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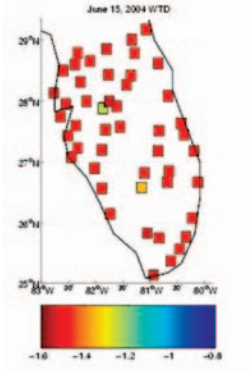

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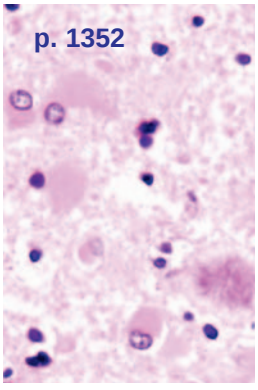

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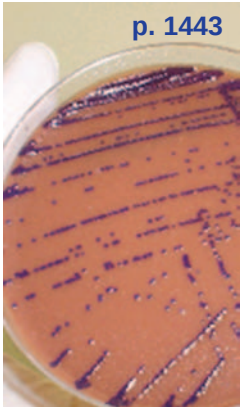

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Canine Rabies Ecology in Southern Africa

John Bingham*

Rabies is a widespread disease in African domestic dogs and certain wild canine populations. Canine rabies became established in Africa during the 20th century, coinciding with ecologic changes that favored its emergence in canids. I present a conceptual and terminologic framework for understanding rabies ecology in African canids. The framework is underpinned by 2 distinct concepts: maintenance and persistence. Maintenance encompasses the notion of indefinite transmission of infection within a local population and depends on an average transmission ratio ≥1. Maintenance in all local populations is inherently unstable, and the disease frequently becomes extinct. Persistence, the notion of long-term continuity, depends on the presence of rabies in ≥1 local population within the canine metapopulation at any time. The implications for understanding rabies ecology and control are reviewed, as are previous studies on rabies ecology in African canids.

The ecologic persistence of pathogenic viruses has been the focus of many studies (1–4). Rabies virus, a lyssavirus that causes a lethal neurotropic infection of mammals, is a pathogen for which ecologic persistence cannot be explained adequately by pathogenetic mechanisms. Death of the host implies that the virus depends on transmission to new susceptible hosts to survive. However, epidemics, a frequent manifestation of rabies, deplete the number of susceptible hosts, which leads to the decline or extinction of the virus in the affected population. How, then, does the virus persist?

In southern Africa, rabies virus affects many host species, but rabies cycles are sustained by carnivore hosts (5,6), particularly by canine species (family *Canidae*), which are the focus of this paper, and by mongooses (family *Herpestidae*) (5,7), which will not be considered here. Domestic dogs (*Canis familiaris*) are hosts of rabies virus in most of Africa; they cause most human rabies cases and contacts that require medical intervention. In southern Africa, jackals (*C. adustus*, *C. mesomelas*) are also hosts, although their role has been controversial; some studies indicate that they can support rabies cycles (8), and other studies indicate that they cannot maintain rabies independently of the disease cycle in dogs (9–12). Although rabies is a prominent disease of African canids, the mechanisms and hosts responsible for sustaining it have not been clearly elucidated.

I review the ecology of canine rabies in southern Africa, particularly with the goal of resolving the controversies on mechanisms of persistence. A conceptual and terminologic framework to understand the long-term ecologic survival of rabies virus in African canine hosts is proposed.

Rabies Virus Biology

Rabies virus is transmitted in saliva through the bite of an infected animal. After gaining entry to the central nervous system by peripheral nerves, it causes encephalitis, leading to fulminant, progressive neurologic disease, characterized by excitement, muscular paralysis, impaired responses to social and environmental signals, and other abnormal neurologic signs. The incubation period is unusually variable and can be long; clinical disease and virus shedding are not seen during this period. Infection of the salivary glands during the clinical stage leads to shedding of virions in saliva (13).

Rabies virus has a broad mammalian host range. However, in any ecologic zone, a single species, the maintenance host, is usually principally responsible for supporting the virus cycle. The virus variant of the maintenance host is intimately adapted to the host's physiology and biochemistry to ensure effective transmission (14). Maintenance hosts are usually extremely sensitive to their variant but relatively resistant to rabies virus variants of other species. In maintenance hosts, the probability is high that the virus will establish infection, will induce the appropriate behavioral changes, such as aggressive biting behavior, and will prolong the clinical survival period in which salivary virus shedding takes place; all of these factors lead to maximal virus transmission (14).

For a virus cycle to be successfully maintained, the average transmission ratio (the average number of new

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cases caused by each infected host) must be ≥1. At the beginning of an epidemic, this number is expressed mathematically as the basic reproductive number, R_0, which is defined as the number of new infections generated from an existing infection, when that infection is introduced into a population composed entirely of susceptible hosts (3,15). R_0 is usually treated as a constant that only applies at the beginning of an epidemic, when the ratio of susceptible to infected hosts is at a maximum. R_0 will not be used in this article, as it precludes variability in space and time (16,17); the term average transmission ratio, as defined above, will generally be used instead.

Individuals of species other than the maintenance host may also become infected; they are usually dead-end hosts because of low transmission ratios, which are caused by factors such as the failure to induce biting behavior, inefficient salivary shedding, and absence of other hosts with which to interact. Occasionally, nonmaintenance hosts successfully transmit the infection to conspecifics, which may lead to the establishment of a new cycle if conditions for continual, effective transmission to conspecifics are favorable. The emergence of a new cycle requires some genetic adaptation of the virus in the new host. Lyssaviruses can probably adapt with relative ease because their broad host range allows adaptive selection to take place, as evidenced by the emergence of many new cycles in the last 100 years. A mechanism to explain how such adaptation may arise is the quasispecies concept, where the inherently high mutation rates of RNA viruses produce variant populations of viruses through which selection can act (18).

Definitions and Concepts

Two working definitions will be cited. A local population is a "set of individuals that live in the same habitat patch and therefore interact with each other" (19). A metapopulation is a "set of [discrete] local populations within some larger area, where typically migration from one local population to at least some other patches is possible" (19). The demographic trends of local populations are asynchronous, particularly where migration between them is relatively low. In this article, the definitions of local population and metapopulation may apply to either the host or the virus. To use an analogy borrowed from ecology, the host local population may be viewed as a resource patch for the pathogen.

Maintenance is the notion of indefinite transmission of virus through members of a host population. (In this context "indefinite" transmission does not mean "permanent" but rather denotes an open-ended cycle that is dependent on availability of susceptible hosts.) The average transmission ratio must be ≥1 for virus maintenance to be successful. A maintenance host is a member of a population of susceptible individuals that can replicate, shed, and transmit virus efficiently to conspecifics. Maintenance hosts live in local populations, which support indefinite transmission of virus independently of other local populations.

Individual local populations are unlikely to maintain rabies continuously because of the inherently high instability of the disease in any single local population; the disease is normally reintroduced from other infected local populations. Persistence encompasses the concept of long-term and continuous presence of disease within a metapopulation. Successful persistence requires that virus is maintained in ≥1 local population at any time.

Many viral pathogens depend on a constant supply of susceptible hosts because the viral infection causes either host death or durable immunity. The unstable pattern caused by depletion and renewal of host local populations is a prominent feature of all carnivore rabies cycles, particularly when studied at a relatively fine geographic resolution (6,8,20–24).

Mathematical models have shown the importance of features such as host population heterogeneity and mixing. If a spatially heterogeneous host metapopulation experiences a degree of movement between local populations, a pathogen can persist over the long term even though it may frequently become extinct in local populations (4,25–28). Metapopulation heterogeneity may be in terms of density, demographic structure, social interactions, and other characteristics that influence transmission ratios.

Maintenance and persistence of rabies are affected by population immunity, which in effect lowers the average transmission ratio. In carnivores, population immunity against rabies is almost exclusively caused by vaccination rather than natural infection, which is usually fatal.

Canine Rabies in Southern Africa

In addition to the domestic dog, 3 wild canids have been implicated as independent maintenance hosts of rabies in southern Africa: the side-striped jackal (*C. adustus*), the black-backed jackal (*C. mesomelas*), and the bat-eared fox (*Otocyon megalotis*) (5,6,8,29). Rabies cases have also been reported in other African canids, such as African wild dogs (*Lycaon pictus*) (30) and Ethiopian wolves (*C. simensis*) (31), but these species do not appear to support extended virus cycles independent of other hosts.

In Africa, dogs are intimately dependent on humans for food and shelter (32,33), and this association means that dog populations can be correlated, in size as well as distribution, with human populations. During the 20th century, the human population of Africa expanded enormously, and the dog population expanded in parallel (33). Social changes, such as urbanization, resulted in an increase in human and dog movement. Rabies persistence would have

been enhanced in such a dog metapopulation consisting of more numerous local populations and greater movement of infected dogs.

Such a prediction is borne out by historical records. Rabies in sub-Saharan Africa is a disease of modern times; no firm evidence exists of its occurrence before the late 19th century. The first confirmed outbreaks, in South Africa in 1893 and Southern Rhodesia (now called Zimbabwe) in 1902, were in domestic dogs, and their origin was traced to distant lands (5). The initial outbreaks were temporally and spatially sporadic, and rabies apparently could not become established. These outbreaks were followed by increasingly frequent, but initially sporadic, outbreaks, until the disease was continuously present in national records, as can be seen in the annual reports of the departments of animal health of various African countries from 1892 to 1960.

Rabies in jackal species appeared in southern and eastern African countries after the introduction of the disease in dogs. In Zimbabwean *C. adustus* populations, rabies occurred in large, dense, moving epidemics in commercial farming areas (8). The jackal index cases of the epidemics were usually associated with cases in dogs, which indicates that these epidemics were initiated by dog rabies cycles; once initiated, however, the epidemics were maintained independent of dogs. The moving epidemics terminated at the geographic limits of the *C. adustus* population dominance.

Both jackal species reach high densities in commercial farming environments. Jackal rabies occurs predominantly in these areas, but it is virtually absent from most national parks, despite substantial jackal populations (8). Commercial farming practices appear to provide ecologic conditions that are highly favorable for jackals and jackal rabies. What these conditions are is unclear, but they may include abundant resources, increased demographic turnover, and the absence of competitors such as dogs and wild scavengers.

In southern Africa, rabies in *C. mesomelas* and *O. megalotis* predominantly occurs in the absence of domestic dogs or rabies in other carnivores. Given the general lack of associated rabies cases in other species, *C. mesomelas* and *O. megalotis* are likely maintenance host populations, capable of maintaining the virus cycle independent of other species. In *C. mesomelas* and *Otocyon* populations, the mechanisms of rabies maintenance are poorly understood; because surveillance is scanty, discerning true spatial and temporal disease patterns is difficult (5,8,29).

Molecular epidemiology studies have indicated that, in Zimbabwe and South Africa, rabies viruses from dogs and jackals are phylogenetically similar and do not fall into host-distinguishable lineages, while viruses of *O. megalotis* are closely related but distinct (34). In addition, viruses all fall within the cosmopolitan lineage that includes many other dog and wild canine virus variants from other regions of the world (35). This finding supports the epidemiologic observations that all present-day dog, jackal, and *O. megalotis* rabies viruses in southern Africa stem from a single, recently introduced virus derived from domestic dogs. However, molecular techniques are not precise enough to indicate which species, dogs or the 3 wildlife species, maintains the virus cycles that are found in wildlife. Epidemiologic evidence is required to determine this (5,8,29).

Review of African Canine Rabies Studies

I propose that African canine rabies ecology must be understood through the distinct concepts of maintenance and persistence. An alternative mechanism proposed for rabies persistence in African domestic dogs includes an infectious healthy carrier status (10,36). One study proposes this mechanism for rabies in spotted hyenas (*Crocuta crocuta*, Carnivora: *Hyaenidae*), on the basis of rabies virus RNA detected in saliva of healthy hyenas by polymerase chain reaction, although virus isolation, arguably the more important test, was unsuccessful from these samples (37). A second mechanism by which rabies may persist is the concept of long incubators, which carry infection through quiescent periods to restart epidemics when the host density has recovered. Long incubators have been reported mainly in humans and other nonmaintenance species (38,39). No evidence currently shows that carrier animals or long incubators play a role in the persistence of rabies cycles in canine hosts, perhaps because they do not occur frequently enough to be obvious. Although they should not be ignored, these concepts are difficult to demonstrate scientifically and even more difficult to quantify in terms of their ecologic importance for virus persistence.

Previous studies have questioned the ability of jackals to support rabies virus cycles (9–11). These studies have not distinguished between the ability of species to support pathogen cycles (i.e., maintenance) and the concept of long-term persistence. Acknowledging this distinction would show that local populations of canine species may maintain epidemics independently and may be free of rabies for periods, often long periods, between epidemics. At the level of the local population, this pattern is essentially similar in domestic dogs, jackals, and other canids (e.g., African wild dogs [30]). Domestic dogs may appear to support rabies infection endemically, whereas jackals do not (11), simply because more numerous discrete local dog populations are within the study area than are jackal populations.

Considering rabies ecology through the concepts presented here would clarify some of the confusion created by

these studies (9–11). For example, a study (11) that used a mathematical model concluded that "the side-striped jackal population itself does not seem able to support rabies infection endemically, i.e., without frequent reintroduction from outside sources of infection." While reintroductions of infection are certainly an important feature in *C. adustus* rabies, reintroduction is probably infrequent, given that most *C. adustus* cases reported in 46 years followed 5 dog-to-jackal initiation events (8). The contradiction is resolved once the concepts of maintenance and persistence are applied. Hence, *C. adustus* is capable of maintaining rabies cycles independent of other species, but rabies cycles have not been persistent.

Terms such as reservoir and endemic do not provide the conceptual clarity necessary to understand rabies ecology (10,11). In a study that acknowledges the complexities of defining the term and uses canine rabies as an example, Haydon et al. (12) state that a reservoir is a population "in which the pathogen can be permanently maintained." However, this definition is problematic because the term "permanently maintained" is ambiguous. This study implies that dogs, but not jackals, are reservoirs because they are permanent hosts, yet many dog populations, as with many jackal populations, do not permanently support rabies cycles. Such definitions fail to provide a convincing argument that essential distinctions exist between dogs and jackals in their ability to support rabies cycles. The study by Haydon et al. also defines a reservoir in relation to a target population, which is "the population of concern or interest to us" and which requires protection. Such anthropocentric definitions of pathogen behavior, although having some conceptual value for protecting human health and interests, are unhelpful for understanding the biologic mechanisms of pathogen emergence and persistence.

The scale at which ecologic systems are examined has implications, since disease frequency is less stable in local populations than in metapopulations (40). Hence, a farmer will perceive rabies on his property as epidemic in nature, with intense outbreaks separated by long periods of absence, while a national epidemiologist may claim that the disease is endemic in his country. Both observations are correct, but observers perceive the epidemiology differently because they view the disease frequency at different scales. The lump analysis of national rabies case data of Zimbabwe (10,11) led to a blending and masking of disease patterns, giving these researchers the erroneous impression that rabies frequency in dogs was more stable than it was in jackals.

While the transmission ratio, as influenced by host density, for example, is the only determinant of maintenance, it is not the sole determinant of persistence. In discussing rabies in Zimbabwean jackal populations, Rhodes et al. (11) suggest that the "average jackal population density is too low to maintain the chain of infection." However, the dense moving epidemics, which lasted several years and occurred in the absence of cases in other species (8), imply that host density was not a limiting factor in jackal epidemics. Once again, we can resolve this apparent contradiction by considering these populations of jackals to be capable of cycle maintenance but not persistence. Transmission is efficient within local populations, but high transmission ratios are transient. The jackal populations of Zimbabwe and the rabies viruses they support should not be considered metapopulations because they do not have, as dog populations do, the spatial separation or interpopulation migration that would be necessary for them to be considered metapopulations. This absence of a metapopulation structure explains the failure of rabies persistence in jackal populations, and this absence, rather than their inability to maintain virus, distinguishes jackals from dogs as hosts of rabies.

Control of Rabies in Africa

Applying the metapopulation principle to canine rabies in Africa reinforces conventional principles of control: employing vaccination or culling in affected host populations and minimizing movement of infected hosts. For many decades, controlling rabies in Africa, has been the mandate of governments. Vaccination programs for dogs generally consisted of periodic visits by a government vaccination team to communities, which were seen as compliant recipients. While in some cases such government-initiated control efforts arguably had some effect on reducing rabies, they did not cause any long-term trend in reducing rabies in maintenance hosts or humans. African governments have been unable to sustain the level of resource commitment needed to maintain effective levels of vaccination coverage. The metapopulation principle indicates that with increasing dog population density, size, and movement, rabies control will require ever-increasing resources. Traditional methods that have not worked well in the past are likely to be even less effective in the future. Instead, a completely different approach to controlling rabies is needed. Perhaps this approach should be based on community-driven initiatives, where the role of governments focuses on support activities such as surveillance, information dissemination, and legislation. Since dogs are an integral and dependent part of human communities, community-driven initiatives for rabies control may be more sustainable than those directed by governments.

Conclusions

In recent decades, the frequency of rabies has increased in Africa. Controlling this disease will require a deeper understanding of its biology. When interpreting rabies case data for epidemiologic analysis, we must distinguish

between the concepts of maintenance (the ability of local populations to support a disease cycle) and persistence (the presence of ≥1 infected local population in a host metapopulation). To clearly conceptualize the ecology of canine rabies, we must use lucid, appropriate definitions for virus-host interactions and epidemiologic patterns.

The ecology of many ecosystems has changed dramatically in recent centuries because of the increase in human populations, the introduction of large-scale commercial agriculture, urbanization, loss of biodiversity within the human biosphere, and other changes. The new ecologic landscapes have been exploited by species that can adapt favorably to them, including many of the prominent rabies maintenance hosts. Rabies viruses have recently become prominent in the African ecosystem because of transmission in mammals that have exploited ecologic changes that have occurred in much of the continent. Such change is set to continue into the future, and those species that can flourish under the new conditions will be candidate hosts for the maintenance of pathogens.

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Dr Bingham completed his PhD dissertation on jackal rabies in Zimbabwe while working at the Central Veterinary Laboratories in Harare. His interests include the ecology and evolution of infectious diseases.

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Achieving Operational Hydrologic Monitoring of Mosquitoborne Disease

Jeffrey Shaman* and Jonathan F. Day†

Mosquitoes and mosquitoborne disease transmission are sensitive to hydrologic variability. If local hydrologic conditions can be monitored or modeled at the scales at which these conditions affect the population dynamics of vector mosquitoes and the diseases they transmit, a means for monitoring or modeling mosquito populations and mosquitoborne disease transmission may be realized. We review how hydrologic conditions have been associated with mosquito abundances and mosquitoborne disease transmission and discuss the advantages of different measures of hydrologic variability. We propose that the useful application of any measure of hydrologic conditions requires additional consideration of the scales for both the hydrologic measurement and the vector control interventions that will be used to mitigate an outbreak of vectorborne disease. Our efforts to establish operational monitoring of St. Louis encephalitis virus and West Nile virus transmission in Florida are also reviewed.

Operational systems that can accurately monitor and predict mosquitoborne disease transmission continue to be needed. Because mosquitoes and the pathogens they transmit are sensitive to environmental conditions, 1 approach has been to use our ability to monitor and predict environmental variability and our understanding of mosquito and mosquitoborne pathogen response to that variability to monitor and predict mosquitoborne disease transmission. This reasoning assumes that we can monitor and predict environmental variability over large areas at the scale at which it affects mosquitoborne disease transmission and that the relationships between environmental variability and mosquitoborne transmission response are clear and stationary. Here we review the recent developments of operational systems that use hydrologic variability to monitor mosquitoborne disease transmission.

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Rainfall and Mosquitoborne Disease

Many mosquito species depend on the availability of water. The first 3 stages of the mosquito life cycle (egg, larvae, and pupae) are aquatic. Consequently, mosquito abundance and the transmission of many mosquitoborne pathogens can be affected by hydrologic variability, in particular, fluctuations in the water cycle that alter the availability of suitable aquatic habitats. To explore these effects, researchers have long looked for associations between rainfall variability and mosquito abundance (1–4) and mosquitoborne disease incidence (5–12). Although using rainfall as an explanatory hydrologic variable is convenient, the physical effects of precipitation on surface conditions are multiple, and the responses of different mosquitoes and mosquitoborne pathogens to these effects are varied. As a result, establishing statistically significant and stationary relationships between precipitation and mosquito abundance or mosquitoborne disease transmission is difficult.

Rainfall has 2 principal influences on the mosquito life cycle: 1) the increased near-surface humidity associated with rainfall enhances mosquito flight activity and host-seeking behavior, and 2) rainfall can alter the abundance and type of aquatic habitats available to the mosquito for oviposition. The first influence can increase mosquito abundance by accelerating the reproductive cycle, which requires mating, host-seeking, and blood-feeding flights. The second influence, however, has less certain consequences. Rainfall increases the wetness of soil near the surface and can expand saturated lowland areas. As a result, the moist, humid habitats preferred by many mosquito species for oviposition, such as swamps and floodwaters (e.g., puddles, water-filled divots), may increase in abundance. This change may favor an increase of mosquito species abundance in these habitats. Such changes in mosquito species composition, abundance, and age structure may then lead to an increase in local disease transmission.

However, the availability of suitable mosquito habitats is not a simple linear function of rainfall. Surface wetness depends on a number of environmental conditions other than precipitation, including antecedent wetness, soil type, and rates of evapotranspiration (i.e., combined evaporation and transpiration). Furthermore, excessive rainfall can decimate some mosquito populations by flushing larval habitats. Other mosquito species can benefit from drought conditions such as when streams dry up and pools more suitable for oviposition form in riverbeds, or when standing waters become eutrophic with increased organic content, which provides additional food for mosquito larvae.

Further complications arise when attempts are made to associate rainfall with mosquitoborne disease incidence. Mosquitoborne disease transmission is related most directly to the number of infected mosquitoes able to transmit disease and not to the total number of biting mosquitoes present in a population (13,14). As a consequence, increased mosquito abundance does not necessarily increase mosquitoborne disease transmission. As mosquito abundance increases, mosquito infection rates must also increase if disease transmission risk is to increase substantially; this requires that newly emerged mosquitoes acquire pathogens and become infective.

Monitoring Mosquito Breeding Habitats

The response of mosquito populations to changes in precipitation and the effects of such changes on mosquitoborne disease transmission are quite complex and variable. However, these responses might be better elucidated if mosquito-breeding habitat availability, the variable for which precipitation is principally a proxy, could be monitored directly.

Several studies have examined how water management practices (e.g., irrigation, damming) affect anopheline density and malaria incidence. Surface waters on rice-cultivated land were associated with *Anopheles gambiae* density in the Ivory Coast (15); anopheline densities in Thailand were associated with rice paddy fields (16); and in Peru, irrigation around villages and houses played a role in determining human malaria risk (17). In Tanzania, *An. arabiensis* densities were 4 times higher in villages with rice cultivation, but malaria exposure was lower because of greatly decreased sporozoite rates among this mosquito population (18). This last study again illustrates some of the complexity underlying the relationship between mosquito abundance and disease transmission risk.

Although the effects of irrigation and water control are clearly important, for many disease systems natural surface water variability is likely an even greater determinant of vector density and mosquitoborne disease transmission rates. However, because monitoring surface water has traditionally been difficult, relatively few studies have explored these relationships. In Uganda, malaria incidence among children was associated with the proximity of their homes to swamps and streams that served as mosquito breeding sites (19). In Sri Lanka, the abundance of the primary malaria vector, *An. culicifacies*, has been linked to the drying of riverbeds (20,21).

