus* ("alahuate," n = 22); Cichlidae: *Cichlasoma trimaculatum* ("charra," n = 62), and Ariidae: *Cathorops caerulescens* ("cuatete," n = 62). The highest prevalence and mean abundance values (number of larvae per fish) were found in *E. pictus* (31.81%, 0.82 ± 1.99); in the other host species values were ≤7.22 and 0.072 ± 0.26, respectively. *E. pictus* mean abundance values differed significantly from those of the other host species (nonparametric Kruskal-Wallis test, H = 27.125, 4 g.l., n = 337, p <0.0).

The intermediate host transmitting the infection to humans in Mexico had previously been identified only in the Rio Papaloapan Basin, in Veracruz and Oaxaca (7,8). The presence of *Gnathostoma* $AdvL_3$ in the muscle of fish species frequently eaten by humans in Acapulco suggests that these fish may have been the main source of infection in the 98 recorded cases of gnathostomosis (3,4). The popularity of "ceviche" (raw fish marinated in lime juice), prepared with the most commonly caught fish (including the three species of eleotrids studied), strongly supports this possibility. The identification of the source of human infection allows local health authorities to implement public information campaigns about the risk of eating raw or undercooked fish (in the form of sushi or ceviche) in this region. After this initial step in the study of this parasitic disease, the worm species must be accurately identified. In addition, understanding the parasite's life cycle is important for control of a parasitic disease.

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References

1. Peláez D, Pérez-Reyes R. Gnatostomiasis humana en América. Rev Latinoam Microbiol 1970;12:83-91.
2. Lamothe AR, Osorio SD. Estado actual de la gnatostomiasis en México. Anales del Instituto de Biología de la Universidad Nacional Autónoma de México. Serie Zoología 1998;69:23-37.
3. Rojas-Molina N, Pedraza-Sánchez S, Torres-Bibiano B, Meza-Martínez H, Escobar-Gutiérrez A. Gnathostomosis, an emerging disease in Acapulco, Mexico. Emerg Infect Dis 1999;5:264-6.
4. Vargas OF, Alarcón RE, Alvarado AFJ. Human gnathostomiasis in Mexico. Int J Dermatol 1998;37:433-53.
5. Diego P, Lozada L. Estudios florísticos de Guerrero No. 3. Laguna de Tres Palos. Facultad de Ciencias, Universidad Nacional Autónoma de México; México, D.F., 1994. p. 29.
6. Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). J Parasitol 1982;68:131-3.
7. Almeyda AJ. Hallazgo de *Gnathostoma binucleatum* n. sp. (Nematoda: Spirurida) en felinos silvestres y el papel de peces dulceacuícolas y oligohalinos como vectores de la gnathostomiasis humana en la cuenca baja del Río Papaloapan, Oaxaca-Veracruz, México. Anales del Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México 1991;18:137-55.
8. Lamothe-Argumedo R, Medina-Vences R, López-Jiménez S, García-Prieto L. Hallazgo de la forma infectiva de *Gnathostoma* sp., en peces de Temascal, Oaxaca, México. Anales del Instituto de Biología de la Universidad Nacional Autónoma de México. Serie Zoología 1989;60:311-20.

First Report of Human Granulocytic Ehrlichiosis from Southern Europe (Spain)

To the Editor: Human granulocytic ehrlichiosis (HGE) is a tickborne zoonosis described in the United States several years ago (1) and in Europe recently (2). Several hundred cases

have been reported in the United States (3). In Europe, nine cases have been reported, six in Slovenia (2,4-6), and three in Sweden (I. Eliasson, http:\\www.healthnet.org/programs/promed.html). We report a serologically confirmed case of HGE in La Rioja, a Lyme disease-endemic area in northern Spain (7-9).

On August 7, 1999, a 16-year-old man from La Rioja, who had been bitten by a tick 15 days before, was seen in an emergency room and treated with 100 mg of doxycycline twice a day. On August 9, he was hospitalized with a 3-day history of malaise, myalgias, headache, and fever (39ºC). The fever abated in the next 36 hours. The patient had not noticed any signs of inflammation or skin rash, and no signs of neurologic injury were evident. He had abdominal pain when the liver was palpated. Chest radiographs were normal, and abdominal ultrasonography showed no abnormalities. Laboratory studies showed a level leukocyte count (3,001/mm^3 [normal range, 4,500-11,000] with 4.3% band forms, 72.3% neutrophils, 4.7% monocytes, 16.7% lymphocytes, and a platelet count of 114,000/mm^3 [normal, 160,000-410,000]). The hemoglobin level was normal. No inclusions (morulae) suggestive of *Ehrlichia* or *Babesia* spp. were seen on blood smears. The erythrocyte sedimentation rate was normal. The aspartate aminotransferase level was 72 U/L [normal, 5-40]; alanine aminotransferase, 65 U/L [normal, 5-40]; and lactodehydrogenase, 637 U/L [normal, 100-250]. All serologic assays were performed by the same, widely experienced microbiologist, in one laboratory. Serologic test results were negative for *Borrelia burgdorferi* (by enzyme-linked immunosorbent assay [ELISA]); *Rickettsia conorii* (indirect fluorescent-antibody assay [IFA]; *Coxiella burnetti* (IFA); *Ehrlichia chaffeensis* (IFA); the agent of HGE (IFA); and hepatitis A, B, and C viruses (ELISA); and indicated immunity for Epstein-Barr virus. Four weeks later, the aminotransferase levels were normal, and the patient was asymptomatic. A new serum determination showed an HGE antibody titer of 1:64 (HGE IFA IgG MRL Diagnostics, California, USA); the serum tested negative for the other microorganisms tested, including with a new test for *E. chaffeensis*. Another serum sample from the patient taken 8 weeks later showed a titer of 1:256 to the HGE agent. An EDTA-treated sample of whole blood obtained from the patient on day 4 after start of doxycycline treatment was negative for the *E. phagocytophila* genogroup by polymerase chain reaction (PCR). We used a set of primers based on the published sequence of the 16s rNA of *E. phagocytophila* (E1: 5'- GGC ATG TAG GCG GTT CGC TAA GTT - 3' and E2: 5'- CCC CAC ATT CAG CAC TCA TCG TTT A -3') (7). Multiple water samples and a positive blood sample from an experimentally infected lamb were used as controls for PCR amplicon contamination. Doxycycline was administered for 14 days, and the patient's clinical and laboratory abnormalities resolved.

Many tickborne diseases are present in La Rioja. The prevalence of *E. phagocytophila* genogroup in the tick *Ixodes ricinus* is high (24.1% of nymphs, determined by PCR) in La Rioja, and evidence of HGE infection in patients at risk has been reported (10,11). This patient's history of previous tick bite, flulike symptoms, seroconversion to HGE agent, aminotransferase elevation, and response to doxycycline suggest the diagnosis of HGE. As in other reported cases in Europe, no morulae suggestive of *Ehrlichia* infection in the acute phase were visible, the clinical manifestations were moderate, and the fever abated quickly with treatment. Also, as in other cases, the negative PCR result can be explained by the prior treatment with doxycycline.

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References

1. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. J Clin Microbiol 1994;32:589-95.
2. Petrovec M, Furlan SL, Zupanc TA, Strle F, Brouqui P, Roux V, et al. Human disease in Europe caused by a granulocytic *Ehrlichia* species. J Clin Microbiol 1997;35:1556-9.
3. McQuiston JH, Paddock CD, Holman RC, Childs JE. The human ehrlichioses in the United States. Emerg Infect Dis 1999;5:635-42.
4. Weber R, Pusterla N, Loy M, Lutz H. Fever, leukopenia, and thrombocytopenia in a patient with acute Lyme borreliosis were due to human granulocytic ehrlichiosis. Clin Infect Dis 1998;26:253-4.

5. Lotric-Furlan S, Petrovec M, Zupanc TA, Nicholson WL, Summer JW, Childs JE, et al. Human granulocytic ehrlichiosis in Europe: clinical and laboratory findings for four patients from Slovenia. Clin Infect Dis 1998;27:424-8.
6. Laferl H, Hogrefe W, Köck T, Pichler H. A further case of acute human granulocytic ehrlichiosis in Slovenia. Eur J Clin Microbiol Infect Dis 1999;18:385-6.
7. Oteo JA. Ticks: a century as vector. Rev Clin Esp 1995;195:11-2.
8. Oteo JA, Backenson PB, del Mar Vitutia M, García-Moncó JC, Rodriguez I, Escudero R, et al. Use of CRH/he Lyme disease mouse model for the recovery of a Spanish isolate of *Borrelia garinii* from erythema migrans lesions. Res Microbiol 1998;149:39-46.
9. Oteo JA, Martínez de Artola V, Eiros JM. Human ehrlichiosis in Spain. Some epidemiological considerations. Enferm Infecc Microbiol Clin 1995;13:265.
10. García-Pérez AL, Mandaluniz N, Barral M, Juste RA. Microscopic and PCR findings in sheep after experimental infection with *Ehrlichia phagocytophila*. Small Ruminant Res 1999. In press 2000.
11. Oteo JA, Blanco JR. Human granulocytic ehrlichiosis. An emerging zoonosis in our medium? Enferm Infecc Microbiol Clin 1999;17:267-8.

Phylogenetic Analysis of the Chinese *Rickettsia* Isolate BJ-90

To the Editor: Five species of tick-associated rickettsiae have been identified in China; of these, three are human pathogens and two are of unknown pathogenicity (1). In 1990, one isolate, BJ-90, was first obtained from a *Dermacentor sinicus* tick, a newly recognized vector collected in a Beijing suburb, an atypical location for *Rickettsia sibirica* (2). Several taxonomic studies of the phenotype, antigenicity, and genotype of BJ-90 have been performed, with inconsistent results (2-6). Recently, phylogenetic analysis based on several gene comparisons has enabled the phylogenetic classification of this rickettsial species (7-11). To confirm the phylogenetic relationships between the BJ-90 strain and other rickettsiae, the 16S rRNA, *gltA*, and OmpA encoding genes were amplified and sequenced. Phylogenetic relationships between the BJ-90 strain and other rickettsia in the GenBank database were inferred by the parsimony and neighbor-joining methods (9). Bootstrap analyses were used to assess the reliability of the phylogenetic analysis.

Both methods showed a high degree of similarity between BJ-90, *R. sibirica* and "*R. mongolotimonae*," which were grouped in the same cluster in three inferred dendrograms. The data from the 16S rRNA and *gltA* sequences showed low statistical significance in the cluster (bootstrap values for the nodes 50% and 33%, respectively). However, data from the *rompA* gene sequence showed highly significant similarity in the cluster (bootstrap value 100%), confirming the reliability of the phylogenetic analysis. The results of this phylogenetic analysis are consistent with those of previous phenotypic, genotypic, and phylogenetic analyses (2,3,5-11), as well as taxonomy derived from direct antigenic comparison of the species (4). The sequences of 16S rRNA, *gltA*, and OmpA have been assigned the following GenBank accession numbers: AF178036 for 16S rRNA, AF178035 for *gltA*, AF179365 for the 611-bp sequence of ompA, and AF179367 for the 3174-bp sequence of ompA. According to previous genotypic and antigenic studies and our phylogenetic analysis, in which the BJ-90 strain is closer to *R. sibirica* than *R. mongolotimonae* in the dendrogram inferred from comparison of the ompA encoding gene sequences, the BJ-90 strain should be considered a variant of *R. sibirica*.

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References

1. Fan MY, Zhang JZ, Chen M, Yu XJ. Spotted fever group rickettsioses in China. In: Raoult, D, Brouqui P, editors. Rickettsiae and rickettsial diseases at the turn of the third millennium. Paris: Elsevier; 1999. p. 247-57.
2. Yu XJ, Fan MY, Bi DZ. Primary identification of spotted fever group Rickettsiae isolated from *Dermacentor sinicus* in the Beijing area. Chin J Microbiol Immunol 1991;11:305.
3. Yu XJ, Jin Y, Fan MY, Xu GM, Liu QH, Raoult D. Genotypic and antigenic identification of two new strains of spotted fever group Rickettsiae isolated from China. J Clin Microbiol 1993;31:83-9.
4. Xu WB, Raoult D. Taxonomic relationships among spotted fever group Rickettsiae as revealed by antigenic analysis with monoclonal antibodies. J Clin Microbiol 1997;36:887-96.