Because ground observations of surface water prevalence are time-consuming and difficult to carry out over large areas, an attractive alternative has been to use remote sensing measurements of land surface wetness. Many such top-down studies have associated the abundance of vectors or vectorborne disease incidence by using satellite imaging (22–28). Such investigations have generally used vegetation classification or the Normalized Differential Vegetation Index (NDVI), which measures vegetation greenness, as proxies for soil moisture and land surface wetness.

A more recent approach has been to simulate land surface wetness conditions by using a hydrology model. Such models can represent the hydrologic cycle at the land-atmosphere interface and track the movement of water and energy between the soil, vegetation, and atmosphere. By accounting for soil type, vegetation type, topography, evapotranspiration rates, and precipitation, one may continuously simulate surface pooling in space and time.

In some sense, the use of a hydrology model is a hybridization of bottom-up (ground observation) and top-down (satellite imaging) approaches and can be developed in conjunction with both through data assimilation. This approach has several advantages: 1) it models the actual aquatic environment used by the mosquitoes, not a filtered proxy; 2) it offers continuous real-time prediction of hydrologic conditions, that is unconstrained by orbital patterns, cloud cover, or vegetation; 3) it resolves the whereabouts of the potential breeding habitats at a very fine scale (areas as small as 10-m cells); and 4) hydrologic models are readily coupled to global climate models, allowing additional medium- and long-range forecast of hydrologic conditions.

Several studies have employed such models. Patz et al. (29) used a water balance model to hindcast weekly soil moisture levels in the Lake Victoria basin. These soil moisture levels were then associated with local human biting rates and entomologic inoculation rates. Shaman et al. (30) used a more detailed hydrology model to predict flood and swamp water mosquito abundances in New Jersey. Mosquito species were found to respond differently to changing local wetness conditions, and these differences were consistent with known breeding behavior and habitat preferences. For example, swamp and flood water mosquito abundance increased during wet conditions, while mosquitoes that preferred eutrophic breeding habitats increased during dry periods. Thus, hydrologic variability was able to

differentially predict mosquito species abundances. In a separate study in Florida, amplification and transmission of St. Louis encephalitis virus (SLEV) were associated with changing modeled land-surface wetness conditions (31).

Developing Early Warning Systems

Irrespective of the hydrologic variable measured (e.g., measured rainfall, water management, or irrigation effects; satellite measurements of land-surface wetness; or modeled hydrologic conditions), if the variable is associated with mosquitoborne disease transmission in a stationary and robust manner, it may be used to monitor that disease. A few such monitoring systems have recently been developed in which climatic conditions (monthly rainfall and temperature) appropriate for mosquitoborne disease transmission are used to develop risk maps of the geographic distribution of the disease. In Africa this risk assessment has been applied on a large scale and used to develop maps of malaria risk and distribution (32,33). Where precipitation is found to precede malaria transmission and the data are readily available, these maps could be used as part of a malaria early warning system. Such maps are an important step toward achieving operational monitoring and forecasting of malaria transmission.

Issues of Scale

Before hydrologic factors are used to monitor and forecast mosquitoborne disease transmission, the scales at which the disease system responds to hydrologic variability, as well as the scales at which hydrologic variability can be monitored, must be considered. For instance, empirical findings may demonstrate that a particular disease vector responds to local variations in precipitation (i.e., hydrologic changes in its immediate environment). To monitor this vector population, one would like to keep informed of local rates of rainfall, preferably over the entire geographic range of the vector. However, in the tropics, precipitation rates can differ greatly between locations just a few kilometers apart, but meteorologic stations are much more sparsely distributed. The mismatch between the scales at which a disease vector responds to hydrologic variability and the scales at which hydrologic variability can actually be monitored limits operational application of such empirical findings and underscores the need to develop systems that monitor and forecast hydrologic variability at scales corresponding to disease system ecologies.

Operational monitoring and forecasting of any vectorborne disease also requires consideration of how information is to be used. Empirical relationships derived from either microenvironment- or macroenvironment-level scales must be relevant at the scales at which vector control interventions are applied. For example, modeling and mapping of individual mosquito oviposition sites (e.g., herbivore hoof prints) may prove too detailed, heterogeneous, and computationally expensive to be of practical use as an aid for vector control efforts. However, if the flooding and drying patterns of such hoof prints are highly covariable over a large spatial scale, for example, several square kilometers, then modeling flooding and drying at these sites might prove feasible. That is, rather than attempt to monitor and control oviposition sites individually, the herbivore hoof prints would instead be monitored collectively on a larger spatial scale. This information could then be used to help make vector-control intervention decisions at that larger spatial scale by focusing the application of larvicide to selected areas wet enough to support many hoof print pools. This approach would reduce unneeded control efforts in regions too dry to support such pools.

Similarly, seasonal climate model predictions may lack the temporal and spatial resolution needed to discern local disease transmission patterns. Resources may be wasted if control efforts are blanketed over a large region of which only small portions are “hot zones” of vector and disease activity. Yet if stable relationships exist between grid-scale and subgrid scale variability, useful information for intervention might be gleaned from such models. For instance, climate models often have a resolution (i.e., grid scale) of $2.5° \times 2.5°$, about 75,000 km^2. This is a very large area over which to adopt a single vector-control intervention strategy. Clearly, variable levels of vector and disease activity will exist within such an area. Local understanding of subgrid scale (i.e., scales <75,000 km^2) variability and how it relates to grid-scale variability is needed to focus control efforts. This understanding could be as simple as knowing that during drought the rivers within a given $2.5° \times 2.5°$ area pool and serve as mosquito-breeding sites and that control efforts should be focused in the pools that form along these rivers.

Thus, the issue of scale has to be considered from both scientific and operational vantages. Not only must empirical relationships between mosquitoborne disease systems and hydrology be robust, they must also be capable of being monitored and used with vectorborne disease intervention programs. Monitoring hydrologic variability must be possible both at the scale at which it affects mosquitoes and mosquitoborne disease transmission as well as at a scale at which interventions can feasibly and effectively be applied. Such considerations, of course, are inextricably linked to an understanding of the biology and ecology of vectorborne disease systems.

Beginning Operational Application: Florida

We now present an example from our own efforts to establish, real-time operational hydrologic monitoring of West Nile virus (WNV) in Florida. We begin with the

scientific basis for this system then describe operational application of the system in 2004. Further development of the monitoring system is then discussed, and issues of scale are considered.

Since its appearance in New York City in 1999, WNV has spread to every state in the continental United States and has emerged as an important infectious disease threat to the general public. WNV was first detected in Florida in 2001; since then human cases of WNV illness have occurred sporadically throughout the state. WNV is closely related to SLEV. Both WNV and SLEV are maintained in the environment through enzootic transmission between avian amplifying hosts and vector mosquitoes (34). In a series of articles, we have demonstrated that a specific sequence of hydrologic conditions, spring drought followed by continued summer rainfall, is critical for the amplification and transmission of both SLEV and WNV in south Florida (31,35–38). Amplification involves a cascade of enzootic virus transmission between vector mosquitoes and wild avian hosts and results in a rapid increase in the number of infected and infective vector mosquitoes. Drought enhances amplification by restricting vector mosquito activity to selected habitats in south Florida, specifically densely vegetated hammocks where mosquitoes rest and wild birds nest and roost. Increased contact between vector mosquitoes and avian amplification hosts in these hammocks facilitates early season viral amplification by forcing the interaction of mosquitoes and birds.

When drought-induced, early season viral amplification in the initial transmission foci is followed by high amounts of summer rainfall that increases near surface humidity levels and the availability of oviposition sites, infective mosquitoes are able to disperse and initiate secondary transmission foci away from the original amplification site. This dispersal and secondary amplification in urban and suburban habitats dramatically increase the number of infective mosquitoes in the environment and place them into greater contact with humans during the late summer months, when WNV and SLEV epidemics typically occur in south Florida. In addition, the mosquito population can increase dramatically in response to the increased water resources. Because of the increased viremic rate among the wild bird population, these newly emergent mosquitoes are themselves more likely to acquire infection.

Our analyses cited above demonstrate that high transmission rates of SLEV and WNV to humans are more likely to occur after a spring drought that is followed by continuous summer rainfall. Public health and vector control personnel are greatly concerned about the likelihood of a major WNV epidemic in Florida. A widespread spring drought followed by continued summer wetting, conducive for epidemic transmission, last occurred in Florida in 1990. During that year, 226 human cases of SLE were reported throughout the southern half of the state. Since the first appearance of WNV in Florida during 2001, the hydrologic pattern of spring drought followed by summer wetting has not occurred. Because of the similarity of SLEV and WNV transmission cycles (38), such a hydrologic pattern will likely result in a major WNV epidemic in the human population of south Florida.

To combat this threat, during 2004 we established real-time monitoring of hydrologic conditions in south Florida. Simulations using the topographically based hydrology (TBH) model (39,40) were made with National Climate Data Center meteorologic data from 49 station sites in south Florida through the end of 2003 (see [38] for further details). These simulations were then extended in real time during 2004 by using real-time, hourly data of land surface meteorologic conditions distributed at 0.125° resolution available from the National Oceanic and Atmospheric Administration (NOAA) through its Global Energy and Water Cycle Experiment (GEWEX) Continental-Scale International Project Land Data Assimilation System (LDAS) Project. Using these data and the TBH model, we produced maps of land-surface wetness conditions (Figure 1). These maps were made available to personnel at the Florida Medical Entomological Laboratory, as well public health and mosquito control officials throughout Florida. These data were used to evaluate ongoing WNV transmission and the threat of virus transmission to humans. The risk for human infection in Florida was reported in a series of risk maps that were published at http://eis.ifas.ufl.edu and updated as changing conditions warranted. The land surface wetness maps were especially helpful in evaluating WNV transmission in Miami, where sporadic human WNV disease cases were reported from June through September 2004.

During 2004, Florida did experience intense, widespread drought; however, the drought persisted through much of the summer. Exceedingly dry conditions existed throughout most of south Florida during late June, July, and early August (Figure 1). This severe drought prevented the infectious mosquito population, which had developed during amplification in May and early June, from dispersing and initiating secondary transmission and amplification foci. This situation resulted in limited WNV transmission to humans in south Florida, where cases were reported in Hillsborough (3 cases), Brevard (4 cases), Broward (3 cases), and Dade (21 cases) Counties. Only with the arrival of Hurricanes Charley and Frances in mid-August and early September (Figures 2 and 3) did land surface conditions in south Florida become considerably wetter. This occurrence, however, was too late to allow the dispersal of infective mosquitoes and the establishment of secondary amplification and transmission foci in time to

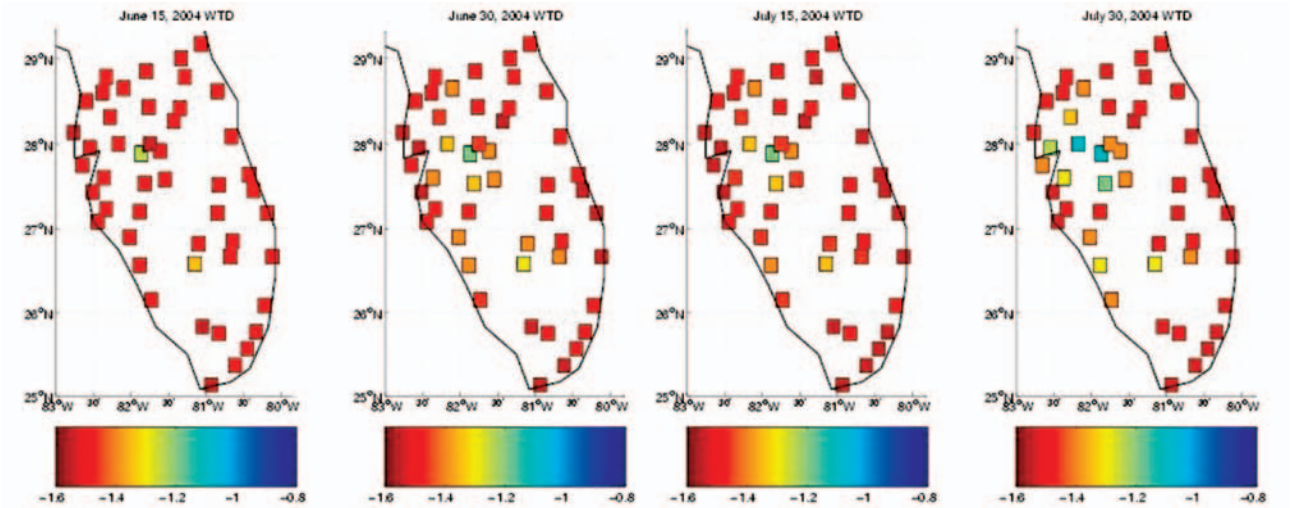

Figure 1. Map of early summer 2004 hydrologic conditions as modeled with the topographically based hydrology model at 49 sites throughout south Florida. Daily averaged conditions are shown for June 15, June 30, July 15, and July 30, 2004. Red colors are drier soil conditions, supporting less surface pooling; blue colors are wetter.

produce infected mosquitoes on a level that would result in epidemic transmission of WNV.

Future Prospects and Issues of Scale in Florida

In 2005, we plan to turn operation of this real-time monitoring over to personnel at the Florida Medical Entomological Laboratory. Sustained real-time use of this information will require continual evaluation of TBH model performance, as well as the empiric relationship between modeled land surface wetness conditions and WNV transmission. Our analyses of WNV and SLEV transmission over the last 25 years in south Florida indicate that this empiric relationship is robust and stationary. The spring maps of hydrologic conditions indicate where drought is occurring and WNV amplification is most likely to occur. The summer maps demonstrate where mosquito dispersal and the establishment of secondary transmission foci are likely to occur. The late summer maps indicate where the risk for WNV transmission to humans is greatest in south Florida.

A geographically large-scale WNV epidemic is of primary concern to public health workers in Florida. Large-scale hydrologic events are easily monitored by using records from the current network of meteorologic stations to model land-surface wetness. However, a denser network of stations may be necessary to comprehensively

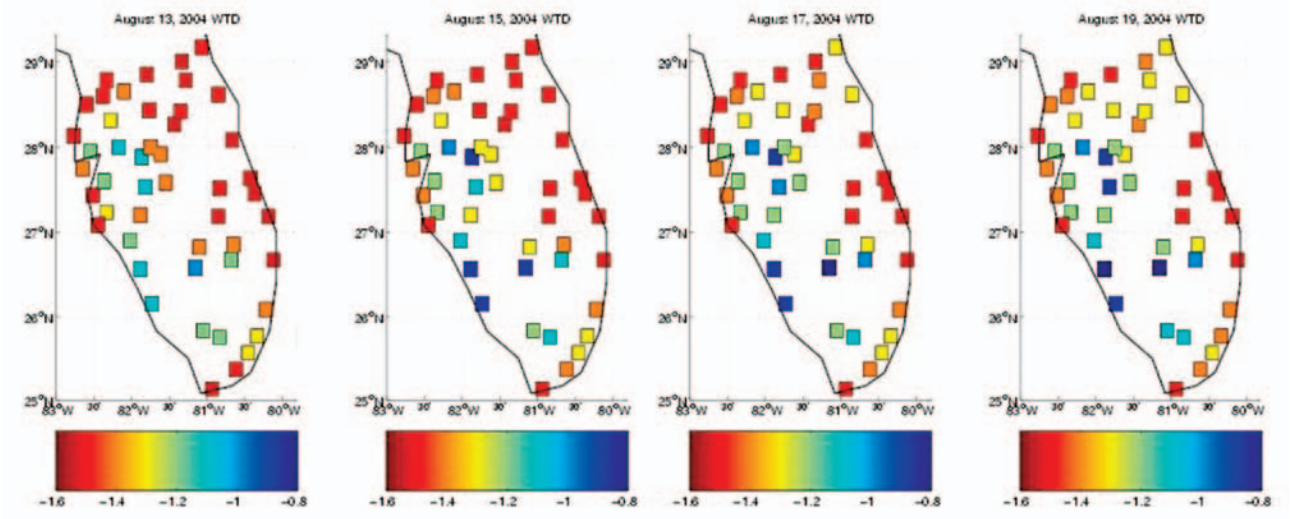

Figure 2. Map of hydrologic conditions during the landfall of Hurricane Charley (August 13) as modeled with the topographically based hydrology model at 49 sites throughout south Florida. Daily averaged conditions are shown for August 13, 15, 17, and 19, 2004. Red colors are drier soil conditions, supporting less surface pooling; blue colors are wetter.

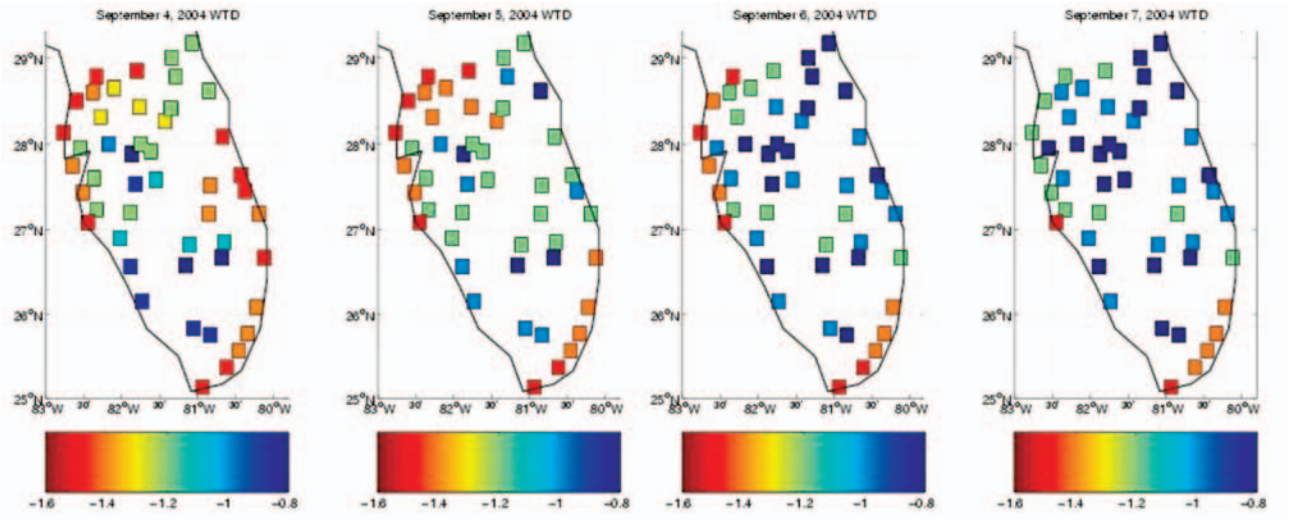

Figure 3. Map of hydrologic conditions during the landfall of Hurricane Frances (September 4) as modeled with the topographically based hydrology model at 49 sites throughout south Florida. Daily averaged conditions are shown for September 4–7, 2004. Red colors are drier soil conditions, supporting less surface pooling; blue colors are wetter.

monitor and predict small-scale focal and sporadic WNV transmission in south Florida. To first order, hydrologic conditions in subtropical Florida covary at the spatial scales at which storms organize. With the exception of irrigation, precipitation is the sole source of water to near-surface soils. Because much of the rainfall in Florida is convective, land-surface wetness can vary over short spatial scales (<10 km). The network of meteorologic stations in south Florida is too sparse to detect this small-scale variability. In the future, we plan to use Doppler radar measurements of rainfall and GEWEX LDAS data to run the TBH model at 0.125° resolution throughout south Florida. Such higher resolution monitoring of drought conditions will enable more comprehensive depiction of the small-scale hydrologic variability associated with focal and sporadic WNV amplification and transmission and may enable such events to be detected. Interventions, such as public health warnings or more intense mosquito control efforts, could then be effected at this scale (0.125° resolution).

In addition to monitoring hydrologic conditions, quantitative maps of WNV transmission risk can be constructed at a scale that is not presently available. Risk maps are generated by combining the empirical relationships derived for the dependence of WNV transmission to sentinel chickens and humans on TBH-modeled wetness conditions in south Florida (38) with the real-time TBH-model-simulated hydrologic conditions. These predictions provide likelihoods of future WNV transmission to sentinel chickens and to humans. As such, these forecasts do not provide all-or-none predictions, which are right or wrong. Rather, the information allows vector-control experts and public health officials to examine shifts in the likelihood of WNV transmission in both space and time. A cost-benefit analysis might show that a small increase in the likelihood of WNV transmission in an area warrants radical changes in local vector-control efforts. Such an analysis could determine the optimal allocation of public health monies in response to these probabilities. Further investigation of such issues is needed for scientists and public health officials to determine fully the utility and limitations of hydrologic predictions.

Our hope is that an operational WNV and SLEV monitoring system will anticipate the next large arbovirus epidemic in Florida. Results will be even better if timely vector control measures are initiated in response to the real-time modeled hydrologic conditions and avert an epidemic. Whether vector control efforts can mitigate a major WN epidemic remains to be seen; however, vector-control efforts at the front end of an epidemic, during amplification, are far more valuable than control efforts attempted after the epidemic has peaked. The key will be to focus the intervention accurately in space and time to the scale of the problem.

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Variant Creutzfeldt-Jakob Disease Death, United States

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The only variant Creutzfeldt-Jakob disease (vCJD) patient identified in the United States died in 2004, and the diagnosis was confirmed by analysis of autopsy tissue. The patient likely acquired the disease while growing up in Great Britain before immigrating to the United States in 1992. Additional vCJD patients continue to be identified outside the United Kingdom, including 2 more patients in Ireland, and 1 patient each in Japan, Portugal, Saudi Arabia, Spain and the Netherlands. The reports of bloodborne transmission of vCJD in 2 patients, 1 of whom was heterozygous for methionine and valine at polymorphic codon 129, add to the uncertainty about the future of the vCJD outbreak.

Variant Creutzfeldt-Jakob disease (vCJD) was first reported in 1996 in the United Kingdom and has been causally linked to eating cattle products contaminated with the bovine spongiform encephalopathy (BSE) agent (1–3). Both vCJD and BSE are rapidly progressive neurodegenerative disorders classified as transmissible spongiform encephalopathies (TSEs) or prion diseases. TSEs are characterized by 1) incubation periods measured in years, 2) presence in the brain of an unconventional agent called a prion, 3) absence of inflammatory reaction, and 4) a neuropathologic feature consisting typically of spongiform lesions, astrogliosis, and neuronal loss. vCJD is distinguished from the more common TSE in humans, sporadic CJD, by the younger median age (28 years and 68 years, respectively) of the patients and by its clinical and neuropathologic manifestations.

In 2002, the likely occurrence of vCJD was reported in a 22-year-old woman living in Florida and represented the first detection of the disease in North America (4). In this report, we describe this patient's illness and provide an update on the epidemiologic features of the ongoing vCJD outbreak worldwide, including recent reports of bloodborne transmission of vCJD.

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Case Report

In early November 2001, the patient in Florida was evaluated for depression and memory loss that adversely affected her work performance and may have contributed to a traffic ticket she received for failure to yield the right of way at a traffic sign. In December 2001, the patient developed involuntary movements, gait disturbances, difficulty dressing, and incontinence. The following month, she was taken to a local emergency department; a computed tomographic scan of her brain showed no abnormalities, a diagnosis of panic attack was made, and antianxiety medications were prescribed.