5. Chen M, Fan MY, Bi DZ, Zhang JZ. Sequence analysis of PCR products amplified from five strains of spotted fever group Rickettsiae with Rr with Rr 190. 70p and Rr 190. 602n primers. Acta Virol 1998;42:91-3.
6. Zhang JZ, Fan MY, Bi DZ. Isolation and identification of spotted fever group Rickettsiae BJ-93 strain. Chin J Microbiol Immunol 1997;1:28-33.
7. Fournier PE, Roux V, Raoult D. Phylogenetic analysis of spotted fever group Rickettsiae by study of the outer surface protein OmpA. Int J Syst Bacteriol 1998;48:839-49.
8. Roux V, Raoult D. Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. Res Microbiol 1995;146:385-96.
9. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the Rickettsiae. J Syst Bacteriol 1997;47:252-61.
10. Stothard DR, Fuerst PA. Evolution analysis of the spotted fever and typhus group of *Rickettsia* using 16S rRNA gene sequences. Syst Appl Microbiol 1995;18:52-61.
11. Weisburg WG, Dobson ME, Samuel JE, Dasch GA, Mallavia LP, Baca O, et al. Phylogenetic diversity of the Rickettsiae. J Bacteriol 1989;171:4202-6.

Specimen Collection for Electron Microscopy

To the Editor: As virologists whose specialties include diagnostic electron microscopy (EM), we read with interest the discussion on bioterrorism scenarios (1,2) and the subsequent note by Marshall and Catton (3) on the rapid EM diagnostic process used for smallpox (1). EM diagnostics for viral agents offer an open, undirected view; a catch-all method; and speed. A negative stain preparation may be made and a result could be obtained within 5 minutes of the specimen's arrival in the EM laboratory. As suggested by Marshall and Catton, however, success depends as much on the quality of the sample collected as on the method of preparation and skill of the microscopist.

The Konsilarlaboratorium für die Elektronenmikroskopische Erregerdiagnostik in the Robert Koch Institut, Berlin, Germany, provides EM viral diagnostic services for up to 800 specimens per year and counsels other German diagnostic units. The Electron Microscope Unit for the Department of Medical Microbiology and Infectious Diseases, University of Manitoba, is used for EM viral diagnostics by both the major health-care facility in Manitoba, Canada, and the Manitoba Provincial Laboratories; it examines approximately 2,300 clinical specimens annually. Our two facilities examine 70 to 90 vesicular specimens of suspected viral origin annually. In our experiences, the most effective methods of specimen collection from virus-induced blisters (or ulcers) involve opening the vesicle with a 26-gauge needle. The exudate may then be collected and prepared for examination in one of three ways: 1) Draw lesion aspirates into the barrel of the needle with a tuberculin syringe and cap the needle (4); 2) touch a light microscope slide to the vesicle fluid; or 3) touch a 400-mesh, plastic-coated specimen grid directly to the base of the lesion (5). The samples may then be transported to an EM facility for preparation and examination. With the first two sample types, the sample is resuspended in approximately 20 µL of 0.2-µ pore-filtered, bidistilled water; this suspension is used to prepare a standard drop preparation on a 400-mesh, carbon-reinforced, plastic-coated grid. In all cases, the specimens are then negatively stained and examined.

Because of safety concerns about HIV infection, many health officials view transport of vesicle aspirates in capillary pipettes or needles as unacceptable. Glass slides are considered more acceptable, but still a risk. Since examination facilities or wards usually do not have the material to do direct touch preparations onto EM grids, many health officials advocate placing samples into transport medium. Alternatively, swabs may be used to prepare smears on glass slides for subsequent EM examination (6). Swabs in transport medium may be of value for culture or polymerase chain reaction procedures. However, in our experience these samples are not acceptable for EM diagnostics. Marshall and Catton suggest skin scrapings as an alternative to swabs (3). We find that these samples are preferable to swab specimens but not ideal. Our success rates in identifying herpesvirus and orthopoxvirus by drop method preparation (7-9) of vesicle aspirates are 62% to 80%, annually. The advent of sample transport as swabs has made additional procedures necessary to improve sensitivity and has delayed results. In Manitoba, direct centrifugation of samples to EM grids with the Beckmann Airfuge (Palo Alto, California, USA) is used as a nonspecific method of concentrating virus in sample preparations. This method increases the yield of viral particles by three or

more orders of magnitude (8,10). In spite of this concentration method, the success rate in EM diagnostics using swab specimens has declined to <10%, while viral agents continue to be identified in >60% of lesions in submitted aspirates.

Because concentration methods are not always available, and in view of the sample problems identified by Marshall (3), we reviewed, in Winnipeg, whether collection of lesion fluids directly onto EM sample grids (5) improved sensitivity over aspiration into 26-gauge needles on tuberculin syringes (4). While neither method increased the number of cases identified in matched samples, the yield of virus seen in samples taken by touching the EM sample grid directly to the base of the lesion did increase, making it easier to identify viral agents in the samples (Hazelton and Louie, unpub. data). In Berlin, we also routinely find higher particle numbers on grids that have been prepared by the direct touch method. Sample preparation on EM grids is conducive to prolonged storage and transport of samples over long distances (5) and removes the risk of needle-stick accidents.

We continue to recommend examining grids touched directly to the lesion or vesicle aspirates. Where possible, infectious diseases and infection control staff contact the EM unit when a sample needs to be collected to receive instructions about methods and ensure that staff are available to conduct the examination. When the specimen needs to be transported some distance, such as between cities, smears on individually packaged glass slides or on sample grids are an alternative method for submitting vesicle aspirates. Glass slides allow the collection of samples for both polymerase chain reaction and EM examination (Charles Humphrey, personal communication). An additional advantage of smears is that interfering background proteins can be removed by drying the sample on the slide and then resuspending the viral agent. Proteins such as mucus, which interfere with staining and visualization, remain insoluble. We understand that other major viral EM diagnostic units also prefer aspirates, smears on glass slides, or lesion exudate on the final sample grid as preferred methods of submission of suspected blister material because of ease in handling and higher efficiency in examination.