In late January 2002, the patient was transported to the United Kingdom, where her mother resided. During the ensuing 3 months, the patient's motor and cognitive deficits worsened, which caused falls resulting in minor injuries; she had difficulty taking care of herself, remembering her home telephone number, and making accurate mathematical calculations. She also experienced confusion, hallucination, dysarthria, bradykinesia, and spasticity. An electroencephalogram evaluation showed no abnormalities, but a brain magnetic resonance imaging (MRI) study showed the characteristic signal abnormalities in the pulvinar and metathalamic regions suggestive of vCJD. Western blot and immunohistochemical analyses of tonsillar biopsy tissue demonstrated the presence of protease-resistant prion protein, which supported the diagnosis of vCJD. By September 2002, the patient was bedridden. An experimental treatment with quinacrine was given to the patient for 3 months, but she showed little

improvement. She remained in a state of akinetic mutism and died in June 2004, ≈32 months after illness onset.

The patient was born in Great Britain in 1979 and immigrated to the United States with her family in 1992. She had no history of surgery or receipt of blood or blood products, and she was never a blood donor. Consistent with findings for vCJD patients in the United Kingdom associated with potential foodborne exposure, this patient was homozygous for methionine at polymorphic codon 129 of the prion protein gene. A full autopsy was performed, and neuropathologic examination of brain tissue showed the presence of florid plaques and severe cortical atrophy (Figure 1). Immunohistochemical staining for the prion protein showed numerous plaquelike formations along with a synaptic staining pattern similar to that previously described in other vCJD patients (Figure 2).

This patient is the only US resident with a confirmed diagnosis of vCJD. She was likely exposed to BSE while growing up in the United Kingdom from 1980 to 1992, which suggests an incubation period of 9–21 years (Table). The illness duration in this patient (≈32 months) was much longer than the median illness duration for patients in the United Kingdom with vCJD (14 months, range 6–40 months).

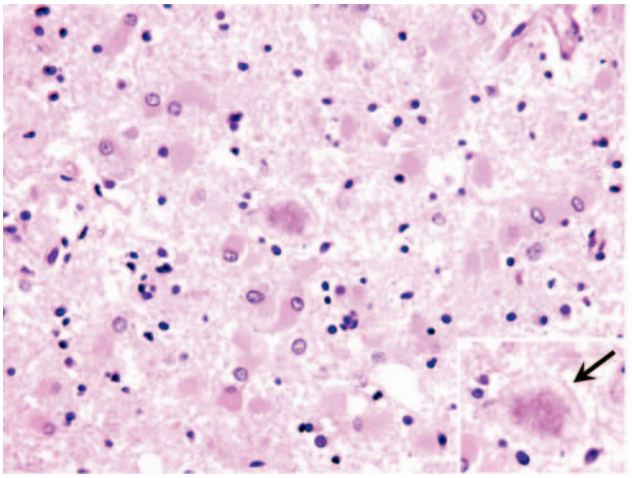

Figure 1. Histopathologic changes in frontal cerebral cortex of the patient who died of variant Creutzfeldt-Jakob disease in the United States. Marked astroglial reaction is shown, occasionally with relatively large florid plaques surrounded by vacuoles (arrow in inset) (hematoxylin and eosin stain, original magnification ×40).

Updates on vCJD

As of early August 2005, 157 vCJD patients were reported from the United Kingdom: 13 have been reported from France, 3 from Ireland, and 1 each from Canada, Italy, Japan, Portugal, Spain, the Netherlands, and the United States (Figure 3). Similar to the vCJD patient from the United States, 1 patient from Ireland and the patients from Canada and Japan were likely exposed to the BSE agent during their residence in the United Kingdom. Preliminary information indicates that the Japanese patient spent only ≈24 days in the United Kingdom. In addition, the US National Prion Disease Pathology Surveillance Center confirmed a vCJD diagnosis by analyzing a brain biopsy sample from a 33-year-old Saudi man admitted to a hospital in Saudi Arabia. Although detailed information on this patient was not available, he may have visited the United Kingdom, if at all, only for several days. Thus, the patient likely contracted the disease in Saudi Arabia after eating BSE-contaminated cattle products imported from the United Kingdom.

Certain characteristics distinguishing vCJD from classic CJD raised early concerns about possible secondary bloodborne spread of vCJD, especially in light of the lack of experience with this newly emerged disease. Of specific concern was the detection of the agent in lymphoid tissues and the possibility of prionemia as the agent spreads from the gut to the brain. In 1997, to monitor for the possible bloodborne transmission of vCJD, researchers in the United Kingdom began investigating recipients of blood components obtained from donors who subsequently died of vCJD (5). As of February 2004, 48 recipients were identified, including 18 who had survived for ≥4 years after transfusion. Two of these 18 recipients had evidence of bloodborne transmission of vCJD. One of the 2 recipients was 62 years of age and had received 5 units of erythrocytes in 1996 (5). One of these units was traced to a 24-year-old donor in whom vCJD developed >3 years after the blood was donated. In 2002 (6.5 years after the transfusion), vCJD developed in the recipient, who died 13 months after illness onset. An autopsy confirmed the diagnosis of vCJD.

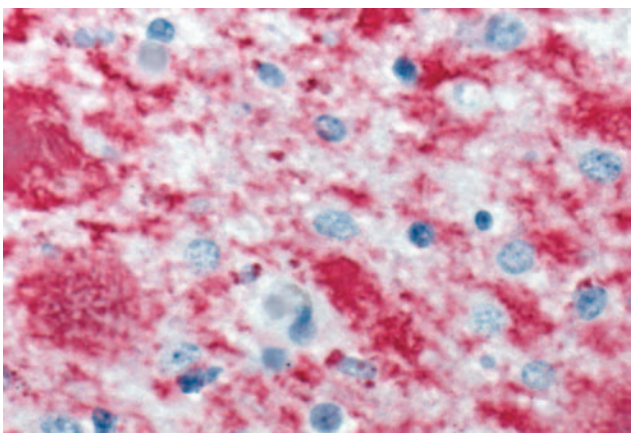

Figure 2. Immunohistochemical staining of cerebellar tissue of the patient who died of variant Creutzfeldt-Jakob disease in the United States. Stained amyloid plaques are shown with surrounding deposits of abnormal prion protein (immunoalkaline phosphatase stain, naphthol fast red substrate with light hematoxylin counterstain; original magnification ×158).

Table. Estimated range of incubation periods for variant Creutzfeldt-Jakob disease cases with presumed route and year of exposure*

Presumed route of exposure and country of residence†	Period of potential BSE/ vCJD exposure	Estimated range of incubation period (y)
Foodborne		
Canada	1987–1990	11–14
Ireland	1989–1995	5–10
Japan‡	1989	12
United Kingdom (Leicestershire, England)	1980–1985	10–16
United States	1980–1992	9–21
Bloodborne		
United Kingdom	1996	6.5
United Kingdom§	1999	>5

*BSE/vCJD, bovine spongiform encephalopathy/variant Creutzfeldt-Jakob disease.
†Estimated range of incubation periods are based on a single vCJD case except for Leicestershire, England, where the Leicestershire Health Authority reported the estimated incubation period for a cluster of 5 vCJD cases.
‡Patient was reported to have stayed in the United Kingdom ≈24 days.
§Patient with presumed bloodborne transmission died of a nonneurologic disease. The presence of the vCJD agent was detected in the spleen and cervical lymph node; the patient was heterozygous for methionine and valine at the polymorphic codon 129 of the prion protein gene.

The second patient potentially linked with bloodborne transmission of vCJD was an elderly person who received a unit of erythrocytes in 1999. vCJD developed in the donor of the unit 18 months after blood was donated (6). The recipient died of a ruptured aortic aneurysm 5 years after the transfusion. Tests of the patient's spleen and cervical lymph node detected protease-resistant prion protein with a Western blot mobility pattern and glycoform ratio similar to those seen in other vCJD patients. These results and the absence of neurologic symptoms and brain pathologic findings indicated that this patient had a subclinical vCJD infection. Prion protein gene sequencing showed heterozygosity for methionine and valine at codon 129, which indicated that persons not homozygous for methionine (>50% of the population) can be infected by the vCJD agent.

In the United States, the risk of bloodborne transmission of vCJD is low because of the absence of indigenous vCJD and the geographic-based donor deferral policy instituted by the Food and Drug Administration in 1999. This policy excludes from donating blood in the United States persons who resided in or had extended visits to the United Kingdom or other European countries during periods of greatest concern for BSE exposure (7).

The exact incubation period for foodborne vCJD is unknown. However, a range of possible incubation periods was estimated for 4 vCJD patients who likely acquired the disease during their residence in the United Kingdom and for 5 vCJD patients reported as part of a cluster in Leicestershire, England (Table). The median of the estimated range of incubation periods for these 9 vCJD patients was 13 years. The incubation period for the vCJD patient linked to bloodborne transmission was shorter (6.5 years). This finding could be the result of direct transmission of the agent into the bloodstream and adaptation of the agent to the human population, thus reducing or eliminating the species barrier (Table).

Conclusions

Patients with vCJD can be distinguished from patients with the more common sporadic CJD by their younger median age at death (28 years and 68 years, respectively), predominantly psychiatric manifestations at illness onset, delayed appearance of frank neurologic signs, absence of a diagnostic electroencephalographic pattern, presence of the pulvinar sign on MRI, and a longer median illness duration (<6 and 14 months, respectively) (3,8). Almost all vCJD patients have died before 55 years of age, compared with only ≈10% of sporadic CJD patients. The most striking early neurologic manifestation in some vCJD patients was painful sensory symptoms (dysesthesia or paresthesia). Other neurologic signs, such as chorea, dystonia, and myoclonus, commonly develop late in the course of vCJD. An MRI result with symmetrically increased signal intensity in the pulvinar region relative to the signal intensity in other deep and cortical gray mater areas has been reported in >75% of vCJD patients. The presence of this MRI feature, known as pulvinar sign, may suggest a vCJD diagnosis in the appropriate clinical context. A prominent, early involvement of lymphoid tissues has enabled a reasonably accurate premortem diagnosis of vCJD, using tonsillar biopsy. However, a more definitive

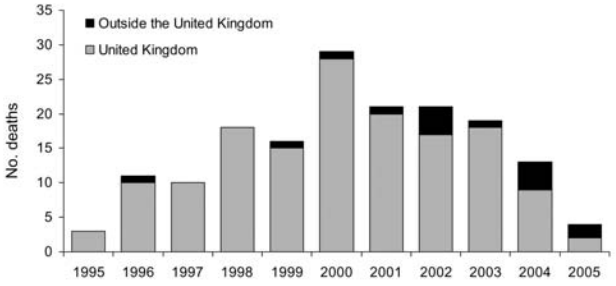

Figure 3. Number of deceased variant Creutzfeldt-Jakob disease patients worldwide (150 from the United Kingdom and 15 outside the United Kingdom) by year of death, June 2005.

diagnosis of vCJD may require testing autopsy brain tissues.

In June 2005, the US Department of Agriculture confirmed BSE in an ≈12-year-old cow born and raised in Texas. This is the first time an indigenous BSE case was detected in the United States. A previous BSE-positive cow identified in Washington State was imported from Canada (3) where, to date, 4 additional BSE cases have been identified. The identification of BSE in North America and the likelihood of bloodborne transmission of vCJD underscore the need to continue surveillance to monitor the occurrence of vCJD in the United States (3). The case-patient described in this report illustrates the need for physicians to remain vigilant for the possibility of vCJD in patients with the signs and symptoms described. Physicians should report suspected vCJD cases to local and state health departments. Because the clinical manifestation of vCJD can overlap that of sporadic CJD, brain autopsies should be sought in all suspected cases to establish the diagnosis and to help monitor the occurrence of vCJD and other potentially emerging forms of CJD. Free state-of-the art prion disease diagnostic testing is available from the National Prion Disease Pathology Surveillance Center (http://www.cjdsurveillance.com), which was established to facilitate autopsy performance and testing (8). Physicians are encouraged to use the services of the surveillance center to evaluate neuropathologic changes in their patients with suspected or clinically diagnosed prion disease.

Acknowledgments

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Potential Impact of Antiviral Drug Use during Influenza Pandemic

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The recent spread of highly pathogenic strains of avian influenza has highlighted the threat posed by pandemic influenza. In the early phases of a pandemic, the only treatment available would be neuraminidase inhibitors, which many countries are considering stockpiling for pandemic use. We estimate the effect on hospitalization rates of using different antiviral stockpile sizes to treat infection. We estimate that stockpiles that cover 20%–25% of the population would be sufficient to treat most of the clinical cases and could lead to 50% to 77% reductions in hospitalizations. Substantial reductions in hospitalization could be achieved with smaller antiviral stockpiles if drugs are reserved for persons at high risk.

Recent outbreaks of highly pathogenic avian influenza in poultry in East Asia (H5N1), Canada (H7N3), and the Netherlands (H7N7), and their subsequent transmission to humans, have intensified concern over the emergence of a novel strain of influenza with pandemic potential. Three influenza pandemics occurred during the 20th century, with varying degrees of severity; outcomes ranged from the high levels of illness and death observed during the 1918 Spanish flu pandemic (estimates of deaths range from 20 to 100 million [1]) to the much lower levels observed during the pandemics of 1957 and 1968 (≈1 million deaths each [2]). While recognizing that the characteristics of future influenza pandemics are difficult to predict, the World Health Organization (WHO) has recommended that nations prepare pandemic contingency plans (3). Several have been drafted, and some have been published (4–7), although all are subject to continuous refinement. Surveillance, on both a local and global scale, will enable policy makers and practitioners to act during the early phases of a pandemic. However, the likely rapid global spread of a pandemic strain will limit the time available to implement appropriate mitigating strategies, and preemptive contingency planning is needed.

A number of intervention strategies can reduce the impact of influenza pandemics. During interpandemic years, influenza vaccination is used to reduce deaths and disease. However, vaccine is unlikely to be available in time or in sufficient quantities for use during a pandemic (8,9). Other, nontherapeutic, disease control options may be used, such as those used during the outbreak of severe acute respiratory syndrome (10).

However, 2 groups of antiviral drugs are available for the treatment and prophylaxis of influenza. These are the adamantanes (amantadine and rimantadine) and the neuraminidase inhibitors (oseltamivir and zanamivir). The adamantanes may be effective against pandemic strains, but concern exists about adverse reactions and the development of antiviral resistance. Resistance to amantadine has been demonstrated in a number of avian H5 strains (11) and its use for treatment of influenza is not recommended (12).

The neuraminidase inhibitors (NIs) reduce the period of symptomatic illness from both influenza A and B viruses (13) and both are recommended for use in the United Kingdom for treatment of at-risk adults who are able to begin treatment within 48 hours of onset of symptoms. Oseltamivir is also recommended for the treatment of at-risk children >12 months of age (12). The development of antiviral resistance has been reported for NIs, particularly related to oseltamivir use in children (14), although current evidence suggests that resistant strains are pathogenically weakened (15). The use of NIs for treatment of pandemic influenza remains an option since they may improve individual disease outcomes and the effect of the disease in the population.

An influenza pandemic is likely to increase demands on healthcare providers, especially in hospitals. Except in Japan, current levels of NI use are low. Any strategy involving NI use would require stockpiles of these drugs.

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The potential use of antiviral agents for prophylaxis has been investigated elsewhere and may be of greatest use in the earliest phases of a pandemic to retard the spread of the virus (16,17). Earlier pandemic influenza modeling studies have also focused on the economic effect of vaccination (18) and the use of NI prophylaxis for disease control (19). We assessed the potential effect of using NIs for treatment on the estimated number of influenza-related hospitalizations likely to occur during a pandemic. Unlike in previous studies (20), we have also taken into account the reduction in infectivity that antiviral treatment may have on community transmission.

Methods

Our models focused on using NIs to treat different age and risk groups and the potential effects treatment might have on influenza hospitalizations. These effects have been quantified by using the mathematical model described in the online Appendix (available from http://www.cdc.gov/ncdod/EID/vol11no09/04-1344_app.htm). The length of the latent, noninfectious period was assumed to be 2 days (19), and the infectious period was assumed to be 4 days (19,21). Hospitalization rates for the baseline scenario were calculated by using data from interpandemic influenza and are given for different and age risk groups (Table 1).

To be effective, NI treatment must be administered within 48 hours of symptom onset. The efficacy of NI treatment appears to prevent 50% of hospitalizations, mirroring efficacy rates against developing complications; this efficacy rate is approximately the same for oseltamivir and zanamivir (13). Symptoms were also reduced by ≈1.5 days; treatment was assumed to produce the same decrease in the infectious period.

The population was stratified as for seasonal influenza; persons were considered to be either at high risk for severe outcome or at low risk (22). The at-risk group included those with chronic respiratory disease, chronic heart disease, chronic renal failure, diabetes mellitus, and immunosuppression; this group also included all persons living in long-term care facilities, such as nursing homes (23), and all those >65 years of age (24).

Demographic data used in the model were based on age-specific distribution of the UK population (Office for National Statistics, http://www.statistics.gov.uk). The model was used to simulate a number of scenarios, on the basis of contingency plans and previous pandemics, to investigate the effect of targeting NIs to different age and risk groups on the expected number of hospitalizations during a pandemic.

Results

The baseline scenario for this study was that advocated by WHO (3) and was also used previously by Meltzer et al. (18). This scenario assumes a clinical attack rate, in the absence of interventions, of 25% of the population, which occurs during a single wave. Assuming that half of infections are nonclinical or asymptomatic (i.e., a serologic attack rate across the population of 50%) (25), a value for the basic reproduction number, R_0, of 1.39 can be calculated. When these parameters are used in the model in the online Appendix, the effect of different-sized antiviral stockpiles on the overall clinical attack rate can be estimated.

Table 1. Hospitalization rates for clinical patients for different age and risk groups based on data from interpandemic years

	Hospitalization rates per 100,000	
Age, y	High risk	Low risk
≤4	3,562	509
5–14	274	39
15–64	873	125
65–74	4,235	605
≥75	8,797	1,257

The outputs from the first set of simulations are shown in Figure 1. The baseline scenario is shown alongside a range of other clinical attack rates (20%–40%) (i.e., varying R_0 from 1.28 to 2.0) in the absence of interventions. For these scenarios, antiviral treatment is assumed to be possible within 48 hours of onset for all symptomatic patients until the stockpile is exhausted, with the exception of those <1 year of age, who are not treated at any stage (treatment for this age group is contraindicated [12]). The points on the curves in Figure 1, where the gradients change from vertical to horizontal, indicate the points at which the stockpile is sufficient to treat all patients; increasing the stockpile size would produce no additional

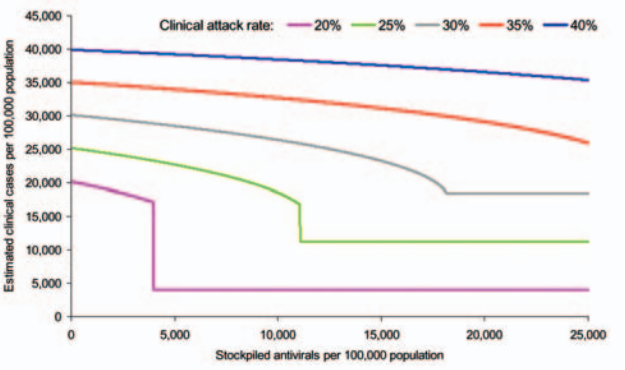

Figure 1. Estimated impact of different sizes of antiviral stockpiles on the number of clinical cases at the end of the pandemic. Depicted are clinical attack rates before interventions of 20%, 25%, 30%, 35%, and 40%, with corresponding values for the basic reproduction number (R_0) of 1.28, 1.39, 1.53, 1.72, and 2.0 respectively. The precipitous decreases observed with the 20% and 25% attack rates result at the points at which the stockpile becomes large enough to last long enough to prevent a recrudescence of the epidemic by suppressing the effective reproduction number.

benefit and would therefore result in a surplus of antiviral treatments.

For the baseline scenario, a stockpile large enough to treat 12% of the population (i.e., a 12% stockpile) would be sufficient to treat all patients, even if the clinical attack rate in the absence of treatment is 25%. This difference is due to a reduction in the effective reproduction number of the disease, R_ε, caused by shortening the infectious period of those treated by 1.5 days. Across the different attack rates, stockpiles sufficient to treat <1% of the population are unlikely to result in major changes to disease dynamics. Outputs are most sensitive to the clinical attack rate when the reduction in the infection period caused by treatment is sufficient to bring R_ε <1. When R_ε is <1, the number of secondary cases produced by each person is <1, and incidence, therefore, decreases. The value of R_ε can be calculated as

$$R_\varepsilon = R_0 S$$

where S is the proportion of the population susceptible. With treatment, this equation can be rewritten as

$$R_\varepsilon = R_0 S\left(1 - \frac{I_t}{I_p}\sum_i c_i\right)$$

where I_t is the decrease in the infectious period due to treatment, I_p the infectious period, and c_i the proportion of infections in each of the different population subgroups, i, that are treated. For the scenarios in Figure 1, I_t = 1.5 days, I_p = 4.0 days and c_i = 0.5 for all groups except those <1 year of age, who only constitute 1.1% of the population. Therefore, the term within the brackets for this scenario can be calculated as 0.81. At the start of the pandemic, S is assumed to be 1; therefore, if R_0 is <1.23, the outbreak can be controlled by treating all patients. For pandemics in which R_0 is >1.23, depletion of susceptible persons through infection is also required before R_ε decreases to <1, which is equivalent to $S = (0.81R_0) - 1$.

The effect of different treatment strategies on hospitalization rates was generated from the baseline scenario: treating all patients, only at-risk groups, only children and the elderly (1–14 and ≥65 years of age), and only the working population (15–64 years of age). These scenarios were of potential interest to public health planners; outputs are shown in Figure 2. Given a large enough stockpile, the best option to minimize hospitalizations would be to treat all patients; for this scenario, a 12% antiviral coverage would reduce hospitalizations by up to 77%. An alternative strategy of treating the whole working population reduces the hospitalization rate by up to 40% but requires a similar antiviral stockpile size, and treating the working population consistently fails to reduce the number of hospitalizations below the number that would be expected if everyone were treated, regardless of stockpile size. This increase is

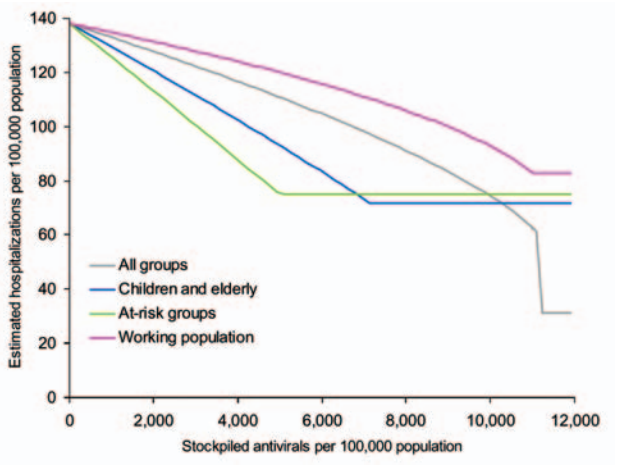

Figure 2. Estimated number of hospitalizations per 100,000 population when different antiviral treatment strategies are applied. Baseline scenario is when the clinical attack rate in the absence of interventions is 25% of the population.

because the hospitalization rate for the working population is less than the average in the population and also because treating a smaller proportion of the population has less effect on the overall transmission rate. For stockpile sizes only large enough to treat <5% of the population, the best strategy would be to treat at-risk groups; this strategy is also best for stockpile sizes up to 7%, with hospitalizations at this level reduced by up to 45%. For stockpile sizes from 7% to 10%, the best strategy is to treat children and the elderly (reducing hospitalizations by up to 48%) and for stockpile sizes >10%, to treat everyone.