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References

1. O'Toole T. Smallpox: An attack scenario. Emerg Infect Dis 1999;5:540-6.
2. Henderson DA. Smallpox: clinical and epidemiologic features. Emerg Infect Dis 1999; 5:537-9.
3. Marshall JA, Catton MG. Specimen collection for electron microscopy. Emerg Infect Dis 1999;5:842.
4. Burtonboy G, Lachapelle JM, Tennstedt D, Lamy ME. Detection rapide des virus au microscope electronique. Interet de la methode de coloration negative pour le diagnostic de certaines dermatoses d'origine virale. Ann Dermatol Venerol 1978;105:707-12.
5. Van Rooyen CE, Scott MA. Smallpox diagnosis with special reference to electron microscopy. Can J Public Health 1948;39:467-77.
6. Flewett TH. Some recent contributions from the electron microscope laboratories. BMJ 1972;1:667-70.
7. Gelderblom HR, Renz H, Özol M. Negative staining in diagnostic virology. Micron Microscopica Acta 1991;22:435-77.
8. Hammond GW, Hazelton PR, Chuang I, Klisko B. Improved detection of viruses by electron microscopy after direct ultracentrifuge preparation of specimens. J Clin Microbiol 1981;14:210-21.
9. Biel SS, Gelderblom HR. Electron microscopy of viruses. In: Cann A, editor. Virus cell culture—a practical approach. Oxford: Oxford University Press;1999:111-47.
10. Gelderblom H, Reupke H. Rapid viral diagnosis using the Airfuge. IV. (Abstract W50A/9). International Congress on Virology. The Hague; 1976: p. 630.

Antimicrobial Resistance

To the Editor: Davis et al. offered four reasons why local antimicrobial selection pressure in cattle may not play an important role in the dissemination of multidrug-resistant *Salmonella* from cattle to humans (1). Their conclusions differ from those of other recent studies (2-6).

The authors' first two arguments relate to the high levels of chloramphenicol resistance in the United States, despite a relative lack of

chloramphenicol use in livestock. In industrialized countries, chloramphenicol use in humans is also low because of medical and legal concerns about aplastic anemia. In Australia, the total average annual human use of chloramphenicol from 1992 to 1997 was 208 kg (6). This is lower than the annual use for most other antibiotics (e.g., sulphonamide 22,331 kg in humans and 24,869 kg in animals; tetracycline 12,677 kg in humans and 77,619 kg in animals) (6). Despite this low use in humans, chloramphenicol resistance can be common in many human pathogens, e.g., multidrug-resistant *Staphylococcus aureus* (7) and *Pneumococcus* (8). Even though tetracyclines are not used in children, children's pneumococcal isolates are often tetracycline resistant (8). With these bacteria, the use of other antibiotics (e.g., penicillins, macrolides, and cephalosporins) appears to drive chloramphenicol (and other) resistance, which is often a part of gene clusters that encode for multidrug resistance. The situation in animals for *Salmonella* is likely to be similar. In the United States, chloramphenicol resistance is higher in isolates from cattle (73% in 1995-97) than from humans (47% in 1997). Therefore, chloramphenicol resistance seen in cattle isolates is very unlikely to have come from the human use of chloramphenicol. Also, chloramphenicol-resistant isolates increased suddenly in both human and animal isolates just after 1990; resistance in cattle isolates rose from 2% to 62% (1). These points suggest that just after 1990 the same chloramphenicol-resistant strains (presumably new clones) were being shared rapidly between cattle and people. This spread is very unlikely to be from people to cattle but rather to people from cattle through food.

The third argument by Davis et al. relates to the spread of resistant strains by wildlife. Even though these strains can move easily around the world, they need to be amplified to cause a serious problem. One of the best ways to amplify resistant bacteria is to give them a selective advantage (e.g., when *Salmonella* is ingested in feed or water by animals that receive in-feed antibiotics).

The authors' fourth argument is that there is still broad dissemination of antibiotic-susceptible strains. So what? In hospitals, despite the overuse of antibiotics, we still see cross-infection with relatively sensitive strains of *S. aureus*, even when these hospitals have a high incidence of multidrug-resistant *S. aureus*. This does not mean that antibiotic use in humans is not one of the important factors in the amplification and spread of multidrug-resistant *S. aureus*.

As Davis et al. point out, antibiotic-resistant bacteria spread worldwide in many ways, including by wild animals and human travel. We need to prevent this spread; however, the central issue is antibiotic use in animals and how it amplifies resistant bacteria (e.g., *Salmonella enterica* serovar Typhimurium DT104). For every antibiotic Davis et al. tested, the level of resistance was higher in *Salmonella* isolates from cattle than from humans (1). The figures supplied by the authors clearly show that antibiotic resistance in cattle and human isolates is related and that resistance in *Salmonella* is and has been more of a problem in cattle than in humans, presumably as a result of widespread use of antibiotics in cattle.

Antibiotic resistance over the medium- to long-term is an inevitable consequence of antibiotic use. Ciprofloxacin and similar fluoroquinolones are the most effective drugs for treating many serious infections in humans, including some *Salmonella* infections (such as bacteremia or osteomyelitis). The prevalence of resistance to fluoroquinolones in human infections acquired from animals through the food chain is increasing (2,4). We should therefore avoid entirely the use of "last-line" human antibiotics such as fluoroquinolones (i.e., antibiotics for which there may be no alternatives if resistance develops) in livestock. All other antibiotics should be used only when there is no other way to prevent or treat infections.

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References

1. Davis M, Hancock D, Besser T, Rice D, Gay JM, Gay C, et al. Changes in antimicrobial resistance among *Salmonella enterica* serovar typhimurium isolates from humans and cattle in the northwestern United States, 1982-1997. Emerg Infect Dis 1999;5:802-6.
2. Molbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydendahl K, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. N Engl J Med 1999;341:1420-5.

3. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. N Engl J Med 1998;338:1333-8.
4. Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. Investigation team. N Engl J Med 1999;340:1525-32.
5. European Commission (1999). Opinion of the Scientific Steering Committee on Antimicrobial Resistance (SSCOAR). European Commission Directorate General XXIV, Consumer Policy and Consumer Health protection, 28 May 1999.
6. Report of the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR). The use of antibiotics in food-producing animals: antibiotic-resistant bacteria in animals and humans. Australian Commonwealth Department of Health and Aged Care and the Commonwealth Department of Agriculture, Fisheries and Forestry; 1999 Sep; Canberra.
7. Turnidge J, Nimmo G, Francis G. Evolution of resistance in *Staphylococcus aureus* in Australian teaching hospitals. Australian Group on Antimicrobial Resistance (AGAR). Med J Aust 1996;164:68-71.
8. Collignon P, Bell J. Drug-resistant *Streptococcus pneumoniae*: the beginning of the end for many antibiotics? Australian Group on Antimicrobial Resistance (AGAR). Med J Aust 1996;164:64-7.

Changes in Antimicrobial Resistance in *Salmonella enterica* Serovar Typhimurium

To the Editor: The conclusion by Davis and colleagues (1) that use of antimicrobial agents in agriculture is unlikely to have contributed to the emergence of multidrug-resistant *Salmonella* serotype Typhimurium DT104 (MR-DT104) is contrary to available evidence. Use of antimicrobial agents in aquaculture in Asia may have contributed to the emergence of DT104. The resistant determinants of MR-DT104 reside on the chromosome, apparently within a transferrable element (2-4). Chloramphenicol resistance in MR-DT104 is due to *floR*, a florfenicol resistance gene (5); florfenicol is a veterinary antimicrobial agent that, although not approved in the United States until 1996, has been used in aquaculture in Asia since the early 1980s. *FloR* was first identified in *Photobacterium damsela,* a bacterium found in fish (5). Furthermore, tetracycline resistance in MR-DT104 is due to a class G resistance gene first identified in *Vibrio anguillarum*, a pathogen of fish (4,6). The molecular sequence where the class G and *floR* determinants reside on the DT104 chromosome is closely related (94% identity) to a plasmid in *Pasteurella piscicida*, another pathogen of fish (7). These data suggest that the resistance determinants of MR-DT104 may have emerged among bacteria in aquaculture and been horizontally transferred to *S.* Typhimurium DT104.

Spread of MR-DT104 between regions during international travel, as Davis and colleagues suggest, is unlikely because in industrialized countries *Salmonella* is seldom transmitted from person to person (8). Once MR-DT104 emerged, it spread rapidly to many regions through unknown means. The rapid emergence of MR-DT104 suggests a means of spread more efficient than person-to-person transmission. Possibilities include movement of infected breeding or "multiplier" stock or shipment of contaminated feed ingredients; such movements may not be as limited as Davis et al. suggest. For example, the international spread of *Salmonella* serotype Agona was traced to the global distribution of contaminated fish meal from Peru (9).

Once MR-DT104 is introduced into food animals in a region, use of antimicrobial agents in animals would contribute to further dissemination of MR-DT104 (8). If MR-DT104 is present on a farm, the use on the farm of any antimicrobial agent to which MR-DT104 is resistant would contribute to its persistence. An example of such use in cattle in the United States is the tetracycline-containing milk "replacement" commonly fed to dairy calves. This product could kill susceptible gastrointestinal flora while allowing tetracycline-resistant flora such as MR-DT104 to survive and proliferate. Once MR-DT104 proliferates on a farm, dissemination to other farms in the region is facilitated, particularly if the other farms are using an antimicrobial agent to which MR-DT104 is resistant.

Increasing antimicrobial resistance in *Salmonella* contributes to its spread and threatens the use of clinically important antimicrobial agents. To slow the emergence and dissemination of resistant *Salmonella,* measures should be implemented to ensure that antimicrobial agents are used prudently in food-producing animals (10).

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References

1. Davis MA, Hancock DD, Besser TE, Rice DH, Gay JM, Gay C, et. al. Changes in antimicrobial resistance among *Salmonella enterica* serovar Typhimurium isolates from humans and cattle in the Northwestern United States, 1982-1997. Emerg Infect Dis 1999;5:802-6.
2. Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol Let 1998;160:37-41.
3. Ridley A, Threlfall EJ. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella typhimurium* DT104. Microb Drug Resist 1998;4:113-8.
4. Briggs CE, Fratamico PM. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob Agents Chemother 1999;43:846-9.
5. Bolton LF, Kelly LC, Lee MD, Fedorka-Cray PI, Maurer H. Detection of multidrug-resistant *Salmonella enterica* serotype *typhimurium* DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J Clin Microbiol 1999;37:1348-51.
6. Zhao J, Aoki T. Nucleotide sequence analysis of the class G tetracycline resistance determinant from *Vibrio anguillarum*. Microbiol Immunol 1992;36:1051-60.
7. Kim EH, Aoki T. Drug resistance and broad geographical distribution of identical R plasmids of *Pasteurella piscicida* isolated from cultured yellowtail in Japan. Microbiol Immunol 1993;37:103-9.
8. Cohen ML, Tauxe RV. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science 1986;234:964-9.
9. Clark GM, Kaufmann AF, Gangrosa EJ. Epidemiology of an international outbreak of *Salmonella agona*. Lancet 1973;490-3.
10. Centers for Veterinary Medicine, U.S. Food & Drug Administration. Proposed framework for evaluating and assuring the human safety of the microbial effects of antimicrobial new animal drugs intended for use in food-producing animals. Washington: FDA; 1999 Jan 6. Available from: URL:http//www.fda.gov/cvm/fda/infores/vmac/antim18.htm

Reply to Drs. Angulo and Collignon

To the Editor: Drs. Angulo and Collignon point out that exposure to one antimicrobial drug (e.g., tetracycline) can confer a selective advantage to a multiresistant organism (e.g., R-type ACSSuT) over nonresistant organisms. However, tetracycline use would not be expected to favor one tetracycline-resistant organism (MR-DT104) over other tetracycline-resistant organisms, and most bovine Typhimuriums before the MR-DT104 epidemic were tetracycline-resistant R-type ASSuT. Since neither florfenicol nor chloramphenicol was then available for use in livestock in the United States, the evidence suggests that the emergence of MR-DT104 in cattle populations was not driven by antibiotic selection pressure. The references Drs. Angulo and Collignon cited to establish the importance of antimicrobial use in livestock for the dissemination of multiresistant clones either do not address the issue of dissemination (1-2) or present evidence to the contrary: the dissemination of fluoroquinolone-resistant MR-DT104 despite the lack of fluoroquinolone use in the herds in question (3).