The optimum treatment strategy is therefore dependent on treating those at highest risk for hospitalization. The simulations for the baseline scenario were based on a uniform age-specific attack rate and on age- and risk-specific hospitalization rates from interpandemic years because of the uncertainty over the precise characteristics of a future pandemic. Since the age-specific clinical attack rate has varied between pandemics, we repeated the analysis above, as far as possible, using the age-specific attack rates from previous pandemics (26–28) (Table 2) for comparison with the baseline scenario.

The 1957 UK pandemic began with imported cases in July 1957; deaths peaked in November 1957, with a reported overall clinical attack rate of 31% (26). The proportion of infections resulting in clinical illness was calculated from a small serologic survey of general practitioners; only 46% of the general practitioners surveyed with a positive antibody titer actually had symptoms (26). The serologic attack rate was calculated as 67%, which would require R_0 = 1.65. The epidemic curve that this figure would generate is shown in Figure 3A, with the curve scaled to fit the 1957 epidemic curve for deaths (26).

Table 2. Reported age-specific clinical attack rates (%) for different scenarios

Scenario	Attack rates by age class, y			
	≤4	5–14	15–64	≥65
Baseline (uniform attack rates)	25	25	25	25
1957 (26)	26	42	22	10
1968 (27)	16	11	49	24
1918 1st wave (28)	16	32	43	9
1918 2nd wave (28)	27	31	29	14
1918 3rd wave (28)	24	22	29	24

The only additional change from the baseline scenario is the 1957 hospitalization rate, which was reported to be 188/100,000 population (26). Using the age-specific attack rates for 1957 (Table 2) in the model, we scaled hospitalization rates to achieve an overall hospitalization rate of 188/100,000 (Table 3).

The results (Figure 3B) show that a 20%–25% antiviral stockpile would be sufficient to treat all patients during the first wave, a figure that is larger than that seen for the baseline scenario, as both the clinical and serologic clinical attack rates were higher. However, qualitatively, the results are similar in spite of the differences in attack rates between different age groups. With a stockpile as large as 20%–25%, an estimated reduction in hospitalizations of ≈67% could be expected. As in the baseline scenario, effective targeting of smaller stockpiles to at-risk groups can also be used to produce large reductions in hospitalization rates. For stockpiles <11%, the best strategy is to treat those at risk, which results in a reduction of 36%. For stockpiles sizes from 11% to 17%, the best strategy is to treat the young and elderly, which results in a 39% reduction. The highest reduction from treating the working population is 31% and remains a suboptimal strategy for any stockpile size.

The implications of different treatment strategies on the hospitalization rates with a 10% stockpile are shown in Figure 3C. Strategies with larger proportions of the 10% stockpile had the greatest effect on the epidemic, steadily delaying, but not diminishing, the peak of hospitalizations. Treating only the working population results in a 15% decrease in hospitalizations, treating all patients results in a 22% decrease, and treating children and the elderly a 32% reduction. With each of these strategies, the antiviral stockpile is exhausted before the end of the pandemic, whereas the fourth strategy of treating at-risk groups reduces hospitalizations by 36% and only requires a 5% stockpile. Therefore, treating those at risk is the most efficient strategy, but further targeting may be considered to avoid surplus treatments.

The 1968 pandemic was characterized by 2 waves, the first relatively small, occurring from February to April 1969; the larger wave occurred from November 1969 to January 1970 (27). We predominately considered the second wave. A confounding factor is that a proportion of the population would have been immune because of the first wave. Weighting age-specific clinical attack rates (Table 1) by age-group sizes from census data, we calculated the

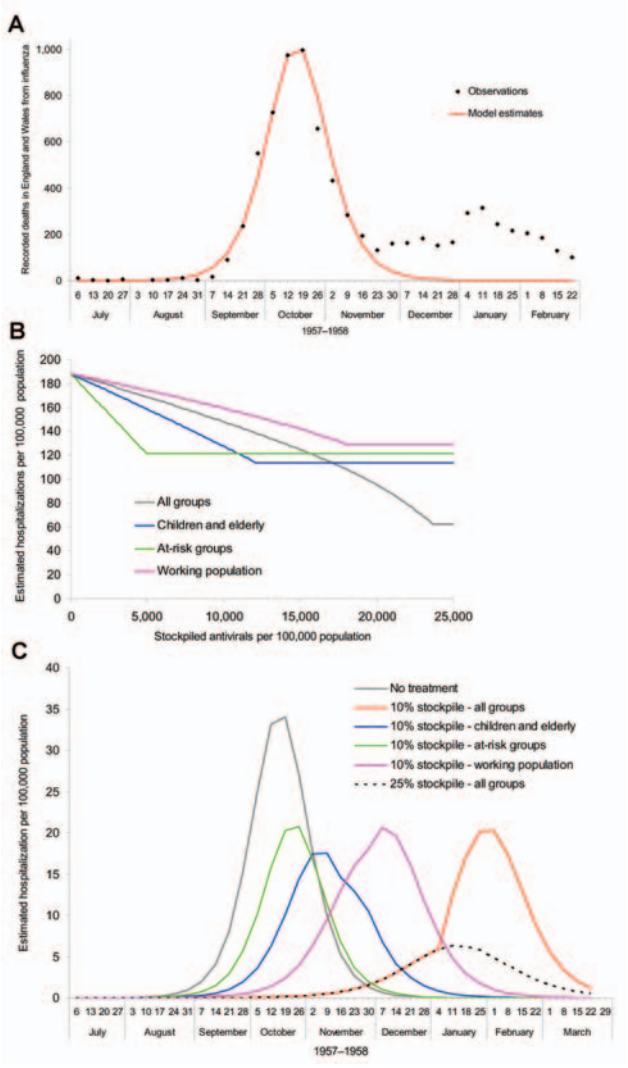

Figure 3. A), Output from the model fitted to the first wave of the 1957 pandemic scaled to fit observations from the 1957 pandemic (26). B), Estimated hospitalization rates from a simulated pandemic with available parameters from the 1957 pandemic, as influenced by stockpile size and treatment strategy. C), Impact of treatment strategy on the time course of hospitalizations when the stockpile size is fixed at 10% of the population, the stockpile is fixed at 25% of the population and all clinical cases are treated, and when no treatment is administered.

Table 3. Parameters required for scenario specific simulations

	Baseline	1957	1968	1918 1st wave	1918 2nd wave	1918 3rd wave
Overall hospitalization rate per 100,000 population	138*	188†	144†	–	–	–
Overall clinical attack rate, %	25†	31†	21‡	5§	9%§	4§
Overall serologic attack rate, %	50‡	67‡	65§	79§	61§	69§
% immune at start of wave	0¶	0¶	15‡	0¶	0¶	0¶
R_0	1.39‡	1.65‡	2.2‡	2.00†	1.55‡	1.70‡
Case-fatality rate, %	–	–	–	0.70†	3.25†	2.70†

*Derived from model simulations.
†Reported values.
‡Calculated directly from data.
§Calculated by fitting the model to data.
¶Assumed values.

overall clinical attack rates for the first and second waves to be 6% and 21%, respectively (27; Office for National Statistics [http://www.statistics.gov.uk]). The serologic attack rate was derived by fitting the model to the data for the second wave from the Royal College of General Practitioners (provided by Douglas Fleming; http://www.rcgp.org.uk); we assumed a similar proportion of asymptomatic cases in both waves. The fit of the model to the data is shown in Figure 4A, from which is derived a 15% residual immunity from the first wave and a 65% serologic attack rate for the second wave, which produces an effective reproduction number of 1.85 for the second wave. The overall hospitalization rate for the second wave was reported as 144 per 100,000 (29), and using the age-specific attack rates for 1968 in Table 2, we adjusted the values in Table 1 to fit this value.

The size of the stockpile required to treat all patients is ≈18% (which is relatively small compared to the 1957 pandemic because of the lower clinical attack rate), which leads to fewer patients being treated and less reduction in overall transmission. If all persons whose infections resulted in clinical illness (i.e., patients) were treated, the hospitalization rate would drop by ≈56% (Figure 4B). For the 1968 pandemic, the effects of the different antiviral targeting strategies were different than in the previous scenarios as a result of the different age-specific attack rates, which are shifted more towards the working population (Table 2). Thus, relatively small stockpiles are required to treat either the at-risk group or the young and elderly group (≈3% for each group), since most patients are in the working population and neither of these 2 groups. For stockpiles of up to 12%, treating the at-risk group is marginally better than treating the young and the elderly (37% reduction in hospitalization as opposed to 32%), and for stockpiles >12%, treating all clinical patients would be the best strategy.

The effects of the different treatment strategies with a 10% stockpile are shown in Figure 4C. Hospitalizations would drop by ≈29% if all patients were treated and by 16% if the working population were treated; both treatment strategies would lead to the stockpiles' being exhausted. As above, treating those at risk would reduce hospitalizations by 37%, whereas treating only children and the elderly would reduce hospitalizations by 32% and only require a 3% stockpile per group. Of these 4 strategies, treating the at-risk groups is the most efficient, but given surplus stockpile, further extension of the groups to be targeted may be considered.

The characteristics for the 1918 pandemic differ substantially from the other 2 in that 3 distinct waves occurred; the age-specific attack rates were highest for those in their teens, 20s, and 30s; and the mortality rates were higher (2). In addition, age-specific attack rates and mortality rates differed for each of the 3 waves (28). Modeling based on the 1918 pandemic was therefore considerably less straightforward than for the previous 2 pandemics, and an approach was taken to fit the transmission model to each of the 3 waves, separately. No cross-immunity was assumed between different waves since studies suggested only weak effects; indeed, some studies suggested greater susceptibility in the third wave if a person had had influenza in the first pandemic wave (28). Clinical attack rates were calculated from reported weekly mortality data and clinical case-fatality rates (28). Serologic attack rates were then fitted separately to each of the curves (Figure 5), from which values of R_0 = 2.0, 1.55, and 1.7 were derived from each of the respective waves. The estimate for the second wave is lower than other estimates of ≈3 (30) derived from US cities and is probably because our estimates were derived from data from throughout England and Wales, thereby incorporating spatial heterogeneity.

Since hospitalization rates were not available for any of the 3 waves, we considered the effect of antiviral treatment on death. The potential efficacy of antiviral treatments in preventing death between waves may have differed, but it was assumed to provide 50% protection against death. This estimate was based on the assumption that 50% protection from the more serious outcomes of influenza can be translated to equivalent protection from death (20).

A pandemic with the characteristics of that in 1918 would, without antiviral treatment, produce an estimated number of deaths equivalent to ≈0.5% of the population across all 3 waves. However, a 20% stockpile sufficient to

treat all patients across the 3 waves would result in ≈53% reduction in deaths. With a smaller stockpile of 10%, the reduction in deaths was only 17% because the stockpile becomes exhausted during the second wave, before most of the deaths occur (Figure 6).

Discussion

The baseline scenario with an overall clinical attack rate of 25%, as currently advised by WHO (3), is roughly in accordance with data from previous pandemics. The general conclusion from our study is that antiviral treatments for 20% to 25% of the population are likely to be sufficient to treat all patients for pandemics with characteristics that have been observed to date. The size of the stockpile required will depend on the clinical attack rate of the pandemic and the R_0 value.

However, with smaller stockpile sizes, substantial reductions in hospitalizations can be achieved through targeting. For the smallest stockpiles, the best strategy was to treat conventional influenza at-risk groups. Treating the young and elderly is only slightly less effective. Treating the working population may have benefits beyond reducing hospitalizations, such as reducing illness-related absenteeism, but it consistently fails to be the best strategy for reducing hospitalizations. For large stockpiles, treating all patients is consistently the best strategy in reducing hospitalization and transmission. When all patients are treated, the marginal effect of treatment on reduced transmission increases with the number of patients treated, until all patients have been treated.

Further studies regarding the effects of antiviral treatments would improve the robustness of the parameter estimates. In particular, better estimates on the efficacy of NI treatment against hospitalization and death rates for different age and risk groups and estimates on the reduction in the infectious period are required. Also, the issue of antiviral resistance needs to be resolved since it could compromise NI effectiveness.

The scenarios above assume that clinical patients were treated within 48 hours of onset of symptoms; however, in reality, some cases will be diagnosed or reported too late, and other patients will be administered drugs mistakenly. To maximize the benefits of antiviral treatment, patients should be strongly encouraged to seek treatment and treatment should be supported by sound clinical judgment and

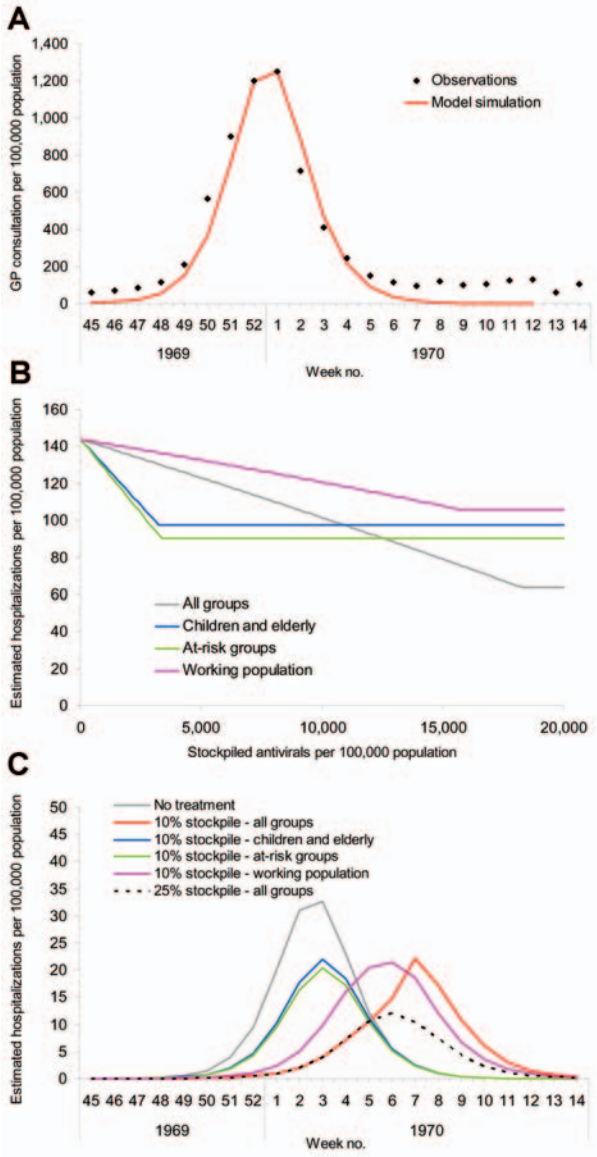

Figure 4. A), Output from the model fitted to the second wave of the 1968 pandemic scaled to fit observations from general practitioners (GPs) from the 1968 pandemic (29). B), Estimated hospitalization rates from a simulated pandemic with available parameters from the 1968 pandemic as influenced by stockpile size and treatment strategy. C), Impact of treatment strategy on the time course of hospitalizations when the stockpile size is fixed at 10% of the population, the stockpile is fixed at 25% of the population and all clinical cases are treated, and when no treatment is administered.

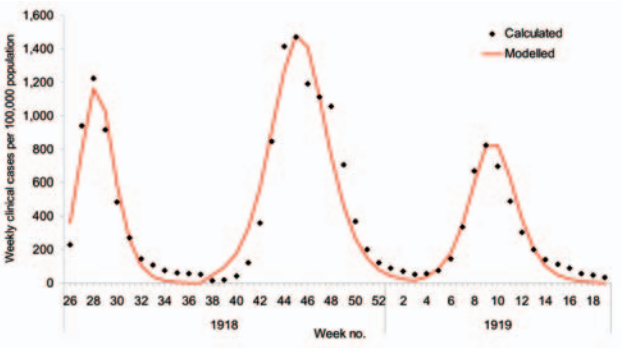

Figure 5. Clinical cases per week estimated by using the clinical case-fatality rates and weekly mortality statistics for the 1918 pandemic and by fitting the basic reproduction number (*R0*) to data from each of the waves by using the transmission model (28).

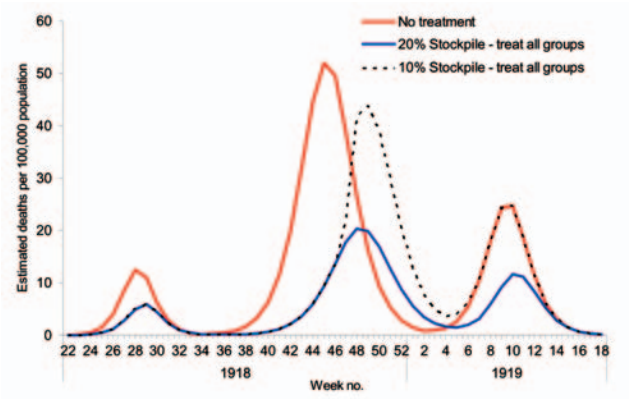

Figure 6. Estimated number of deaths from the 3 waves of the 1918 pandemic when there is no treatment, a 20% antiviral stockpile, and a 10% antiviral stockpile.

diagnostic capability. If high levels of treatment are not achievable, disproportionately higher hospitalization rates than those calculated here would ensue. In addition, identifying groups with higher transmission rates for targeting treatment would result in greater reductions in transmission than reported here.

Assessments will need to be recalculated in the earliest phases of a pandemic with real-time data to confirm or update the assumptions used and ensure that the model parameters are appropriate. Therefore, were a pandemic to occur, intensive analysis of its dynamics would be required at its start.

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Fluoroquinolone-resistant *Escherichia coli*, Indonesia

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In a recent, population-based survey of 3,996 persons in Indonesia, fluoroquinolone (FQ)-resistant *Escherichia coli* was prevalent in the fecal flora of 6% of patients at hospital admission and 23% of patients at discharge, but not among healthy relatives or patients visiting primary healthcare centers (2%). Molecular typing showed extensive genetic diversity with only limited clonality among isolates. This finding suggests that independent selection of resistant mutants occurs frequently. FQ-resistant isolates exhibited a higher rate of spontaneous mutation, but sparser virulence profiles, than FQ-susceptible isolates from the same population. The resistant isolates belonged predominantly to phylogenetic groups A (57%) and B1 (22%) but also to the moderately virulent group D (20%). Hypervirulent strains from the B2 cluster were underrepresented (1%). Because FQ-resistant *E. coli* can cause disease, especially nosocomial infections in immunocompromised patients, spread of such strains must be stopped.

Escherichia coli is a common constituent of the gastrointestinal flora of most vertebrates, including humans, and may be isolated from a variety of environmental sources. While most strains are nonpathogenic, certain ones can cause a variety of intestinal and extraintestinal infections. Pathogenicity is largely determined by gene-encoding virulence factors (VFs), such as adhesins, toxins, and polysaccharide surface coatings (1). Phylogenetic analysis showed that most *E. coli* strains fall into 4 main phylogenetic groups, designated A, B1, B2, and D (2). *E. coli* strains that cause extraintestinal infections derive predominantly from group B2 and, to a lesser extent, group D. Strains of groups A and B1 represent most commensal strains and are largely devoid of virulence determinants (3). Although strains harboring a robust extraintestinal VF repertoire cluster predominantly in groups B2 and D, isolates within each phylogenetic group can be further classified as extraintestinal pathogenic *E. coli* (ExPEC) or non-ExPEC depending on whether specific virulence traits are present (4,5).

The fluoroquinolones (FQs) are potent antimicrobial agents used for the treatment and prophylaxis of infections caused by gram-negative bacteria, including *E. coli*. FQ-resistant *E. coli* has been reported increasingly during the last decade in both the hospital environment and the community, which may ultimately limit the utility of these broad-spectrum agents (6–8). Moreover, FQ-resistant *E. coli* strains often show resistance to other drugs, such as ampicillin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and gentamicin (7,9). Recent reports have suggested that clinical FQ-resistant *E. coli* actually tends to be less virulent than susceptible isolates. FQ-resistant *E. coli* from hospitalized Dutch patients derived predominantly from the low-virulence phylogenetic groups A and B1. None of the 13 invasive isolates derived from phylogenetic group B2 (10). In addition, evidence suggests that clinical FQ-resistant *E. coli* isolates from humans in Iowa were associated with a shift toward non-B2 phylogenetic groups and to a lower overall virulence

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genotype (4). FQ resistance may also be associated with strains that intrinsically have a higher overall mutation rate, since the resistance to FQs in *E. coli* involves the accumulation of multiple spontaneously occurring point mutations in several genes (9,11). These associations, however, may depend on the strains' geographic or clinical origin.

In our study, we investigated these putative associations in a well-defined collection of isolates from Indonesia. A population-based survey of ≈4,000 people in 2 cities on the island of Java (Surabaya and Semarang) was initiated in 2000 by the Antimicrobial Resistance in Indonesia, Prevalence and Prevention study group to investigate the level of carriage of resistant microorganisms. FQ-resistant *E. coli* was prevalent in the fecal flora of 6% of patients at hospital admission and 23% of patients at discharge but not among healthy relatives or patients visiting primary healthcare centers (2% in both groups) (ES Lestari, unpub. data). In our study, we analyzed these FQ-resistant *E. coli* isolates to elucidate their molecular epidemiology and virulence. To define clonal relatedness, we performed enterobacterial repetitive intergenic consensus (ERIC) polymerase chain reactions (PCR). The phylogenetic background and virulence profile of these isolates were determined by PCR methods and compared with similar data for FQ-susceptible *E. coli* isolated from the same population. Finally, we examined the link between FQ resistance and the intrinsic mutation rate.

Materials and Methods

Strains

The study group program surveillance was initiated to determine the prevalence of antimicrobial resistance in Indonesia. Four different groups of persons in Surabaya and Semarang were studied for carriage of resistant microorganisms in their stools. The 4 groups were patients on the day of admission to the hospital (group 1), patients on the day of discharge after ≥5 days of hospitalization (group 2), patients visiting a primary healthcare center (group 3), and healthy relatives or household members of group 1 patients (group 4). In groups 1 and 2, rectal swabs were taken from patients in the internal medicine, surgery, gynecology/obstetrics, or pediatrics departments. The specimens were collected from July to October 2001 in Surabaya and from January to May 2002 in Semarang. Further details on the methods of culturing will be published elsewhere. A total of 5,535 *E. coli* isolates from 3,284 patients were cultured. Antimicrobial susceptibility testing was performed for 1 isolate per patient. The overall by-isolate prevalence of resistance to ciprofloxacin as determined by disk diffusion was 8%. The prevalence of resistance was highest among patients on the day of discharge (18% in Surabaya and 27% in Semarang) and lowest among patients visiting primary healthcare centers and among family members of patients admitted to the hospital (2% in both groups). The prevalence of FQ-resistant *E. coli* among patients who were tested on the day of admission was 8% in Surabaya and 4% in Semarang. We studied 196 FQ-resistant isolates in more detail. Seventy-five (38%) of these were from Surabaya (19, 48, 4, and 4 isolates from stated population groups 1, 2, 3, and 4, respectively) and 121 (62%) from Semarang (13, 92, 11, and 5, respectively). The FQ-resistant isolates were recovered from patients from all 4 hospital departments in both cities. In Semarang, 43% of these isolates were from surgery departments and 41% were from internal medicine departments. In Surabaya, 43% of the isolates were from the internal medicine department. All 196 ciprofloxacin-resistant *E. coli* and 200 ciprofloxacin-susceptible *E. coli* (20 randomly chosen isolates from groups 1, 2, and 3 and 40 from group 4, from each city) were confirmed by Vitek 2 (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions and included in the molecular analyses.

DNA Isolation

Bacterial DNA was isolated by using the MagNA Pure LC with the MagNA Pure LC DNA Isolation Kit III for bacteria and fungi (standard protocol; Roche Molecular Biochemicals, Mannheim, Germany). DNA concentration was assessed spectophotometrically. Samples were frozen at –20°C until used.

Bacterial Typing by ERIC-PCR

ERIC-PCR was conducted with primers ERIC-1R and ERIC-2 as described previously (8,12,13). The amplification products were subjected to electrophoresis in a 1% agarose gel and were stained with ethidium bromide (50 μg/mL). The ERIC-PCRs were performed by 1 technician within 1 month. Profiles were visually analyzed by 2 microbiologists. Single-band differences in profiles among strains led to the definition of separate genotypes. Ambiguous isolates were retested and analyzed by 2 other microbiologists.

Phylogenetic Analysis and Virulence Typing

Isolates were assigned to 1 of 4 main *E. coli* phylogenetic groups (A, B1, B2, and D) according to an established triplex PCR assay, in which the 4 phylogenetic groups yield distinct combinations of 3 possible PCR products, *chuA* (heme transport), *yjaA* (unknown function gene from *E. coli* K-12 genome), and TSPE4.C2 (anonymous fragment identified by subtractive hybridization) (2,14). All isolates were screened for 5 ExPEC-defining virulence markers, *papA*/*papC*, *sfa*/*focDE*, *afa*/*draBC*,

kpsM II, and *iutA*. Based on previous statistical analyses of similar data, from collections within which each isolate's ExPEC status could be inferred based on ecologic source or experimental virulence, isolates were classified as ExPEC if positive for ≥2 of these 5 defining virulence markers (4). All isolates were also screened for *hlyD* (hemolysin), another ExPEC-associated VF. Subsequently, all isolates that satisfied molecular criteria for ExPEC were screened for 32 additional virulence markers[1]. These virulence genes were detected by a combination of multiplex PCR and dot-blot hybridization with primers specific for internal or flanking sequences and probes generated and labeled with these primers; this method was previously validated by using dot-blot hybridization with defined control strains (15). A VF score was calculated for each strain as the sum of all VF genes for which the strain tested positive. In all of these PCR assays, the identity of the PCR products was deduced by comparing their size to molecular size standards in ethidium bromide–stained agarose gels. Appropriate positive and negative controls were included in each run.

Mutation Rate Analysis

The mutation rate was determined for 20 randomly selected isolates from phylogenetic group A (10 FQ-susceptible and 10 FQ-resistant) by monitoring the isolates' capacity to generate mutations conferring resistance to rifampin, as described previously (9,16). Forty independent cultures of each of the 20 strains were set up in Luria broth. After overnight incubation, equal concentrations of cultures were suspended in 0.85% NaCl. The suspensions were spread on Luria agar plates containing 100 μg/mL rifampin and incubated overnight. For each strain, the proportion of cultures giving no resistant mutants was used to calculate the mutation rate per cell per generation according to the fluctuation test of Luria and Delbrück. To avoid confounding by variation in phylogenetic background, only phylogenetic group A isolates were investigated. For comparisons of results, we used the relative mutation rate, which was defined as the rate relative to the rate for *E. coli* strain Nu14 (5×10^{-9} per cell per generation) (9).

Statistical Analysis

All data were analyzed by using the statistic software packages SPSS version 10.0 (SPSS, Chicago, IL, USA) and EpiInfo version 5.00 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Chi-square or Fisher exact tests (2-tailed) were used when appropriate for comparisons of proportions. Comparisons involving VF scores and relative mutation rates were analyzed by using the Mann-Whitney *U* test. The criterion for statistical significance was a p value <0.05.

Results

Spread of FQ-resistant *E. coli*

Genetic heterogeneity among the 196 FQ-resistant *E. coli* was assessed by ERIC-PCR. We documented 158 different patterns, designated types 1–158, which indicated a genetically diverse collection of strains. Twenty pairs of isolates with identical profiles were identified, and 9 distinct multiple-isolate clones were represented by isolates from 3 patients each. The limited number of shared genotypes was mainly recovered from group 2 patients, i.e., patients at the time of discharge from the hospital, 49 (73%) of 67 isolates. Among the total number of 140 isolates from group 2, we identified 119 different ERIC-PCR profiles.

Type 37 occurred in 3 patients from the internal medicine department in Surabaya; all 3 patients were present within this department on the same day. The finding of this unique isolated cluster can be explained by patient-to-patient transmission or a nonpatient-associated environmental source. This explanation was not further examined in this study. Type 90 was isolated from 2 patients on the day they were discharged from the internal medicine department in Semarang. Samples were collected on consecutive days. An isolate with an identical ERIC-PCR pattern was found in the same period in the same hospital in a pediatric patient at discharge. No further obvious clustering in time and place was observed among isolates from the 9 multiple-strain clusters.

Phylogenetic Analysis

PCR-based phylotyping showed that the 200 FQ-susceptible isolates were predominantly from phylogenetic groups A (52%) and B1 (30%) (Table 1). The 196 FQ-resistant isolates also mainly derived from phylogenetic groups A (57%) and B1 (22%), but some derived from the moderately virulent phylogenetic group D (20%). Hypervirulent strains from the B2 cluster were underrepresented (1%). Eighteen (67%) of the 27 isolates from the 9

[1]ExPEC: *papEF* (P fimbrial tip pilins); *papG* (P adhesin); *papG* alleles I, II, and III; *sfaS* (S fimbriae); *focG* (F1C fimbriae); *iha* (putative adhesin-siderophore); *bmaE* (M fimbriae); *gafD* (G fimbriae); *F17a* (F17a fimbriae); *clpG* (CS31A adhesin); *afaE8* (afimbrial adhesin VIII); *fimH* (type 1 fimbriae); *cnf1* (cytotoxic necrotizing factor); *cdtB* (cytolethal distending toxin); *ireA* (siderophore receptor); *sat* (secreted autotransporter toxin); *astA* (S-like enterotoxin); *iroN* (siderophore receptor); *fyuA* (yersiniabactin receptor); *kpsM* II, K1, and K2 (*kpsM* II variants; group 2 capsule); *kpsM* III (group 3 capsule); *rfc* (O4 lipopolysaccharide synthesis); *cvaC* (colicin V); *traT* (serum-resistance associated outer membrane protein); *ibeA* (invasion of brain endothelium); *ompT* (outer membrane protease T); *iss* (increased serum survival); *usp* (uropathogenic specific protein); *malX* (marker for pathogenicity-associated island from strain CFT073); and H7 *fliC* variant (flagellin).

Table 1. Distribution of phylogenetic groups and virulence factors*

Group or factor	All isolates (n = 396)	FQ-susceptible (n = 200)	FQ-resistant (n = 196)	p values
A	215 (54)	103 (52)	112 (57)	NS
B1	102 (26)	59 (30)	43 (22)	NS
B2	17 (4)	15 (8)	2 (1)	0.001
D	62 (16)	23 (12)	39 (20)	0.02
papA	28 (7)	27 (14)	1 (1)	<0.001
papC	29 (7)	28 (14)	1 (1)	<0.001
sfa/focDE	8 (2)	8 (4)	0	0.007
afa/draBC	11 (3)	11 (6)	0	0.001
iutA	131 (33)	61 (31)	70 (36)	NS
hlyD	20 (5)	20 (10)	0	<0.001
kpsM II	56 (14)	54 (27)	2 (1)	<0.001
ExPEC	44 (11)	40 (20)	4 (2)	<0.001

*FQ, fluoroquinolone; ExPEC, extraintestinal pathogenic *Escherichia coli*; NS, not significant. Screening for ExPEC was performed for 199 FQ-susceptible and 195 FQ-resistant isolates. Data are no. (%) of isolates.

distinct clones that were represented by 3 isolates each belonged to group A.

Table 1 shows that the resistant isolates were significantly depleted for phylogenetic group B2 and enriched for group D, when compared with the susceptible isolates. These shifts in phylogenetic distribution were significant both overall and specifically in Semarang, whereas a similar but nonsignificant trend was observed in Surabaya.

The phylogenetic distribution of all 396 isolates among the 2 cities was highly similar (data not shown). Comparisons of the distributions among the 4 population groups showed that group D isolates were more often obtained from patients sampled on the day of discharge than from other population groups (37 [21%] of the 180 group 2 isolates belonged to group D versus 25 [12%] of the 216 nongroup 2 isolates, p = 0.01). Stratification showed, however, that this association was due to the excess prevalence of FQ-resistant group D isolates among the group 2 patients. Furthermore, B2 isolates were significantly more prevalent in group 3, patients visiting public healthcare centers (7 [13%] of the 55 group 3 isolates belonged to group B2 versus 10 [3%] of the 341 nongroup 3 isolates, p = 0.004).

Virulence Typing

All *E. coli* isolates were tested for a set of virulence factors to allow an inference as to their pathogenic potential. The overall prevalence of the 5 defining ExPEC VFs ranged from 2% (*sfa/focDE*) to 33% (*iutA*) (Table 1). The FQ-resistant isolates were significantly depleted for *papA*, *papC*, *sfa/focDE*, *afa/draBC*, *hlyD*, and *kpsM II* (Table 1), when compared with the susceptible isolates. Accordingly, 40 (20%) FQ-susceptible *E. coli* isolates, but only 4 (2%) FQ-resistant isolates (2%), were classified as ExPEC, as they exhibited ≥2 of the 5 key ExPEC VFs (p<0.001). Thus, FQ resistance was associated with reduced inferred virulence. All FQ-resistant *E. coli* isolates from the 9 distinct clones that were represented by 3 patients each were found to be non-ExPEC.

The distribution of the 6 screening VFs was also analyzed in relation to the 4 phylogenetic groups (Table 2). Each VF was broadly distributed, occurring in ≥3 phylogenetic groups. However, *papA*, *papC*, *kpsM II*, *hlyD*, and *sfa/focDE* were all significantly associated with phylogenetic group B2. Accordingly, 53% of the phylogenetic group B2 isolates qualified as ExPEC versus 9% of the non-B2 isolates (p<0.001) (Tables 2 and 3).

The 44 ExPEC isolates were studied in more detail (Table 3). The ExPEC isolates derived mainly from phylogenetic groups A (36%) and B1 (32%), with the 4 FQ-resistant ExPEC isolates belonging to groups A (n = 2) and D (n = 2). Many (36%) ExPEC isolates originated from patients on the day of discharge. Both of the FQ-resistant ExPEC isolates from group 2 were from patients in the surgical ward in Semarang. Again, no evidence for clonality was seen. The 4 resistant ExPEC isolates exhibited sparse

Table 2. Distribution of virulence factors

Virulence factor	All isolates (n = 394)	Phylogenetic group, n (%)			
		A (n = 215)	B1 (n = 101)	B2 (n = 17)	D (n = 61)
papA	28 (7)	7 (3)	11 (11)	8 (47)*	2 (3)
papC	29 (7)	8 (4)	11 (11)	8 (47)*	2 (3)
iutA	131 (33)	66 (31)	32 (32)	8 (47)	25 (41)
kpsM II	56 (14)	19 (9)	23 (23)	11 (65)*	3 (5)
hlyD	20 (5)	8 (4)	7 (7)	5 (29)*	0
sfa/focDE	8 (2)	2 (1)	4 (4)	2 (12)*	0
afa/draBC	11 (3)	6 (3)	2 (2)	1 (6)	2 (3)

*p<0.05.

Table 3. Distribution of phylogenetic groups and virulence factors*

Characteristic	Prevalence of associated characteristic, n (%)			
	All isolates (n = 44)	FQ-susceptible (n = 40)	FQ-resistant (n = 4)	p values
Group A	16 (36)	14 (35)	2 (50)	NS
Group B1	14 (32)	14 (35)	0	NS
Group B2	9 (21)	9 (23)	0	NS
Group D	5 (11)	3 (8)	2 (50)	NS
Surabaya	23 (52)	22 (55)	1 (25)	NS
Semarang	21 (48)	18 (45)	3 (75)	NS
Admission	13 (30)	12 (30)	1 (25)	NS
Discharge	16 (36)	14 (35)	2 (50)	NS
PHC	10 (23)	9 (23)	1 (25)	NS
Relatives	5 (11)	5 (13)	0	NS
iha	25 (58)	25 (64)	0	0.025
sat	25 (58)	25 (64)	0	0.025
fyuA	35 (81)	35 (90)	0	0.001
ibeA	3 (7)	1 (3)	2 (50)	0.019
malX	26 (60)	26 (67)	0	0.019

*FQ, fluoroquinolone; NS, not significant; PHC, primary healthcare center. Extended virulence typing was performed for 39 FQ-susceptible isolates and 4 FQ-resistant isolates. Only those virulence factors are shown for which the comparison of FQ-resistant extraintestinal pathogenic *Escherichia coli* (ExPEC) to FQ-susceptible ExPEC was significant.

VF profiles when compared with the susceptible ExPEC isolates. These isolates lacked classic ExPEC VFs such as *focG*, *hlyD*, and *cnf1*. Four other VFs, *iha*, *sat*, *fyuA*, and *malX*, were more prevalent among susceptible, rather than resistant, ExPEC isolates. Only *ibeA* was more prevalent among the resistant isolates. The VF *iutA* was detected in all FQ-resistant ExPEC isolates and in 27 (68%) of the 40 FQ-sensitive isolates. This difference was not significant. Aggregate VF scores were lower among FQ-resistant ExPEC isolates (median 6, range 4–8) than among the 40 FQ-susceptible ExPEC isolates (median 10, range 3–16, p = 0.024).

Mutation Rate

The link between mutation rate and resistance to FQs was studied, as the rate of mutation accumulation might be a factor in the development of FQ resistance. The 10 FQ-susceptible isolates had relative mutation rates of ≤0.52 (median rate 0.32, range 0.03–0.52), whereas the 10 FQ-resistant *E. coli* exhibited relative mutation rates of ≥0.55 (median rate 0.97, range 0.55–4.58) ($p<0.001$) (Figure).

Discussion

In this study, we investigated the epidemiology and virulence characteristics of FQ-resistant *E. coli* collected during a large, population-based survey of ≈4,000 people in 2 cities in Indonesia (Surabaya and Semarang). The overall prevalence of resistance to ciprofloxacin was 8%, but in the fecal flora of patients at time of discharge from the hospital the prevalence was 23%.

Dissemination of FQ-resistant *E. coli* and Mutation Rate

Three possible explanations for the high prevalence of FQ-resistant *E. coli* among patients that had been hospitalized for ≥5 days must be considered: transferable resistance, clonal spread, and mutation-based selection of resistance fostered by the use of antimicrobial agents. Transferable plasmid-mediated quinolone resistance has been described recently in *E. coli* from China (17). Wang et al. found that 6 (8%) of 78 ciprofloxacin-resistant *E. coli* strains from a hospital in Shanghai contained *qnr*. However, from the present study we cannot draw any conclusion about the contribution of this mechanism in Indonesia. As for clonal spread, molecular typing showed extensive genetic diversity among FQ-resistant isolates in Indonesia. We identified a few distinct multiple-isolate clones in the hospital environment. Although all these clonal strains were shown to be non-ExPEC, they may still pose a health threat, especially to immunocompromised

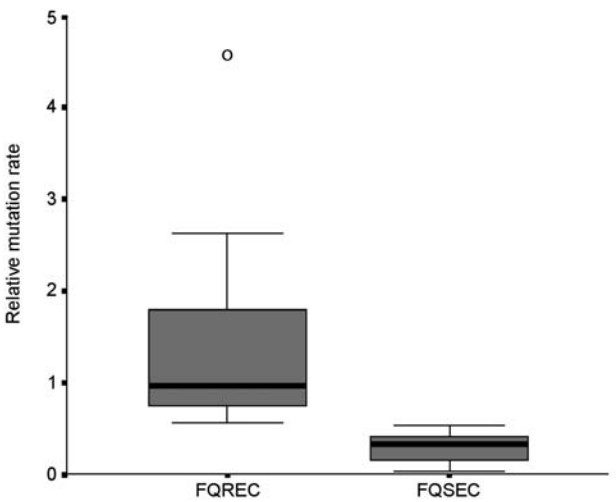

Figure. Box plot of relative mutation rate of 10 fluoroquinolone (FQ)-resistant (FQREC) and 10 FQ-sensitive (FQSEC) *Escherichia coli*.

patients in hospital settings. Nosocomial outbreaks of infections caused by disseminating FQ-resistant clones have already been described (8). However, in our study, limited clonality among isolates was found, which suggests that other factors contribute more to the high prevalence of FQ-resistant *E. coli* among hospitalized patients.

To determine whether mutation-based resistance fostered by selection pressure contributed to the prevalence of FQ-resistant *E. coli* in Indonesia, we performed a mutation rate analysis of selected isolates. We found a strong correlation between resistance and an elevated mutation rate. This finding agrees with a recent report from Komp Lindgren et al., in which high mutation rates of *E. coli* strains from urinary tract infections were strongly associated with FQ resistance (9). To demonstrate that this mutation-based resistance was selected for by the use of FQs, we must know the consumption figures of the quinolones. In other reports, evidence suggests that the use (and misuse) of ciprofloxacin in human and animal medicine may predispose to an increase in infections with resistant *E. coli* (8). As information on the use of FQs in Indonesia is currently not available, we cannot draw any conclusions on a potential link between antimicrobial drug use, selection pressure, and mutation-based resistance. Thus, based on the large clonal diversity of the FQ-resistant *E. coli* and the resistant isolates that have a slightly elevated mutation rate relative to FQ-sensitive isolates, independent emergence of new resistant mutants likely occurs regularly in this setting.

Phylogenetic Typing and Virulence Profiling

Phylogenetic typing and virulence profiling were performed to investigate whether a potential clinical hazard was associated with the presence of these isolates. Our data on the distribution of phylogenetic groups among the 396 *E. coli* isolates are consistent with those of most other studies. In an examination of human commensal *E. coli* strains, the frequencies of B2 strains were found to be 1 (2%) of 55 in Mali, 6 (11%) of 56 in France, and 11 (19%) of 57 in Croatia (18). In our study, 4% of the isolates overall were of B2 origin. However, the results from a report by Zhang et al. do not agree with our data (19). B2 strains accounted for 42 (48%) of 88 commensal rectal strains from healthy college-aged women in Michigan. Likewise, Sannes et al. noted a high prevalence of group B2 among rectal isolates from hospitalized, elderly, male veterans in Minnesota (20). Differences may be due to geographic variation, differences in host population characteristics, or differences in strain characteristics such as antimicrobial resistance.

We did not observe a significant shift toward low-virulence phylogenetic groups for resistant isolates, as was reported by Johnson et al. (10). However, we confirmed that the isolates were notably depleted for phylogenetic group B2 and enriched for group D. We also confirmed that FQ-resistant *E. coli* exhibited sparser virulence profiles. The most prevalent VF was *iutA*, which was detected in 36% of the resistant isolates; however, this VF is less common in virulent group B2 strains (1). Accordingly, only 2% of the resistant isolates were found to be ExPEC. These 4 isolates also lacked the VFs *iha*, *sat*, *fyuA*, and *malX* as compared to the FQ-susceptible ExPEC. Whether ExPEC strains cause infection in humans depends on several other factors, including susceptibility of the host. Therefore, that many (36%) of the 44 ExPEC isolates were from group 2 patients who had been hospitalized for $\geq$5 days is of concern. When patients become colonized with FQ-resistant ExPEC strains in the hospital, they presumably will have an increased risk of acquiring a nosocomial infection and, when discharged with such a strain, also for community-acquired infection; in such case, an optimal therapy will be more difficult to select. Of note, a relationship has recently been shown to exist between ciprofloxacin-resistance in *E. coli* and the production of extended-spectrum β-lactamases, which would further limit therapeutic options (21).

Our observations provide insight into the epidemiology and virulence characteristics of FQ-resistant *E. coli* from stools of patients and healthy participants in Indonesia. The high prevalence of FQ-resistant *E. coli* in the hospital environment seems to be primarily due to a combination of limited clonal spread and the spontaneous emergence of resistant strains, possibly fostered by selection pressure. Transferable resistance, however, cannot be ruled out as an additional explanation in the present study and will be the subject of future investigations. Although the resistant isolates mainly belong to phylogenetic groups A and B1 and show a low virulence profile, similar strains have caused disease in humans (3,10). The data support the need to implement strict infection control measures in hospitals and to promote and monitor the prudent use of antimicrobial drugs. Continued surveillance of the changes of resistance patterns and virulence profiles of clinical and nonclinical *E. coli* isolates is warranted.

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Dead Crow Density and West Nile Virus Monitoring, New York

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New York State used the health commerce system to monitor the number of West Nile virus (WNV) human disease cases and the density of dead crows. In each year from 2001 to 2003 and for the 3 years combined, persons living in New York counties (excluding New York City) with elevated weekly dead crow densities (above a threshold value of 0.1 dead crows per square mile) had higher risk (2.0–8.6 times) for disease caused by WNV within the next 2 weeks than residents of counties reporting fewer dead crows per square mile. This type of index can offer a real-time, relatively inexpensive window into viral activity in time for prevention and control. Changes in reporting, bird populations, and immunity may require that thresholds other than 0.1 be used in later years or in other areas.

Controlling West Nile virus (WNV) and other vector-borne pathogens requires identifying areas of risk as early as possible. Ideally, risk indices should be relatively inexpensive and easy to implement, should provide timely and accurate forecasts of risk, and should not trigger expensive or controversial control measures when the actual risk is low. WNV infections in >200 species of birds (1) and associated bird deaths have been reported from multiple locations in the Western Hemisphere (2–8). Studies indicate that dead crow (*Corvus* spp.) reports have a number of advantages, before or without laboratory confirmation (9–16): 1) crows appear to be extremely sensitive to WNV infection and have a high case-fatality rate (6,17), which makes WNV transmission to crows relatively easy to detect; 2) crows are widely distributed, large, and generally easy to recognize; 3) crows have high mean viremia levels (17) and a high reservoir competence index (1), which indicates that they are a good source of virus for mosquito infections and local disease amplification; and 4) no resources or time are required for bird or sample collection, processing, and testing.