Available data support Dr. Collignon's example of *Campylobacter* as an agent for which fluoroquinolone use in livestock resulted in increasing prevalence of fluoroquinolone resistance (4). However, the epidemiology of resistance in polyclonal commensals such as *Campylobacter* is very unlike that of epidemic, clonal *S.* Typhimuriums. The epidemic, clonal dissemination of *S.* Typhimurium more closely resembles that of methicillin-resistant *Staphylococcus aureus* (MRSA). Epidemic MRSA clones differ genetically from nonepidemic ones, and dissemination of epidemic clones does not necessarily require antimicrobial selection pressure (5). Because antimicrobial usage practices that contribute to the control of MRSA have not been scientifically defined, infection control practices must play the central role in successful MRSA control programs (6-8).

Dr. Angulo's hypothesis that MR-DT104 emerged genetically in Asian fish is plausible, but other credible hypotheses exist. *tet*(G), first described in *Vibrio angullarium*, also occurs in *Pseudomonas aeruginosa* (9). Similarly, *flo*R is closely related to the *P. aeruginosa* chloramphenicol-resistance gene *cmlA* (10), and *pse*-1 encoded beta-lactamase is a common feature of hospital *P. aeruginosa* isolates (11). Thus, the hypothesis that MR-DT104 acquired resistance genes horizontally from nosocomial pseudomonads might also be worthy of consideration.

Salmonella infections acquired in other countries are frequently diagnosed in travelers recently returned to the United States. Although transmission of these infections to other humans may be rare in the United States, human-to-bovine transmission may occur regularly: thousands of cases of bovine *Taenia saginata* cysticercosis occurred immediately before and during the dissemination of MR-DT104. As humans are the only definitive host of the *T. saginata* tapeworm, these cases confirm the large-scale occurrence of human-to-animal transmission of enteropathogenic agents in the United States. Since transmission of *S.* Typhimurium from herd to herd is common in the United States, increased emphasis on *Salmonella* infection control may be an effective method for reducing dissemination of organisms such as MR-DT104.

With or without imposition of stringent controls on antibiotic use in the United States and Europe, the future genetic emergence of new epidemic clones of *S.* Typhimurium somewhere in the world is highly likely, and controlling the dissemination of epidemic clones is essential to avoid increasing problems with multidrug resistance. Certainly we do not disagree with the concept of reducing antimicrobial use, particularly such frivolous use as in calf milk replacers. However, we urge public health officials to consider that infection control is as central to control of agents such as MR-DT104 as it is for epidemic MRSA.

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References

1. Cohen ML, Tauxe RV. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science 1986;234:964-9.
2. Glynn MK, Bopp C, Dewitt MS, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N Engl J Med 1998;338:1333-8.
3. Mølbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydendahl K, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium DT104. N Engl J Med 1999;341:1420-5.
4. Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N Engl J Med 1999;340:1525-32.
5. Papakyriacou H, Vaz D, Simor A, Louie M, McGavin MJ. Molecular analysis of the accessory gene regulator (agr) locus and balance of virulence factor expression in epidemic methicillin-resistant *Staphylococcus aureus*. J Infect Dis 2000;181:990-1000.
6. Wagenvoort JHT. Dutch measures to control MRSA and the expanding European Union. Eurosurveillance 2000;5:26-8.
7. Lipsitch M, Bergstrom CT, Levin BR. The epidemiology of antibiotic resistance in hospitals. Proc Nat Acad Sci U S A 2000;97:1938-43.
8. McGowan JE Jr. Strategies for study of the role of cycling on antimicrobial use and resistance. Infect Control Hosp Epidemiol 2000 Jan;21(1 Suppl):S36-S43.
9. Ng LK, Mulvey MR, Martin I, Peters GA, Johnson W. Genetic characterization of antimicrobial resistance in Canadian isolates of *Salmonella* Serovar Typhimurium DT104. Antimicrob Agents Chemother 1999;43:3018-21.
10. Briggs CE, Fratamico PM. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob Agents Chemother 1999;43:846-9.
11. Pitt TL, Livermore DM, Miller G, Vatopoulos A, Legakis NJ Resistance mechanisms of multiresistant serotype 012 *Pseudomonas aeruginosa* isolated in Europe. J Antimicrob Chemother 1990;26(3):319-28.

Malaria and Global Warming in Perspective?

To the Editor: The two reports from the International Panel on Climate Change (IPCC) (1,2) cited in the letter by Pim Martens (3) are widely regarded as "the standard scientific reference for all concerned with climate change and its consequences," yet the contents of these reports are often misleading. The quoted passage does not acknowledge the devastation caused by malaria in temperate regions. The reassurance that "existing public health resources" would "make reemergent malaria unlikely" ignores the nonclimatic factors that led to its disappearance and continued absence. Moreover, although malaria/climate models are not meant to predict future worlds, the IPCC chapter (1) on human health—one-third of which is devoted to vector-borne disease—makes extensive use of such models to warn of substantial "actual climate-related increases in malaria incidence" and "highly likely" exten-

sions of its distribution. The chapter does include statements that the "predictions" of such models should be viewed cautiously "until they have been validated against historical data sets," and "malaria is most likely to extend its spread…in tropical countries." The past presence of malaria in "southern Europe" is also mentioned, but such qualifiers are applied to predictions of 10- to 100-fold increases in epidemic potential in temperate climates. These predictions are frequently cited as evidence of a major threat to humanity (4,5).

The IPCC reports state "…anopheline mosquito species that transmit malaria do not usually survive where the mean winter temperature drops below 16°-18°C." Similarly, two oft-quoted publications (6,7) define the vector's limit of survival as the 15°C winter isotherm, i.e., in the northern Sahara. However, in the past the limit was the 15°C summer isotherm. In fact, much of Europe and all of the United States are within the 20°C or 25°C summer isotherms, and malaria was once prevalent in parts of southern Canada and up to 64°N in Russia and Siberia. The same publications state that *Aedes aegypti*, the principal urban vector of dengue and yellow fever, cannot survive mean temperatures below 10°C, but with global warming "…dengue could extend into the southern United States." This statement has been repeatedly quoted (5), although *Ae. aegypti* is common where winter temperatures of -15°C are not unusual and epidemics of dengue and yellow fever have occurred as far north as Boston and Dublin. Repeated claims that global warming may have already led to increases in these diseases in the tropics are equally indefensible (8,9).

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References

1. Intergovernmental Panel on Climate Change (IPCC). Impacts, adaptations and mitigation of climate change: scientific-technical analyses. Watson RT, Zinyowera MC, Moss RH, editors. New York: Cambridge University Press;1996. Chapter 18.
2. Intergovernmental Panel on Climate Change. The regional impacts of climate change: an assessment of vulnerability. Working Group II. Intergovernmental Panel on Climate Change. New York: Cambridge University Press; 1998. Chapters 5,8.
3. Martens P. Malaria and global warming in perspective? [letter] Emerg Infect Dis 2000;6:313-4.
4. Ross A. Strange weather: culture, science and technology in the age of limits. London: Verso; 1996.
5. Gelbspan R. The heat is on: the high stakes battle over Earth's threatened climate. New York: Addison-Wesley; 1997.
6. Patz JA, Epstein PR, Burke TA, Balbus JM. Global climate change and emerging infectious diseases. JAMA 1996;275:217-23.
7. Epstein PR, Diaz HF, Elias S, Grabherr G, Graham NE, Martens WJM, et al. Biological and physical signs of climate change: focus on mosquito-borne diseases. Bulletin of the American Meteorological Society 1998;79:409-17.
8. Reiter P. Global warming and vector-borne disease in temperate regions and at high altitude. Lancet 1998;351:839-40.
9. Mouchet J, Manguin S, Sircoulon J, Laventure S, Faye O, Onapa AW, et al. Evolution of malaria in Africa for the past 40 years: impact of climatic and human factors. J Am Mosq Control Assoc 1998;14:121-30.

Workshop Summary

Borna Disease Virus: A Veterinary and Public Health Problem?

March 23–24, 2000
Wales, United Kingdom (UK)

Borna disease virus (BDV), which is endemic in parts of Europe, infects a broad range of species and causes a rare meningoencephalitis in horses and sheep. Several reports have suggested that infection with the virus may be associated with certain neuropsychiatric disorders in humans; however, the methods used in these reports and the significance of the findings are controversial.

The Public Health Laboratory Service, Health and Safety Executive; the Ministry of Agriculture, Fisheries and Food; the Welsh Development Agency; and the Wales Innovation Relay Centre organized a Workshop on Borna Disease Virus, to review information on the diagnosis, pathology, and epidemiology of BDV in humans and animals. Attending the workshop were 66 delegates from 9 countries, including Hong Kong, Australia, and the United States, as well as the disease-endemic countries of Germany, Austria, and Switzerland. Participants discussed the significance of recent findings for veterinary and public health policy in the United Kingdom and addressed certain questions: Is BDV present in United Kingdom animal populations? Is there clinical disease compatible with Borna disease in animals in the United Kingdom? Are human populations in the United Kingdom likely to be exposed, and if so is there any evidence that exposure could lead to clinical illness? Discussion was intended to provide guidance for veterinary and public health policymakers in developing surveillance and research programs.

The workshop reviewed the history of BDV, which has been recognized since the early 1800s, and examined evidence for the presence of clinical disease in horses in Germany, Austria, Switzerland, and Liechtenstein. Data were also presented indicating that clinical BDV infection is present in domestic cats in Sweden and the United Kingdom. With regard to BDV infection of humans, workshop participants concluded that BDV is a potential zoonosis, but that further validation and harmonization of diagnostic tests are needed and can be achieved through collaboration between international laboratories.

Full proceedings of this workshop, including a transcript of the roundtable discussion, will be posted later this year at http://www.cdsc.wales.nhs.uk.

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Upcoming Events

Frontline Health-Care Workers Conference "Partnerships in Prevention"

August 6-8, 2000
Marriott Hotel
Washington, D.C.

Topics to be covered at the conference include Science and Technology for Protecting Health-Care Workers, Advances in HIV and Hepatitis B and C, Postexposure Prophylaxis, Safety in Animal Research and Treatment, Device and Technology Regulations, Approaches for Accident Analysis in Hospitals, and Policy and Economics for Protecting Health-Care Workers. For more information, call 847-566-4566.

15th International Congress on Tropical Medicine and Malaria
Cartagena, Colombia
August 20–25, 2000

The 15th International Congress on Tropical Medicine and Malaria will be held in Cartagena, Colombia, August 20–25, 2000. The conference will cover a broad range of tropical medicine topics and will provide a forum for discussing health policies for the prevention and control of diseases affecting tropical regions. For more information, contact Santiago Nicholls, Instituto Nacional de Salud, Laboratorio de Parasitología, Bogotá, Colombia; telephone: 57-1-222-0577, ext. 422/423; fax: 57-1-222-3055/222-0194; e-mail: rnichols@hemagogus.ins.gov.co. Detailed information is also available at the Congress website: http://wwwprof.uniandes.edu.co/~xviftm/cartagena.htm.

Seventh Western Pacific Congress on Chemotherapy and Infectious Diseases
Hong Kong
December 11–14, 2000

The preliminary program and call for abstracts of the Seventh Western Pacific Congress on Chemotherapy and Infectious Diseases are now available on the conference web site, http://www.mvdmc.com/wpccid. For additional information, contact the Congress Secretariat, MV Destination Management, by e-mail at info@mvdmc.com.hk, by telephone at (852) 2735-8118, or by fax at (852) 2735-8282. The deadline for abstract submission is August 10, 2000.

Erratum Vol 6, No. 2

In the article "The bdr Gene Families of the Lyme Disease and Relapsing Fever Spirochetes: Potential Influence on Biology, Pathogenesis, and Evolution," by Roberts et al., Table 2 on page 113, contained printing errors. A correct version of the table appears below. We regret any confusion these errors may have caused.