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Analyses of data from the northeastern United States in 2000 and 2001 found that counties with high dead crow densities (DCD, dead crows per square mile) early in the season were significantly more likely to have a case of WNV disease in a human (18). In New York in 2000, the number of human disease cases by county was more strongly associated (r = 0.92) with DCD than with the number of WNV-positive birds or with the number of sightings of all bird species (19). In addition, weekly DCD increased several weeks before the onset of cases in humans (19,20). In New York counties with no human cases, DCD never exceeded 0.1. In counties with 1 or 2 cases of human disease, DCD exceeded 0.1 before human case onset and reached 1.4. In Richmond County (Staten Island), with 10 human cases, DCD exceeded 1.5 before disease onset in humans and peaked at 7. This article evaluates New York's real-time use since 2000 of the weekly, county-level DCD index as an indicator of human WNV disease risk, with a signal level of 0.1.

Methods

The New York State Department of Health (NYSDOH) developed a Web-based secure health commerce system that supports all of its information interchange with public health and healthcare communities (21). In the spring of 2000, NYSDOH implemented a statewide, integrated WNV surveillance system on health commerce in response to the 1999 emergence of WNV. The system includes real-time surveillance components for humans, mammals, birds, and vectors and allows local health departments, the state dead bird hotline, and laboratories to enter and retrieve surveillance data in real time for disease tracking (22). Although sightings of dead birds of any species are reported by private citizens and agencies, dead crow sightings are emphasized in automated summary tables and charts based on their utility in previous studies. Because New York City developed its own WNV surveillance

system to monitor dead bird reports, New York City data have not been included in this follow-up study. By using real-time surveillance data, system users can press a button to immediately generate a DCD calculation and graph for each county in the state for a specific period of interest. For this study, the weekly DCD was graphed for each county during a season to monitor trends over time, and human cases were added when they met the 2001 Centers for Disease Control and Prevention (CDC) case definitions for a confirmed or probable case (23).

According to CDC case definitions, New York (excluding New York City) had 6 (1 excluded from study) confirmed or probable human cases of WNV disease in 2001, 53 (3 excluded) in 2002, and 40 (3 excluded) in 2003. Reasons for case exclusion were occupational exposure (2001), infection by blood-transfusion (2002), date of onset after dead bird surveillance had abated (2002, 2003), intrauterine transmission (2002), and travel outside of New York at likely time of infection (2 cases in 2003). The 92 human cases included in the study were widely distributed in New York except for the sparsely populated north-central region (Figure 1).

The Cochran-Mantel-Haenszel (CMH) option of the "freq" procedure in SAS (SAS System for Windows V8, SAS Institute, Cary, NC, USA) was used to calculate point and interval estimates of WNV disease risk, depending on DCD $\geq$0.1 in a person's county of residence. Data from each week with onset of a human case were included in the analysis (8/19–9/22 in 2001, 7/28–10/5 in 2002, and 8/3–9/27 in 2003). For each week, a table was constructed comparing the week's human cases per population in counties with a "signal" of DCD $\geq$0.1 and the human cases per population in counties with no signal. For example, 9 patients had disease onset in week 35 of 2003; 8 of the patients resided in counties with DCD $\geq$0.1 in the previous 2 weeks and 1 of whom did not (Table 1). (Note that in other weeks the total population in the 2 categories is different, depending on which counties had high DCD in the previous 2 weeks.)

The CMH chi-square statistic was used to compare the incidence (risk) of WNV disease in the DCD signal areas with the incidence in the non-DCD signal areas over all the weeks of this study. As implemented by SAS (24), this procedure pools data across strata (in this case, across weeks), determines a p value for the difference in incidence between the 2 exposure categories, and estimates a single relative risk for the exposed versus the unexposed population across all weeks (including those weeks when fewer cases resulted in risk estimates that would not be considered meaningful).

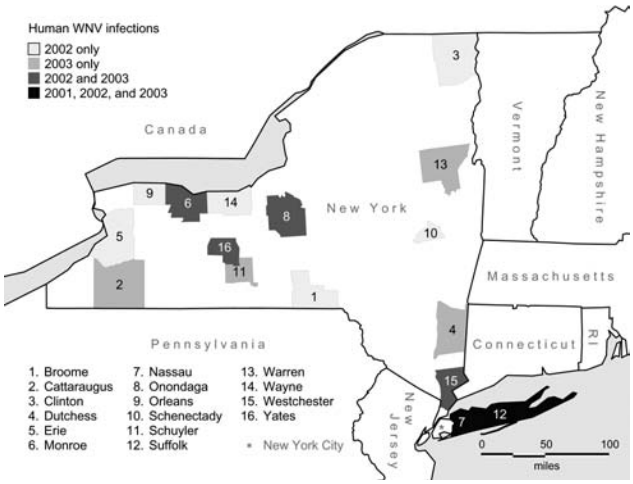

Figure 1. New York counties with laboratory-confirmed cases of human West Nile virus disease, 2001–2003.

In New York's dead crow surveillance system, an increase in a county's crow death reports assumes an increase in the number of infected mosquitoes able to transmit virus to both crows and humans. Since the time between WNV exposure (mosquito bite) and human disease onset is 2–14 days (25), we assumed that $\leq$2 weeks could pass between the exposure of interest (high DCD in the county of residence) and disease onset. For this reason, CMH-pooled risk estimates were calculated separately for 3 exposure periods, defined as the county having a density signal 2 weeks before the onset week of the human case, 1 or 2 weeks before the onset week of the human case, or in the onset week of the human case or 1–2 weeks before.

Results

For the 2 Long Island counties with human cases of WNV disease in 2001, weekly DCD $\geq$0.1 were seen more than 1 month before onset of human cases (Figure 2). DCD increased before the first WNV-positive bird was reported (1–3 weeks after it was found). However, the highest peaks in weekly DCD occurred after viral activity was confirmed and may have been influenced by increased interest in reporting dead crows after the media had reported WNV in the area. For other New York counties, weekly DCD remained lower (<0.1), and no human cases were detected.

Table 1. Example of 2×2 table constructed for chi-square analyses in this study*

Week 35, 2003	Counties with DCD $\geq$0.1 in past 2 wks	Counties with DCD <0.1 in past 2 wks
No. cases with disease onset during week	8	1
Total population	4,422,461	6,545,718

*Data for each week with human West Nile virus disease cases in 2001–2003 were pooled to calculate risk for disease occurring within 2 weeks of elevated dead crow density (DCD) in county relative to risk in counties without elevated DCD in the past 2 weeks.

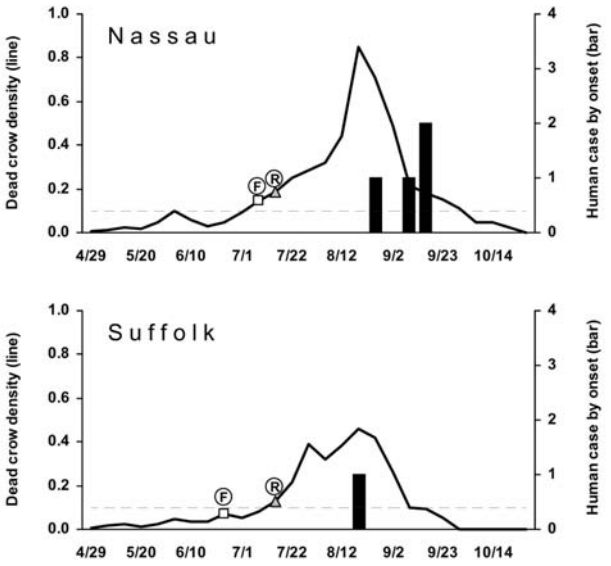

Figure 2. Dead crow densities (DCD, dead crows per square mile) and number of cases of human West Nile virus (WNV) disease, by week, 2001. Horizontal dashed line indicates DCD = 0.1. F, date that the first bird with confirmed WNV infection was found; R, date that the laboratory result of the first bird with WNV infection was reported.

In 2002, Long Island (Nassau and Suffolk counties) had the most human cases (Figure 3). However, almost as many human cases were reported for Erie and Broome counties further north. Eight other counties reported 1–4 cases. In general, DCD ≥0.1 occurred several weeks before the onset week of the first human case in most counties, with the peak density around the period of the human case onset, except in sparsely populated rural counties (Clinton, Orleans, Wayne, and Yates), which reported 1 human case each. Suffolk County is notable for its relatively lower weekly DCD. Two counties had DCD ≥0.1 without human cases. The first WNV-positive bird of the season was typically reported 1–4 weeks after the bird was found. Sharp increases in DCD immediately after the report of the first WNV-positive bird were not generally noted in 2002.

A similar pattern was seen in 2003, with Long Island (Nassau and Suffolk counties) again leading the number of human cases (Figure 4). All 3 counties with >1 human case had DCD ≥0.1 in the week of, or the weeks before, the first human case onset. A similar pattern was seen for Monroe County with 1 human case. Onondaga County, with 1 human case, had a weekly DCD ≥0.1 after the human case onset. The DCD approached 0.1 in Dutchess County 2 weeks before the week of the human case onset. The DCD remained low in the sparsely populated counties (Cattaraugus, Schuyler, Warren, and Yates) with 1 human case. Two counties had DCD ≥0.1 without human cases. In 2003, laboratory confirmation of viral activity in a dead bird was available 1–2 weeks after the bird was found. DCD increases after those reports were observed for some, but not all, counties.

For each year and for the 3 years combined, the CMH pooled estimate of risk for WNV disease among residents of counties with DCD ≥0.1 was ≥2 times the risk among residents of counties with DCD <0.1 (Table 2). Relative risks were highest in 2001; residents of counties with elevated DCD had 7.6–8.6 times the risk of contracting WNV disease than residents of counties with lower DCD. Relative risks were lower in 2002 (2.0–2.3) but increased in 2003 (5.3–6.5). During the 3-year period, residents of counties with elevated DCD had 3.4–3.8 times the risk of contracting WNV disease within the next 2 weeks than residents of counties reporting fewer dead crows per square mile.

Discussion

As in 2000 (19,20), increases in weekly number of dead crows per square mile were found in most New York counties several weeks before onset of human cases in 2001 through 2003. Persons in counties with DCD ≥0.1 were notably more likely (2–9 times) to contract WNV disease in the next several weeks. Applying this signal statewide (including New York City) over all 4 years (2000–2003), we found that 148 (91%) of 163 human patients resided in counties with this signal during or before the week of illness onset. This result supports findings of increased DCD in association with human WNV cases in other areas

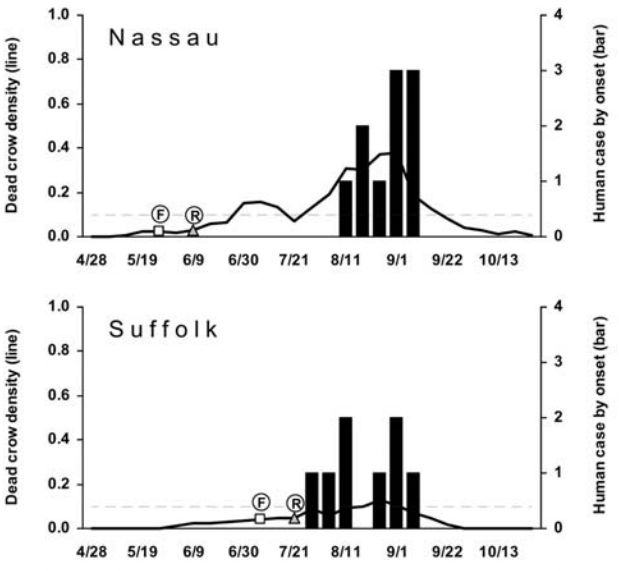

Figure 3. Dead crow densities (DCD, dead crows per square mile) and number of cases of human West Nile virus (WNV) disease, by week, 2002. Horizontal dashed line indicates DCD = 0.1. F, date that the first bird with confirmed WNV infection was found; R, date that the laboratory result of the first bird with WNV infection was reported. A complete version of this figure, with data for other counties with human WNV disease, is available online at http://www.cdc.gov/ncidod/EID/vol11no09/04-0712-G3.htm

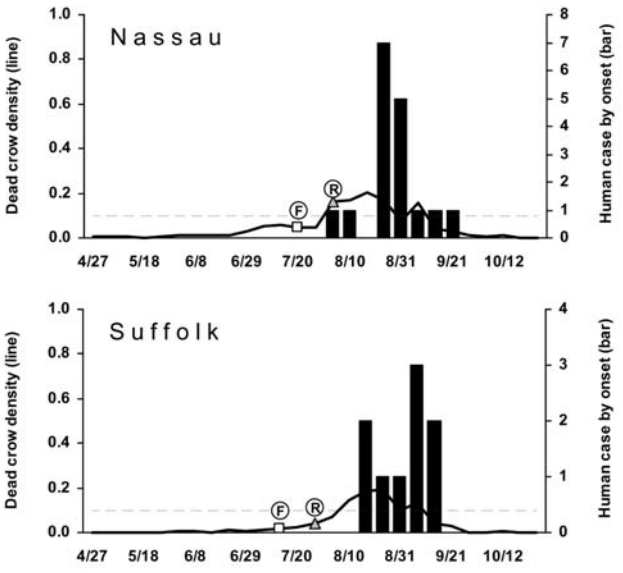

Figure 4. Dead crow densities (DCD, dead crows per square mile) and number of cases of human West Nile virus (WNV) disease, by week, 2003. Horizontal dashed line indicates DCD = 0.1. F, date that the first bird with confirmed WNV infection was found; R, date that the laboratory result of the first bird with WNV infection was reported. A complete version of this figure, with data for other counties with human WNV disease, is available online at http://www.cdc.gov/ncidod/EID/vol11no09/04-0712-G4.htm

(12,15,18). The specific level of the signal (≥0.1 dead crows per square mile per week) may not be applicable in all counties or in New York in the future, if reporting changes, crow populations are reduced, or crows become more resistant to infection.

For counties with >1 patient, the density index rarely failed to forecast the increased risk. Suffolk County in 2002 was a notable exception. If continuous WNV activity led to immunity in crows, this type of signal would be less effective. However, to date the case-fatality rate is still believed to be high, since few crows are found alive with antibody to indicate immunity (6,17). Alternatively, if large-scale death from WNV decreases the crow population, fewer crow deaths would be seen and reported. Data indicate a large die-off of crows in Suffolk County: 5,788 in 2000 and 2,953 in 2001. In 2002, dead crow reports dropped to 883 and were at 939 for 2003.

Weekly county-level DCD was less useful at forecasting the occasional single human case in less-populated counties. Rural areas have fewer persons to report dead crows and fewer persons to become infected with WNV, even when infected mosquitoes and birds are in the area. In any area, including those with few crows, monitoring all dead bird sightings would provide more reports to use for possible WNV tracking, and these sightings should be studied in areas with few crow reports. The value of having more reports may be offset by the lower case-fatality rate in other species. However, even in Florida, where dead birds from the dove family (order Columbiformes) were reported more frequently than dead corvids, the number of dead crow reports, adjusted for human population, was higher in focal areas of WNV transmission, and crow deaths peaked at the same time or before some (but not all) human cases (13).

On rare occasions, the weekly DCD signal provided a false indication of increased risk in a county without human cases. NYSDOH emphasizes reporting and preventing neurologic cases; milder cases of disease such as West Nile fever could have occurred undetected in those counties. A previous study found a pattern of decreasing DCD after mosquito control (19), which indicates that control activities could have reduced the risk, or that differences in mosquito species and their host preferences may have existed. In developing risk indicators that can be relied upon for determining prevention and control actions, occasional false signals may not be a problem if the consequence is increased surveillance and education. If signals trigger resource-intensive control programs, however, false-positive signals may be more problematic.

An important issue is how to maintain the public's interest in reporting if bird testing is reduced or stopped after the virus is confirmed in an area. If dead crows are unreported, because people are either not in the area to find them or not interested in reporting them, DCD will be less useful for monitoring risk. A disadvantage of the county-level approach presented in these analyses is that it does not allow identification within a county of the higher-risk areas. Geographic information system approaches to identify geographic clustering of dead crow or dead bird reports, with or without laboratory confirmation, can help locate areas of risk (14,15,26,27). Such studies are underway in New York (28,29), but they are more resource

Table 2. Risk for West Nile virus disease in counties with elevated dead crow density (DCD) in the 2 weeks before disease onset*

Counties with elevated DCD†	2001 RR (95% CI)	2002 RR (95% CI)	2003 RR (95% CI)	2001–2003 RR (95% CI)
2 weeks before onset	8.6 (1.8–41.8)	2.2 (1.1–4.6)	5.4 (2.1–14)	3.5 (2.0–6.0)
1 or 2 weeks before onset	7.9 (2.9–19.1)	2.3 (1.1–4.8)	6.5 (2.6–16.3)	3.8 (2.2–6.6)
0, 1, or 2 weeks before onset	7.6 (1.6–36.8)	2.0 (0.95–4.4)	5.3 (2.2–12.8)	3.4 (1.9–5.9)

*Versus residents of counties without elevated DCD in the previous 2 weeks; risk is calculated for exposure to elevated DCD only 2 weeks before, 1 or 2 weeks before, and during the same week or 1 or 2 weeks before week of onset. SAS provides risk estimates by 2 methods; because zero values occur in 2×2 tables for some weeks, we report results by the adjusted logit method, which uses a 0.5 correction for zero-value cells. RR, relative risk; CI, confidence interval.
†Weekly DCD ≥0.1.

intensive, and thus more difficult to institute in real time, than the county-level density index.

With the number of human WNV cases increasing to nearly 10,000 in the United States in 2003 (30), the need for surveillance tools to determine when to use costly prevention and control measures has increased. The rapid spread of emerging diseases, such as WNV disease, and the potential use of disease agents for bioterrorism have shown the need to develop real-time surveillance tools, especially for detecting disease activity before laboratory-confirmed cases are reported.

Examples of real-time tools include syndromic surveillance systems (31), most of which rely on indicators other than laboratory-confirmed cases. Although surveillance systems that use laboratory-confirmed cases will continue to have a critical role, a more rapid index of viral activity, such as New York's DCD index, may be useful in some situations. Even if increases in DCD provide an early warning of increasing viral activity and risk to humans, more research is required for systems that function well to share this information in real time, securely and confidentially, with appropriate public health partners who can adjust surveillance and control procedures (32–34).

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Dengue Virus Type 3, Brazil, 2002

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During the summer of 2002, Rio de Janeiro had a large epidemic of dengue fever; 288,245 cases were reported. A subset of 1,831 dengue hemorrhagic fever cases occurred. In this study, performed in the first half of 2002, samples from 1,559 patients with suspected cases of dengue infection were analyzed. From this total, 1,497 were obtained from patients with nonfatal cases, and 62 were obtained from patients with fatal cases. By the use of different methods, 831 (53.3%) cases, including 40 fatal cases, were confirmed as dengue infection. When virus identification was successful, dengue virus type 3 (DENV-3) was obtained in 99% of cases. Neurologic involvement was shown in 1 patient with encephalitis, confirmed by the detection of DENV-3 RNA in the cerebrospinal fluid. This explosive epidemic of DENV-3 was the most severe dengue epidemic reported in Brazil since dengue viruses were introduced in 1986.

Many factors were responsible for the resurgence of epidemic dengue fever (DF) and dengue hemorrhagic fever (DHF) in the final years of the 20th century. Demographic and societal changes such as population growth, urbanization, and modern transportation contributed greatly to the increased incidence and geographic spread of dengue activity (1). The prevalence of the disease is highest in tropical areas of Asia and the Americas, with ≈50–100 million cases of DF and 250,000–500,000 cases of DHF occurring annually worldwide (1–3).

The current epidemiologic situation in Latin America resembles that in Southeast Asia some years ago, with the cocirculation of multiple serotypes in many countries and an increased number of DF and DHF cases. During 2002, Latin American countries reported >1 million cases of DF with >17,000 cases of DHF including 225 deaths (2).

In Brazil, the introduction of dengue virus type 1 (DENV-1) and dengue virus type 2 (DENV-2) in the state of Rio de Janeiro in 1986 and 1990, respectively, resulted in the subsequent spread of these serotypes throughout the country (4). The reintroduction of dengue virus type 3 (DENV-3) in the American continent in 1994 (5) and its rapid spread to the Caribbean Islands in subsequent years (6) resulted in intensified virologic surveillance in the State of Rio de Janeiro, as a response to an imminent threat of DENV-3 epidemics in Brazil. DENV-3 was first isolated in December 2000 in the municipality of Nova Iguaçu, metropolitan region, from a patient with classic DF (7) and initiated a period of cocirculation of DENV-1, DENV-2, and DENV-3 in the state (8). In January 2002, a sudden increase in the number of dengue cases occurred in susceptible populations that had only experienced DENV-1 and DENV-2 epidemics. In the first half of the year, the state reported 288,245 dengue cases, including 1,831 DHF cases and 91 deaths. The metropolitan region including Rio de Janeiro city and surrounding counties reported 246,803 cases and 83 deaths. The number of DHF cases exceeded the total number of cases reported in Brazil from 1986 to the time of the epidemic, and the annual incidence of dengue infection in 2002 in the state reached 1,735 per 100,000 inhabitants (9). We describe laboratory and clinical findings from 1,559 patients, including 62 who died during the largest and most severe epidemic that has occurred in Rio de Janeiro since DENV became endemic in the country in 1986.

Materials and Methods

Study Population

The 1,559 case-patients included in this study had acute febrile illness with ≥2 of the following manifestations: headache, retrobulbar pain, myalgia, arthralgia, rash, and

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hemorrhagic manifestations. A total of 1,497 cases were in outpatients from different healthcare centers, and the remaining 62 were suspected dengue fatal cases in patients hospitalized in private and public hospitals in the metropolitan area of Rio de Janeiro city. The age range (1–73 years) was quite evenly distributed; 10.5% were 1–10 years of age, and 16.9%–19.9% of all patients were in each 10-year age group.

Laboratory Methods

Acute-phase serum specimens, cerebrospinal fluid (CSF), and fresh tissues were stored at –70°C and convalescent-phase serum specimens at –20°C until tested. Dengue infections were confirmed by virus isolation or viral RNA detection by reverse-transcriptase polymerase chain reaction (RT-PCR), by immunoglobulin (Ig) M and/or IgG seroconversion, or by the demonstration of DENV antigen in formalized fixed autopsy tissues by immunohistochemical tests.

Virus Isolation

Virus isolation was performed for all serum samples obtained until day 7 after the onset of disease (n = 927), by infection of clinical specimens into clone C6/36 of *Aedes albopictus* cells. The virus isolates were typed by the indirect fluorescent antibody test with serotype-specific monoclonal antibodies (10).

RNA Extraction and RT-PCR

RT-PCR (11) was performed as a rapid molecular tool to detect and type DENV only in acute-phase sera and fresh tissues from patients who died, hospitalized patients, and outpatients whose disease severity was characterized by thrombocytopenia, hemorrhagic manifestations, or both (n = 282). Viral RNA was extracted from clinical samples (sera, CSF, and tissue) with QIAamp Viral RNA Mini Kits (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol.

Serology

Dengue IgM-capture enzyme-linked immunosorbent assay (ELISA) (PanBio, Brisbane, Australia) was performed according to the manufacturers' instructions in sera obtained after day 5 after onset of disease and in all sera from patients who died (n = 1,060). An in-house IgM antigen capture ELISA (MAC-ELISA) (12) was also performed to confirm dengue infection in sera from patients who died.