Table 2. *Borrelia* Bdr homology groups and gene nomenclature

Bdr subfamily designation	Species/revised gene designation	Accession or TIGR number	Previous gene names	Ref.
Subfamily A				
B. turicatae OZ-1	$bdrA_1$	AF062395	*repA*	(46)
B. turicatae OZ-1	$bdrA_2$,A_3,A_4	AF128445-AF128447	none	(25)
B. hermsii YOR-1	$bdrA_1$,A_2,A_3	AF143473-AF143475	none	(25)
B. hermsii HS1	$bdrA_1$,A_2	AF143457-AF143458	none	(25)
B. hermsii MAN	$bdrA_1$,A_2	AF143465, AF143467	none	(25)
B. parkeri	$bdrA_1$	AF143455	none	(25)
Subfamily B				
B. turicatae OZ-1	$bdrB_1$,B_2,B_3,B_4,B_5	AF128448-AF128452	none	(24)
B. hermsii MAN	$bdrB_1$,B_2,B_3	AF143463, AF143464, AF143466	none	(25)
Subfamily C				
B. parkeri	$bdrC_1$	AF143455	none	(25)
B. hermsii MAN	$bdrC_1$,C_2,C_3,C_4,C_5	AF143468-AF143472	none	(25)
B. hermsii HS1	$bdrC_1$,C_2,C_3,C_4	AF143459-AF143462	none	(25)
B. hermsii YOR-1	$bdrC_1$	AF143476	none	(25)
B. parkeri	$bdrC_2$	AF143456	none	(25)
Subfamily D				
B. burgdorferi B31G	$bdrD_1$,D_2,D_3,	BBL35, BBM34, BBO34	*bdrO, bdrK, bdrM*	(30)
B. burgdorferi B31G	$bdrD_4$,D_5,D_6	BBP34, BBQ42, BBS37	*bdrA, bdrV, bdrE*	(30)
B. burgdorferi B31	$bdrD_7$	X87201	ORF-E (lp50 allele)	(41)
B. burgdorferi B31	$bdrD_8$	X87127	ORF-E (cp30.5 allele)	(41)
B. burgdorferi B31	$bdrD_9$	U42599	ORF-E (cp18 allele)	(41)
B. burgdorferi B31	$bdrD_{10}$	BBN34	*bdrQ*	(30)
B. burgdorferi B31	$bdrD_{11}$	BBR35	*bdrG*	(30)
Subfamily E				
B. burgdorferi B31G	$bdrE_1$,E_2,E_3	BBL27, BBN27, BBO27	*bdrP,* none, *bdrN*	(30)
B. burgdorferi B31G	$bdrE_4$,E_5,E_6	BBR27, BBS29, BBQ34	*bdrH, bdrF, bdrW*	(30)
B. burgdorferi 297	$bdrE_1$,E_2	U45421, U45422	*rep+2.9-1, rep+2.9-2*	(42)
B. burgdorferi 297	$bdrE_3$,E_4	U45423, U45424	*rep+2.9-3, rep+2.9-4*	(42)
B. burgdorferi 297	$bdrE_5$	U45425	*rep+2.9-5*	(42)
B. burgdorferi 297	$bdrE_6$	AF046998	*rep+2.9-8*	(45)
B. burgdorferi 297	$bdrE_7$	AF046999	*rep+2.9-9*	(45)
Subfamily F				
B. afzelii DK1	$bdrF_1$	Y08143	*p21*	(43)
B. burgdorferi B31G	$bdrF_1$,F_2,F_3	BBF03, BBG33, BBH13	*bdrS, bdrT, bdrU*	(30)

Editorial Policy and Call for Articles

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

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Instructions to Authors

Manuscript Preparation

Follow "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Ann Intern Med 1997:126[1]36-47) (http://www.acponline.org/journals/annals/01jan97/unifreqr.htm).

Begin each of the following sections on a new page and in this order: title page, abstract, text, acknowledgments, references, tables, figure legends, and figures.

Title page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Also provide address for correspondence (include fax number and e-mail address).

Abstract and key words. Avoid citing references in the abstract. Include up to 10 key words; use terms listed in the Medical Subject Headings from Index Medicus (http://www.nlm.nih.gov/mesh/meshhome.html).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Type only on one side of the paper and number all pages, beginning with the title page. Indent paragraphs 5 spaces; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use Courier font size 10 and ragged right margins. Italicize (rather than underline) scientific names when needed.

Electronic formats. For word processing, use WordPerfect or MS Word. Send graphics in native format or convert to .TIF (Tagged Image File), or .EPS (Encapsulated Postscript) formats. The preferred font for graphics files is Helvetica. Convert Macintosh files into one of the suggested formats. Submit slides or photographs in glossy, camera-ready photographic prints.

References. Follow the Uniform Requirements style. Place reference numbers in parentheses, not in superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title in full. List the first six authors followed by "et al."

Tables and figures. Create tables within the word processing program's table feature (not columns and tabs within the word processing program). For figures, use color as needed; send files, slides, photographs, or prints. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced. Place figure keys within the figure.

Manuscript Submission

Include a cover letter verifying that the final manuscript has been seen and approved by all authors.

Submit three copies of the original manuscript with three sets of original figures and an electronic copy (on diskette or by e-mail) to the Editor, Emerging Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., MS D 61, Atlanta, GA 30333, USA; e-mail eideditor@cdc.gov.

Types of Articles

Perspectives, Synopses, Research Studies, and Policy Reviews: Articles should be approximately 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch.

Perspectives: Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases or related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses: This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. Use of subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are encouraged.

Research Studies: These articles report laboratory and epidemiologic results within a public health perspective. Although these reports may be written in the style of traditional research articles, they should explain the value of the research in public health terms and place the findings in a larger perspective (e.g., "Here is what we found, and here is what the findings mean").

Policy Reviews: Articles in this section report public health policies that are based on research and analysis of emerging disease issues.

Dispatches: These brief articles are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome. Dispatches (1,000 to 1,500 words) need not be divided into sections. Provide a short abstract (50 words); references, not to exceed 10; figures or illustrations, not to exceed two; and a brief biographical sketch.

Book Reviews: Short reviews (250 to 500 words) of recently published books on emerging disease issues are welcome.

Letters: This section includes letters that give preliminary data or comment on published articles. Letters (500 to 1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

News and Notes: We welcome brief announcements (50 to 150 words) of timely events of interest to our readers. (Announcements can be posted on the journal web page only, depending on the event date.) In this section, we also include summaries (500 to 1,500 words) of conferences focusing on emerging infectious diseases. Summaries may provide references to a full report of conference activities and should focus on the meeting's content.