IgG-ELISA was performed, as previously described (13), in serum samples available from patients with fatal outcomes (n = 37) and in paired serum samples from patients with fatal cases (n = 88). According to the IgG-ELISA criteria, the immune response is defined as primary when acute-phase serum samples obtained before day 5 of illness have IgG antibody titers <1:160 and convalescent-phase sera have titers ≤1:40,960. Infections are considered secondary when IgG titers are ≥1:160 in the acute-phase serum and ≥1:163,840 in convalescent-phase samples.

Immunohistochemical Procedure

Sections of formalin-fixed, paraffin-embedded tissues were processed by using the streptavidin-biotin method, according to the manufacturer's protocol (Kit LSAB, DAKO, Carpinteria, CA, USA). Monoclonal antibodies for DENV-1, -2, and -3 were provided by the Centers for Disease Control and Prevention.

Results

Laboratory Findings

DENV was isolated from 237 (25.6%) of 927 acute-phase serum specimens injected into C6/36 cells and identified as DENV-3 (n = 234), DENV-1 (n = 2), and DENV-2 (n = 1). Of the 927 serum samples, 282 were submitted for virus isolation and RT-PCR. RT-PCR identified 129 (45.7%) of 282 cases as DENV-3. Thus, the overall results obtained with both methods showed that 321 (99.1%) of 324 viruses identified were DENV-3. A total of 171 samples were submitted for both MAC-ELISA and either virus isolation or RT-PCR. When MAC-ELISA results were added to the diagnostic algorithms, case confirmation reached 53.3% (831/1,559) (Table 1).

Dengue infection was confirmed in 40 (64.5%) of 62 patients who died. In 21 of these cases, infection was confirmed by at least 2 methods employed as follows: 2 cases by virus isolation and RT-PCR; 9 cases by MAC-ELISA and RT-PCR; 6 cases by RT-PCR and immunohistochemistry; 2 cases by MAC-ELISA and immunohistochemistry; 1 case by virus isolation, RT-PCR, and immunohistochemistry; and 1 case by virus isolation, MAC-ELISA, and RT-PCR.

The male: female ratio was 1:1.08 in DENV-3 patients and 1:1.6 when only fatal cases were considered. The age range of patients who died was 7–65 years. A total of 103 clinical samples (serum or fresh tissues samples of liver, spleen, lung, kidney, and brain) were available from the 62 patients with fatal outcome. In these samples, we were able to detect viral RNA, by using RT-PCR, in 33 (32.0%) of 103 specimens. DENV-3 RNA was identified from the CSF of 1 patient (Table 2). Of the 99 clinical specimens injected into C6/36 cells, DENV-3 was recovered from 6 specimens; a total of 24 fatal cases were confirmed as DENV-3 infection by using both methods (Table 2).

Immunohistochemical procedures detected DENV antigen in 48% of specimens from patients with fatal cases, mainly in hepatocytes. Among all the tissues analyzed, the liver was the site where DENV was most frequently

Table 1. Monthly distribution of suspected dengue cases investigated January–July, 2002, State of Rio de Janeiro*

Month	Virus isolation positive/ studied (%)	RT-PCR positive/ studied (%)	Serotype detected	MAC-ELISA positive/ studied (%)	IgG-ELISA positive/ studied (%)	Confirmed cases/studied cases (%)	Deaths positive/ studied (%)
January	114/360	47/93	2 DENV-1; 1 DENV-2; 135 DENV-3	203/373	61/67	308/525	6/8
February	61/315	49/89	103 DENV-3	212/356	29/41	279/504	15/20
March	55/173	28/69	72 DENV-3	123/220	15/29	187/375	15/23
April	3/45	5/18	7 DENV-3	38/71	3/8	49/97	4/8
May	2/22	0/7	2 DENV-3	4/26	ND	6/38	0/1
June	2/6	0/6	2 DENV-3	0/9	0/2	2/11	0/2
July	0/6	ND	0	0/5	0/1	0/9	0
Total	237/927 (25.6)	129/282 (45.7)	2 DENV-1; 1 DENV-2; 321 DENV-3	580/1,060 (54.7)	108/148 (73.0)	831/1,559 (53.3)	40/62 (64.5)

*RT-PCR, reverse transcriptase–polymerase chain reaction; MAC-ELISA, immunoglobulin M antigen capture enzyme-linked immunosorbent assay; IgG, immunoglobulin G; DENV, dengue virus; ND, not done.

recovered by using RT-PCR, virus isolation, and immunohistochemistry (Table 3). The pattern of immunoreactivity in all tissues showed cytoplasmic granular positivity.

The histopathologic findings in patients with confirmed fatal cases showed that the liver was the most affected organ, with macro- and microvacuolization and discrete lymphocytic infiltration of the periportal space. Focal necrosis, swelling of hepatocytes, and colestasis were frequently observed. Edema and congestion were the predominant findings in the brain. Microhemorrhagic foci were also present; however, a marked inflammatory reaction was not observed. Meningeal congestion was frequent. Intraalveolar hemorrhaging was seen in the lungs, associated with the inflammatory infiltration of lymphocytes. In the spleen, congestion of the paracortical zone was the most frequent finding.

IgG-ELISA was performed on 37 serum specimens available from patients who died to characterize the immune response, 20 (54.1%) cases were classified as primary infection, 9 (24.3%) cases as secondary, and 8 (21.6%) cases as inconclusive. In 88 nonfatal cases of confirmed DENV-3 infection, 49 (55.7%) were classified as primary infection and 39 (44.3%) as secondary infection.

Clinical Findings

When stratified analysis was conducted on data from the 297 DENV-3 patients who died (131 male and 166 female), confirmed by RT-PCR, virus isolation, or both, the following signs and symptoms were noted: fever (100.0%), headache (96.3%), myalgia (80.8%), prostration (71.4%), nausea/vomiting (70.0%), retroorbital pain (58.9%), and arthralgia (54.9%). Hypotension (8.8%) and abdominal pain (1.7%) were also observed in some patients with severe cases. Neurologic signs were observed in 1.3%, and hepatic involvement was demonstrated by the number of patients with jaundice (5.4%). Thrombocytopenia was noted in 6.1% of patients. The hemorrhagic manifestations in 297 of these patients were metrorrhagia (13.3%), epistaxis (3.7%), melena (5.1%), hematuria (4.0%), hematemesis (2.7%), bleeding gums (1.3%), hemoptysis (0.7%), and ecchymosis (1.0%).

Discussion

During 2002, a total of 771,551 dengue cases were reported in Brazil, mainly in the southeastern and northeastern regions. That number corresponded to 80% of reported dengue cases in the Americas (http://www.paho.org; 21 Nov 2002).

Table 2. Investigation of suspected fatal dengue cases according to available clinical samples*

Clinical specimen	RT-PCR positive/ studied (%)	Virus isolation positive/ studied (%)	Serotype detected	MAC-ELISA positive/studied	Immunohistochemistry positive/studied	Confirmed cases/studied cases (%)
Serum	15/42	4/38	15 DENV-3	18/42	ND	26/42 (61.9)
CSF	1/2	0/2	1 DENV-3	0/2	ND	1/2
Fresh tissues	17/59	2/59	17 DENV-3	ND	ND	17/59(28.8)
Formalin-fixed and paraffin embedded tissues	ND	ND	0	0	23/48	23/48 (47.9)
Total	33/103 (32.0)	6/99 (6.0)	33 DENV-3	18/44 (40.9)	23/48 (47.9)	40/62† (64.5)

*RT-PCR, reverse transcriptase–polymerase chain reaction; MAC-ELISA, immunoglobulin M antigen capture enzyme-linked immunosorbent assay; DENV, dengue virus; IgG, immunoglobulin G; ND, not done; CSF cerebrospinal fluid.
†Total of confirmed fatal cases by any method/total of fatal cases studied.

Table 3. Dengue virus detection according to tissues samples analyzed from patients with laboratory-confirmed fatal cases*

Tissues sample	RT-PCR positive/studied	Virus isolation positive/studied	Immunohistochemistry positive/studied
Liver	7/7	2/6	15/23
Lung	4/4	0/4	2/10
Brain	3/3	0/3	2/7
Kidney	2/2	0/2	0
Spleen	1/1	0/1	4/11

*RT-PCR, reverse transcriptase–polymerase chain reaction.

The State of Rio de Janeiro, with a total population of 14,391,282 inhabitants, is located in an area of 43,696,054 km^2 on the coast of the southeast region of Brazil. Most of the population (11,094,994) inhabit the greater metropolitan region of the state, including the capital Rio de Janeiro and another 18 surrounding municipalities. This region caused 308,125 (87.5%) of 351,959 DENV-1 and DENV-2 cases reported in the state in the last 15 years (9).

The introduction of DENV-3 into Rio de Janeiro in 2000 placed the region at high risk for a new epidemic due to this serotype, since the introduction of a new serotype into a susceptible population with high mosquito densities may produce a large epidemic after a lag period (14). Indeed, 1 year after the DENV-3 introduction, this serotype was responsible for the most severe epidemic in the state's history in terms of the highest number of reported cases, the severity of clinical manifestations, and the number of confirmed deaths. In this DENV-3 epidemic, the number of DHF/dengue shock syndrome (DSS) cases (1,831) and deaths (91) exceeded the total number of DHF/DSS cases (1,621) and deaths (76) in the entire country from 1986 to 2001 (15). The occurrence of 3 confirmed deaths in children <15 years of age could represent a change in the epidemiologic scenario, since DHF/DSS cases in Brazil have been observed almost exclusively in adults (16).

When we analyzed the clinical data on patients with nonfatal cases, the frequency of fever, headache, and myalgias was similar to those observed during the DENV-1 epidemic in 1986 to 1987 (17); however, prostration, hemorrhagic manifestations, and hypotension were observed more often in the more recent DENV-3 epidemic. Furthermore, prostration caused by DENV-3 infection was previously described as a cause for hospital admission during an epidemic in Queensland, Australia (18). Mild and severe forms of the disease were also reported during DENV-3 epidemics in New Caledonia and Tahiti, respectively (19,20).

An increase in unusual manifestations was observed during this epidemic, characterized by the incidence of central nervous system (CNS) involvement and hepatitis. Although CNS involvement has been previously reported during dengue epidemics, including those in Brazil (21,22), it increased during this epidemic, when many patients reported dizziness. In 1 fatal case, this involvement was confirmed by detecting DENV-3 RNA in CSF. Neurologic disorders associated with dengue cases have been referred to as dengue encephalopathy, attributed to immunopathologic responses and not to CNS infection. However, isolating DENV-3 and detecting DENV-2 by using RT-PCR from CSF provide evidence that DENV has neurovirulent properties and can cause encephalitis in both primary and secondary infections (23). Moreover, the breakdown of the blood-brain barrier has been previously demonstrated in fatal dengue cases (24). Data about transaminase levels from dengue patients were not available; however, the impact of DENV infection on liver functions could be demonstrated by patients with jaundice. Alterations in levels of aspartate aminotransferase and alanine aminotransferase were observed in 63.4% and 45% of dengue patients in a study performed during a DENV-3 outbreak in the city of Campos de Goytacazes in the same year (25). Transient derangement of liver functions has been previously demonstrated in dengue patients and in DHF patients with or without hepatomegaly (26,27). In this study, hepatomegaly was reported only in patients who died. A low rate of hepatomegaly due to dengue infection was previously reported in Manila; 1% of patients with confirmed cases had this sign. These levels are considerably lower than the levels observed in Bangkok (80%–90%) and Jakarta (49%) (26). A study on clinical differences observed in patients with dengue caused by DENV-3 showed that they had 3.06 times more risk for abdominal pain than patients with DENV-1 and 6.07 times more risk for shock than patients infected with DENV-2 (28).

A retrospective study of the patients who died (29) in this epidemic showed that warning signs occurred in 88.1% of patients on hospital admission: hypotension (59.5%), abdominal pain (35.7%), and preshock (35.7%). During hospitalization, the proportion with hypotension reached 75.6% and with shock, 61%. The World Health Organization criteria for DHF were fullfilled by 35.5% of the hospitalized patients. Death due to shock occurred in 57.8% of patients, cardiac failure in 17.8%, and massive pulmonary hemorrhage and meningoencephalitis in 2 cases (29).

Liver tissue was the most important tissue for virus detection by using virus isolation, RT-PCR, or immunohistochemistry. Recently, the liver was recognized as a major target organ in the pathogenesis of DENV infection; the active replication in hepatocytes (30,31) could explain

these findings. The virologic confirmation of cases in 24 patients who died was similar to that described in Indonesia (32).

The increased mortality rate has already been related to the general phenomenon of increased dengue incidence and severity. The reintroduction of DENV-3 in Puerto Rico and Queensland did not result in death (14,18); however, in Jakarta the DENV-3 fatality rate was nearly 3 times higher than the fatality rate observed for the other serotypes (33).

In this study, the disease severity and the occurrence of deaths resulting from primary infections could be partially explained by the virulence of the DENV-3 strain. Analysis of the partial nucleotide sequence of the genome showed that Brazilian DENV-3 belongs to genotype III (Sri Lanka/India), similar to the strains currently circulating on the American continent (34). Previous studies have shown that this genotype caused DHF epidemics in Sri Lanka and India and was associated with DHF cases in Mexico (35). Fatal cases resulting from dengue primary infections were described before DENV-3 was introduced in Brazil (36), although the largest number of DHF/DSS cases occurring in the state were due to secondary DENV-2 infections (Southeast Asia-Jamaican genotype) (16). These findings showed that some DENV strains can be more virulent than others and that antibody-dependent enhancement alone does not explain all cases of severe disease (33,37–39). Genotyping studies performed in Sri Lanka and French Polynesia showed that viral strains in themselves are an important risk factor for DHF/DSS (20,40).

The scenario of dengue in Brazil indicates that more emphasis should be placed on efforts to control the vector. An active epidemiologic surveillance laboratory should be supported, and a clearer understanding of the epidemiologic characteristics of dengue transmission is required.

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Trypanosomiasis Control, Democratic Republic of Congo, 1993–2003

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In the Democratic Republic of Congo (DRC), human African trypanosomiasis (HAT) reached unprecedented levels in the 1990s. To assess recent trends and evaluate control efforts, we analyzed epidemiologic and financial data collected by all agencies involved in HAT control in DRC from 1993 to 2003. Funds allocated to control populations, as well as to the population screened, doubled from 1993 to 1997 and from 1998 to 2003. The number of cases detected decreased from 26,000 new cases per year in 1998 to 11,000 in 2003. Our analysis shows that HAT control in DRC is almost completely dependent on international aid and that sudden withdrawal of such aid in 1990 had a long-lasting effect. Since 1998, control efforts intensified because of renewed donor interest, including a public-private partnership, and this effort led to a major reduction in HAT incidence. To avoid reemergence of this disease, such efforts should be sustained.

Human African trypanosomiasis (HAT), or sleeping sickness, is a vectorborne disease caused by the parasite *Trypanosoma brucei*. East African HAT is caused by *T. b. rhodesiense* and West African HAT, the subject of this article, by *T. b. gambiense*. The latter species causes a slowly progressing fatal disease with few specific symptoms or none in its initial stage (1). The only proven effective way to control *T.b. gambiense* HAT is mass population screening and treatment of those infected. Well conducted campaigns reduce the human parasite reservoir and therefore HAT incidence (2).

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Substantial observational evidence from Sudan (3), Uganda (4), Equatorial Guinea (5), and the Bandundu region in former Zaire (6) has shown that intensive screen-and-treat programs effectively reduce HAT incidence. So far, no evidence has shown that adding vector control to active case finding is effective, and vector-control efforts are limited (5). HAT is one of the so-called neglected diseases that afflict the developing world; the term indicates the lack of drug research and development for these conditions (7). In the field of HAT, the situation was so bleak by 1998 that production of sleeping sickness drugs was no longer guaranteed. A public-private partnership was established in 2001 between the World Health Organization (WHO) and Sanofi-Aventis (Paris, France), the main pharmaceutical manufacturer of anti-HAT drugs. Sanofi-Aventis donated the 3 most used anti-HAT drugs (DFMO [difluoromethylornithine], melarsoprol, and pentamidine) for 5 years and also offered funding for disease control and innovative research. Bayer AG (Leverkusen, Germany) has donated a 5-year supply of suramin, another anti-HAT drug. These donations were welcomed by HAT control programs, which used to spend up to 46% of their annual budgets on the purchase of drugs (S. Van Nieuwenhove et al., unpub. data).

The sustainability of HAT control has been a recurrent concern, as exemplified by the postcolonial history of sleeping sickness control in the Democratic Republic of Congo (DRC). By 1960, the year of DRC's independence, HAT was almost completely eliminated, but by 1976, many new cases were diagnosed. HAT control received substantial international aid during the 1980s, which amounted to >90% of DRC's HAT budget. However, this international support was suddenly withdrawn after the massacre of students at the Lubumbashi University in May 1990 (8). Inevitably, sleeping sickness returned to DRC in

full measure. In 1994, donors again allocated financial support for HAT control as humanitarian emergency aid and channeled its implementation through nongovernmental organizations. However, by 1997, the epidemiologic situation seemed little better than in the 1930s (9,10) and showed a rising trend that was cause for concern. Moreover, the HAT problem in DRC was no longer restricted to remote rural districts: urban areas such as Kinshasa were reporting cases (11). In 1998, Belgian bilateral aid for HAT resumed under a 5-year support program, and full screening and treatment programs were restarted. Several authors ascribe the reemergence of HAT in DRC primarily to the interruption of bilateral and multilateral aid that occurred after 1990 (12–14). The drastic reduction in specific control activities at a time when the epidemic was spreading, in the context of overall collapse of the Congolese health infrastructure, most likely contributed to the exponential rise in HAT cases after 1990. We examined the recent trends of HAT in DRC and evaluated the effects and sustainability of the control program.

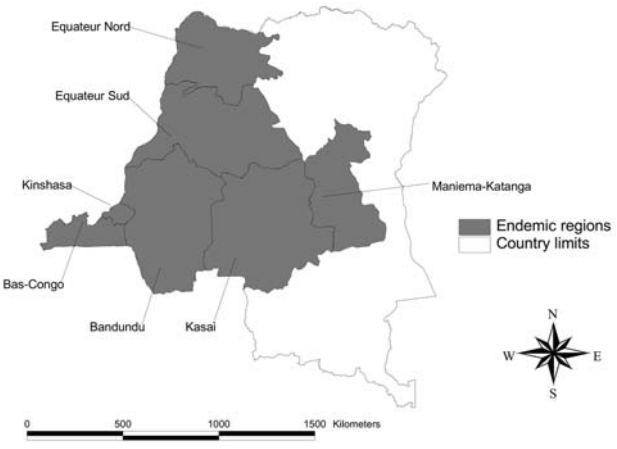

Figure 1. Disease-endemic regions (indicated by shaded areas) in the Democratic Republic of Congo, as managed by human African trypanosomiasis program.

Methods

Context

DRC has a surface area of 2.345.000 km^2 and ≈60 million inhabitants, for a density of 25 inhabitants/km^2. Administratively, DRC is subdivided into 11 provinces, and HAT is endemic in 9 of them (Programme National de Lutte contre la Trypanosomiase Humaine Africaine [PNLTHA], unpub. data). Since 1990, the country has been devastated by political turmoil and civil war (1996–1997 and 1998–2003). The health status of the population has deteriorated because of progressive breakdown of health infrastructures, disease outbreaks, and the reemergence of endemic diseases such as tuberculosis and HAT. The emergence of HIV/AIDS has added to this catalog of health disasters.

HAT control in DRC is organized by a national program, PNLTHA. This program divides HAT-endemic areas into 7 regions, each under the responsibility of a regional coordinator (Figure 1). These regions do not coincide with the administrative divisions (provinces).

The main control strategy of PNLTHA is to actively screen the population at risk by specialized mobile teams (15), who refer patients with confirmed cases to regular health services and specialized centers for treatment. Screening was based on the palpation of cervical glands until 1996, when a serologic screening test (card agglutination test for trypanosomiasis [CATT]) was added to the algorithm (16). Each mobile team screens ≈40,000 persons/year. A considerable amount of passive case finding takes place as well, for example, when the regular health service staff diagnose HAT in a patient who arrives for a consultation. PNLTHA's control strategies also include vector control.

Data Sources

We used the PNLTHA epidemiologic surveillance database that included all HAT cases detected by mobile teams and regular health services since 1926. For 1993–2003, we examined the monthly reports compiled by the regional PNLTHA coordinators, with the exception of those from Maniema-Katanga and the Province Orientale because they were incomplete and fragmentary as a consequence of the ongoing war.

We distinguish 2 discrete periods for the analysis of international aid. From 1993 to 1997, only humanitarian aid budgets were allocated to HAT control, typically lasting for a maximum of 6 months. Because of the political turmoil at that time in DRC, international aid for HAT was given as "indirect aid," i.e., donors would give cash grants to 3 nongovernmental organizations (NGOs)—Fonds Médical Tropical (FOMETRO), Medische Missie Samenwerking (MEMISA), and Médecins sans Frontières (MSF)—and rely on them for implementation. MEMISA and MSF would supplement this indirect aid with funds they had privately raised. Between 1998 and 2003, the Congolese government again benefitted from long-term international aid programs, and the Belgian Technical Cooperation (BTC) launched its own technical assistance program for HAT control. The same NGOs continued to play a major role in implementation, as well as partly funding, these control activities. For the period under study, WHO funds were donated directly to PNLTHA, while those from the European Union were given to FOMETRO.

We obtained financial data on budgets and expenditure for HAT control directly from the various donor agencies

and cross-checked data with all the implementing agencies (PNLTHA, the 3 NGOs, and the BTC engaged in HAT control in DRC during the period under study) (Table 1). Only funds allocated to HAT control were incorporated in our study; we excluded funds earmarked for research. To avoid duplication, we categorized financial resources by donating and not by implementing agency. Over the entire study period, the Congolese government only allocated funds for personnel costs, and those were included in our computations at an average salary of US$12.50 per month per person. All international aid was donated in cash directly to the NGOs or BTC.

Expenditure in Belgian francs was converted into US dollars, according to the exchange rate that applied at time of expenditure. Expenditure in euros was converted at a fixed rate of 40.3399 Belgian francs = 1 euro. The exchange rate between the US dollar and euro was the average exchange rate per year based on Federal Reserve Statistical Release (available at http://www.federalreserve.gov/releases/H10/Hist/). All current dollars were converted to constant 1998 dollars by using the US Office of Labor Consumer price Index. All data were stored and analyzed in an Excel database (Microsoft Corp., Redmond, WA, USA).

Definitions

PNLTHA defines a new HAT patient as a person whose condition has, for the first time, been diagnosed by parasitologic examination as sleeping sickness. Relapse cases are thus not included in this study. The HAT detection rate is the number of newly detected cases, expressed as a proportion of the screened population. We distinguish the active detection rate (ADR), in which data are collected through active case finding, from the overall detection rate, which also includes cases detected at health facilities. The coverage rate of the population is the proportion of the population tested (through active or passive case finding) divided by the population at risk for HAT. The participation rate applies only to active case finding and is defined as the number of persons screened by the mobile teams divided by the target population. The proportion of treated patients is the number of persons who received HAT treatment divided by the number of persons detected with HAT.

Evaluation Method

We structured our evaluation of the HAT control program in the form of input, process, output, and outcome analyses (17) and according to the method of Bouchet et al. (18) (Table 2). Input represents the human and financial resources invested in the program. Drug availability, measured as the number of occasions that the stock ran out during the period under study, was also considered as an input. Process indicators are not reported in this study because they are relevant only to the daily management of the program. Program output was measured through an analysis of coverage of the population at risk, the participation rate in screening, the number of detected HAT cases, the proportion of patients with detected HAT patients who received treatment, and the proportion of patients with treated cases that have been followed-up correctly. We report the annual HAT detection rate, both nationally and for each region, as indicators of program outcome.

Results

Outcome

Figure 2 represents the evolution in the annual number of newly detected HAT cases in DRC from 1926 to 2003. Between 1960 and 1989, the figure shows an increasing trend with 2 small peaks in 1970 and 1986. This trend was interrupted in 1990 and 1991, which coincides with the

Table 1. Financial resources converted to constant 1998 US$ and their origin during the first (1993–1997) and the second period (1998–2003)*†

		1993–1997		1998–2003	
	Type of donor	US$	Percentage	US$	Percentage
Belgian government	Bilateral	4,508,774	69.2	14,566,002	87.1
European union	Bilateral	1,337,946	20.5	656,367	3.9
Congolese government	NA	270,611	4.2	329,441	2.0
WHO	Multilateral	0	0.0	527,698	3.2
Pain pour le Monde‡	NGO	70,430	1.1	68,411	0.4
MSF‡	NGO	0	0.0	104,233	0.6
MEMISA‡	NGO	70,965	1.1	462,906	2.8
AFRICA‡	NGO	0	0.0	6,440	0.1
Caritas–Germany‡	NGO	254,506	3.9	0	0.0
Total		6,513,232	100.0	16,721,496	100.0
Total per year		1,302,646		2,786,916	

*NA., not applicable; NGO, nongovernmental organization; WHO, World Health Organization; MSF, Médecins Sans Frontières Belgique; MEMISA, Medische Missie Samenwerking; AFRICA, Association des Femmes pour les Rencontres Intellectuelles et Culturelles en Afrique.
†Sanofi-Aventis/Bayer in-kind drug donation not included; see text.
‡The amounts mentioned for NGOs are limited to the "own funds," i.e., funds that they had privately raised and spent on sleeping sickness control. Several NGOs were implementing HAT control activities with funds provided by the bilateral or multilateral donors.

Table 2. Indicators for evaluating population screening for human African trypanosomiasis (HAT)*

Indicators	
Input	Financial resources
	Human resources
	Availability of tests and anti-HAT drugs
Process	Identification of villages at risk
	Census of population at risk
	Involvement of population at risk in active case detection
	Lymph node palpation
	CATT test
	Parasitologic test for HAT confirmation
	Lumbar puncture to determine the stage of disease
	Treatment
	Treatment follow-up
Output	Coverage rate of population at risk
	Participation rate
	Identification of suspects
	Identification of HAT cases
	Proportion of HAT cases detected and treated
	Proportion of HAT cases treated and followed
Outcome	Annual HAT detection rate and trend

*CATT, card agglutination test for trypanosomiasis.

sudden arrest of control activities in 1990. When humanitarian aid was launched in 1993, the annual number of detected cases increased markedly. The peak was reached in 1998, when the control program detected 26,318 new HAT cases in a screened population of 1,472,674 persons.

After 1998, a marked decline occurred in the number of HAT cases detected, which was not due to an overall decrease in screening activities; the number of operational mobile teams and number of screened persons continued to increase over that period (Figure 3). The overall HAT detection rate, based on active case finding, declined from 1.1% in 1994 to 0.3% in 2002. However, this overall decline in detected HAT cases masks differences between regions. Figure 4 shows the evolution of the number of HAT cases and active detection rate, by region, from 1993 until 2002.

Input

In 1990, 25 mobile teams covered the population at risk. With the reduction in external financial support, 10 teams remained operational from 1991 to 1993. This number slowly increased to 33 teams in 1998 and to 46 teams in 2002. These increases were concentrated in the regions of Bandundu, Equateur-Nord, and Kasai, where the number of teams rose to 13, 13, and 7, respectively, which accounts for 33 mobile teams of 46. In 1993, PNLTHA staff was 250. This number increased progressively to 580 in 2001 and remained stable at 580 in 2003.

From 1993 to 1997, total annual expenditure amounted to US$1,302,646, while in the period 1998–2003, total annual expenditure doubled to US$2,786,916. Table 1 shows the amount and the origin of the financial resources. The budget breakdown was as follows: functioning costs (fuel, vehicle maintenance, supervision, training, stationery, etc.) (32.0%), personnel time (26.4%), HAT drugs (24.3%), laboratory reagents (7.3%), and other material and equipment (10%). The average expenditure per HAT case detected and treated is shown in Table 3.

From 1993 to 2001, implementing agencies spent, on average, 24% of their budgets in purchasing trypanocidal drugs (range 12%–44%). Though the Sanofi-Aventis/Bayer donation program was established in 2001, in practice, implementing agencies could continue working throughout 2002 with existing drug stocks. For 2003, a detailed analysis of amount of donated drugs versus purchased drugs consumed was not possible, and we therefore ignored the in-kind drug donation in Table 1. The full effect of the donation will only become clear after 2004, although can be estimated by its monetary value (Table 4). PNLTHA records the number of patients who have been treated by drug regimen, and these data allowed us to estimate the quantity of the trypanocidal drugs that were required, as well as the total cost of those drugs, calculated according to the preferential price, which was valid until 2001. We estimated that the total drug cost per year in DRC, for treating ≈14,000 HAT patients/year, corresponded to ≈US$600,000/year. This value is consistent with the previous estimate that 24% of budgets are reportedly used for drugs. Notably, since the public-private partnership was established in 2001, supplies of trypanocides never ran short, whereas this problem was a matter of continuous concern before.

Output

The population at risk in the DRC has been estimated at 12,600,000 persons (PNLTHA, unpub. data). Screening and treatment of the at-risk population is estimated to have risen from 6% in 1993 to 19% in 2003. We observed notable differences between regions. Equateur-Nord had a

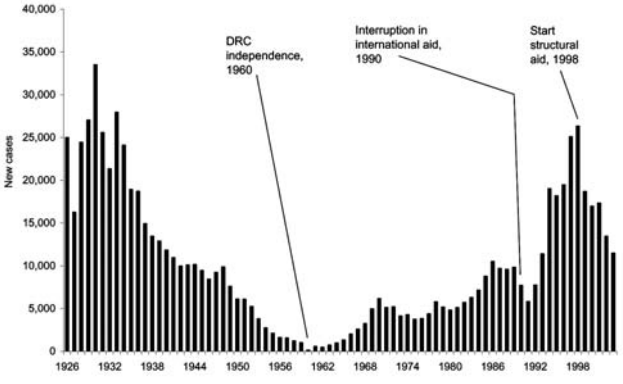

Figure 2. Number of new human African trypanosomiasis new cases in the Democratic Republic of Congo, 1926–2003.

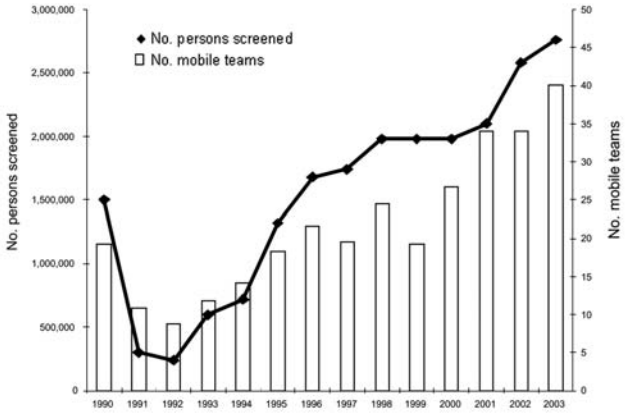

Figure 3. Population screened per year and number of mobile teams operating in the Democratic Republic of Congo, 1990–2003.

coverage rate of >50%, while in the other regions the rate ranged from 10% to 20%. Figure 3 shows that the number of persons screened each year almost tripled from 1993 to 2003.

The participation rate of the population in active case finding was almost 96% in 1998. By 2002, the rate had fallen to 78%. The proportion of new patients who received treatment was ≈95% throughout the whole country but varied from region to region, from 89% to 100%. From 2001 to 2003, a total of 44,247 patients were treated with pentamidine (31.5%), suramin (5.8%), the combination pentamidine-suramin (0.2%), melarsoprol (55.2%), eflornithine (0.85%), nifurtimox (2.6%), and the combination melarsoprol-nifurtimox (3.5%) (because nifurtimox is not registered for use against HAT in DRC, it was given on a compassionate basis when no other drugs were available or when melarsoprol treatment failed).

Discussion

After the number of cases peaked in 1998 with 26,000 new cases, the annual number of HAT cases reported in DRC has decreased to 10,900 cases in 2003. From 1993 to 2003, the annual number of persons screened for HAT, as well as financial resources allocated to HAT control in DRC, has doubled.

The increase in reported cases and in the detection rate observed between 1993 and 1997 can be attributed to increased transmission but also to renewed efforts after several months when active case finding was interrupted. However, the striking decrease in HAT cases from 1998 to 2003 cannot be explained by decreased case-detection efforts because the number of persons screened in the same period doubled. Changes in detection rates through active case finding are difficult to interpret because the population reached is not the same over time. The additional number of persons screened might come from populations that were less at risk in the first place, as happened, for example, in Ville de Kinshasa, where a new mobile team started operating in May 2001 in an area with lower prevalence. Population movements during the war could, in theory, also explain the observed changes in HAT prevalence, but no noteworthy migration from disease-endemic to disease-nonendemic areas or vice versa took place over the study period. We therefore conclude that the decreasing trend in HAT case detection observed in DRC since 1999 is real. Most likely this trend is explained by the intensification of control efforts, the steep increase in resource allocation since 1998, and a major drug donation in 2001. The systematic use of CATT as the serologic screening test in 1996 has probably contributed to a decline in transmission, because it increased screening effectiveness (15).

However, these national figures hide important differences between regions. In the northern and southern Equateur regions and in Kinshasa, the absolute number of HAT cases and detection rates has declined, whereas these indicators remain stationary in the Bas Congo, Kasai, and Bandundu regions. In fact, the decline observed at national level is, to a large extent, based on the decline observed in 1 region, Equateur-Nord, which experienced a major

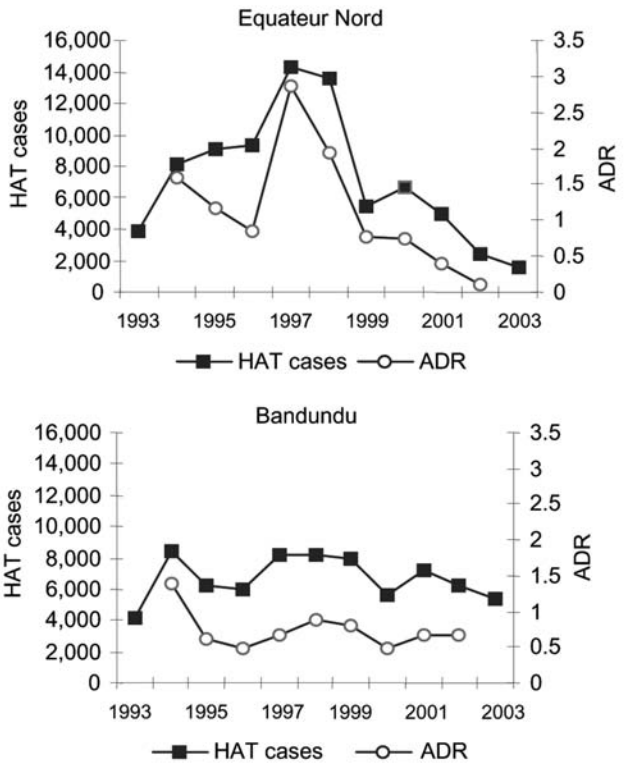

Figure 4. Number of new human African trypanosomiasis cases and active detection rate (ADR) in Equateur Nord and Bandundu, Democratic Republic of Congo,1993–2003 (data from additional regions may be viewed online at www.cdc.gov/ncidod/EID/vol11no09/04-1020-G.htm).

Table 3. Average expense per examined person and average cost per case detected and treated (converted to constant 1998 US$) in active case finding*

	1993–1997	1998–2003
No. screened in population at risk	5,003,477	10,659,210
No. HAT cases detected and treated†	41,500	55,500
Average expenditure per person screened (US$)	1.30	1.57
Average expenditure per case detected and treated (US$)	156.9	301.2

*HAT, human African trypanosomiasis.
†Takes into account only active case finding by mobile teams and the assumption of a treatment rate of 100%.

outbreak but brought it under control by an intensive and well-coordinated campaign.

A similar rapid decrease in the number of HAT cases has been observed by Van Nieuwenhove and Declercq (19) in southern Sudan and by Paquet et al. (4) in Uganda. However, the HAT epidemic reemerged in southern Sudan after control activities were stopped, indicating that disease control efforts should be maintained even when prevalence is low (20–22).

Our analysis showed how HAT control in DRC almost completely depends on international aid and that the interruption of financing from 1990 to 1991 had a long-lasting negative effect on case load. Funding may be discontinued for different reasons, such as changes in donor policies or priorities, so HAT control remains vulnerable. Private NGOs have so far accounted for a minor part of funding in DRC, although they played a role both in advocacy and in program implementation. The recent public-private alliance with pharmaceutical companies not only made continued care for HAT patients possible again but also released substantial financial resources that can be used in the future for operations in DRC. Moreover, through direct financial support to research, training, and rehabilitation, the public-private partnership has contributed to a wider alliance and extension of activities. However, the fact that the 3 main drugs used to treat HAT patients are produced and donated by a single company creates a new type of dependency. Care for HAT patients may be seriously compromised if production or donation stopped for any reason, for example, a company takeover, management changes, or a change in the company's priorities.

The disparities now emerging in disease epidemiology in different parts of DRC call for the adoption of differential control strategies in different regions of the country. Where the ADR has dropped to low levels, screening intervals could be lengthened. Alternatively, and with lower cost, surveillance methods could be used that detect emerging epidemics at an early stage, such as serologic surveys, or that rely on data collection from passive case finding and enhanced diagnosis in the primary health structures (23). Where ADR remains high, the program must identify the reasons for this and find solutions to make control more effective. Furthermore, the increase in treatment failures in the southeastern part of the country should be carefully monitored, and evolving parasite resistance should be thoroughly investigated.

Our analysis shows that successful HAT control is possible, but that it depends on continued financial support and drug availability. Therefore, the governments of disease-endemic countries and the international community must make long-term financial commitments to ensure the continuity of HAT control activities. This necessitates sound financial sustainability planning for HAT control, as is already done, for instance, in childhood immunization (24). Research is necessary on how to rationalize control

Table 4. Estimated costs of anti-HAT drugs consumed in the Democratic Republic of Congo from 2001 to 2003, based on observed number of patients treated by regimen*†

Drug	Observed no. patients treated	Drug quantity/patient required	Total quantity drugs required	Unit price‡ (US$)	Total cost (US$)
Pentamidine	13,957	8	111,656	2	223,312
Suramin	2,604	6	15,624	7	109,368
Pentamidine-suramin	104	8	832	2	1,664
		6	624	7	4,368
Melarsoprol	24,456	9	220,104	7	1,540,728
Eflornithine	377	14	5,278	22	116,116
Nifurtimox§	1,168	100	116,800	0	0
Melarsoprol-nifurtimox	1,581	4	6,324	7	44,268
		64	101,184	0	0
Total	44,247				2,039,824

*HAT, human African trypanosomiasis.
†Source of data: Programme National de Lutte contre la THA (PNLTHA) annual reports. The price of drugs is based on prices reported by the World Health Organization, 1998. Data for the HAT cases retreated in 2003 are not available.
‡Preferential price applicable during the period.
§PNLTHA did not buy nifurtimox in this period.

activities so that control programs can adopt the most effective and efficient strategies.

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Although Sanofi-Aventis indirectly sponsored part of the HAT control activities in DRC, and donated HAT drugs, the company played no role in study design, collection, analysis, interpretation of data, report writing, or the decision to submit the paper.

Dr Lutumba is head of the research unit of the national sleeping sickness control program of DRC. He has extensive experience in sleeping sickness control in DRC and participates in clinical research programs on HAT diagnosis and treatment.

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Persistence of Resistant *Staphylococcus epidermidis* after Single Course of Clarithromycin

Maria Sjölund,*† Eva Tano,* Martin J. Blaser,‡ Dan I. Andersson,† and Lars Engstrand*†

We examined how a common therapy that includes clarithromycin affects normally colonizing *Staphylococcus epidermidis*. Samples from the nostrils of 5 patients receiving therapy were collected before, immediately after, 1 year after, and 4 years after treatment. From each patient and sample, *S. epidermidis* strains were isolated and analyzed for clarithromycin susceptibility and presence of the *erm*(C) gene. We show that macrolide-resistant strains of *S. epidermidis* were selected during therapy and that the same resistant strain may persist for 4 years, in the absence of further antimicrobial treatment.

The emergence and spread of drug-resistant bacteria pose a serious threat to global public health (1,2), and the normal biota constitutes a potential reservoir of resistance genes that can spread to invading pathogens (3,4). A gene (*aphA-3*) that confers resistance to amikacin and kanamycin in *Campylobacter* spp. may have originated from the gram-positive *Enterococcus*, *Streptococcus*, or *Staphylococcus* spp. (4). Similarly, *aadE* and *tet(O)*, which encode streptomycin and tetracycline resistance, respectively, have been found in *Campylobacter* spp. but are considered to have been transferred from gram-positive bacteria (4). Moreover, parts of the mosaic penicillin-binding protein genes of *Streptococcus pneumoniae* that confer penicillin resistance are likely to originate from viridans streptococci, which tend to be more resistant (5), and the *mecA* gene that renders *Staphylococcus aureus* resistant to all β-lactams likely originated in coagulase-negative staphylococci (6).

Staphylococcus epidermidis, a coagulase-negative staphylococcus, is a major component of the normal human biota (7). Large populations (10^3–10^6 CFU/cm^2) of *S. epidermidis* are commonly found in the anterior nares and the axillae (7). Coagulase-negative staphylococci have been increasingly recognized as important nosocomial pathogens (8), affecting immunocompromised patients or those with indwelling devices, such as joint prostheses, prosthetic heart valves, and central venous catheters (8,9). Since the infections associated with *S. epidermidis* are chiefly acquired during hospitalization, it is not surprising that they are increasingly resistant to antimicrobial drugs (10). Macrolide resistance in *S. epidermidis* is commonly caused by *erm* genes (10), whose products dimethylate a 23S rRNA adenine residue, preventing macrolide binding to the 50S ribosomal subunit (11,12). In *S. epidermidis*, *erm*(C), which induces high-level macrolide resistance, predominates (13,14).

In this study, we have assessed how a commonly used therapy that includes clarithromycin affects the normal microbiota of *S. epidermidis*. We show that a 1-week course of clarithromycin selects for macrolide-resistant *S. epidermidis* that may persist up to 4 years after treatment.

Methods

During a cohort study that examined eradication of *Helicobacter pylori* by a combination therapy that included clarithromycin, we chose 5 patients in order to study macrolide resistance in *S. epidermidis*. In the larger study, all patients were colonized with *H. pylori* and had either a duodenal or gastric ulcer, for which a 7-day course of clarithromycin 250 mg twice per day (b.i.d.), metronidazole 400 mg b.i.d., and omeprazole 20 mg b.i.d. was given. We excluded patients who had previously been treated for *H. pylori* or who had received any antimicrobial treatment within the prior 4 weeks. The control group included 5 patients with dyspeptic symptoms who had not received any antimicrobial treatment. During the 4-year course of this study, no other antimicrobial treatment was allowed.

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The study was approved by the human ethics committee at Uppsala University, Uppsala, Sweden.

Samples from the nares of each patient were collected 1 day before treatment, 3–7 days immediately after, 1 year later, and 4 years later. All samples were stored at –70°C until analyzed. From each study patient and each sample, 10 independent colonies of *S. epidermidis* were isolated on Columbia blood agar plates (Difco, Baltimore, MD, USA) and verified by Gram staining, positive catalase, negative DNase, negative mannitol, and negative trehalose testing. DNA was extracted from the bacterial strains with the DNeasy Tissue kit (Qiagen, Hilden, Germany). MIC of clarithromycin was measured with the Etest (AB Biodisk, Solna, Sweden), as recommended by the Swedish Reference Group for Antibiotics.

The *erm*(C) gene was detected as described (13), by using primers ermC1/C2 5′-GCTAATATTGTTTAAATCGTCAATTCC-3′ and 5′-GGATCAGGAAAAGGACATTT-3′ but with the following modifications: each polymerase chain reaction (PCR) contained 25 μL master mix (PCR Master, Roche, Penzberg, Germany), 30 pmol of each primer, 14 μL distilled water, and 5 μL DNA sample. The amplified 572-bp product was separated by electrophoresis on a 1.5% agarose gel.

For pulsed-field gel electrophoresis (PFGE), bacterial cells were harvested by centrifugation from 3 mL overnight cultures in brain-heart infusion broth and resuspended in 3 mL Tris-HCl buffer (pH 7.6). The bacterial suspension (150 μL) was mixed with 150 μL 2% agarose (Sigma, St. Louis, MO, USA) in Tris-HCl buffer and used for making the gel plugs. The plugs were incubated at 35°C overnight in 4 mL Lysis 1 buffer (6 mmol/L Tris-HCl [pH 7.6], 1 mol/L NaCl, 100 mmol/L EDTA [pH 7.5], 0.5% Brij 58, 0.2% deoxycholate, 0.5% sodium lauryl sarcosine [Sarcosyl, Kodak International Biotechno-logies, New Haven, CT, USA], 1 mg/mL lysozyme [Life Technology, Sigma-Aldrich, Steinheim, Germany], and 7 μg/mL lysostaphin [Sigma]), then incubated overnight at 55°C in 4 mL Lysis 2 buffer (1% sodium lauryl sarcosine [Sarcosyl], 0.5 mol/L EDTA [pH 9.5], and 50 μg/mL Proteinase K [Roche Diagnostics Corporation, Indianapolis, IN, USA]). The plugs were washed 3 times for ≥30 min at 35°C in 4 mL of Tris-EDTA buffer. A 3-mm slice of each gel plug was incubated overnight at 25°C with *Sma*I (Life Technology, Invitrogen, Carlsbad, CA, USA) and buffer, then placed in the wells of a 1.0% agarose gel (Ultra pure agarose, Life Technology, Invitrogen), sealed with 1.0 % agarose, and put in 0.5× Tris-borate-EDTA buffer. Electrophoresis (Gene Path Electrophoresis System, Bio-Rad Laboratories, Inc., Hercules, CA, USA) was performed with the following conditions: 5–60 s switch interval with a voltage gradient of 6 V/h at an angle of 120° for 23 h. After electrophoresis, the gel was stained with ethidium bromide for 30 min, destained in distilled water for 1 h, and DNA was visualized under UV light (Gel doc 1000, Bio-Rad Laboratories, Inc.). The restriction fragment profiles were interpreted by comparison with each other, with a reference *S. aureus* strain NCTC 8325, and with a λ phage DNA standard (New England Biolabs, Beverly, MA, USA).

Results

At 1 day before treatment, all 5 patients in the treatment group harbored clarithromycin-susceptible (MIC <0.5 μg/mL) *S. epidermidis* among the 10 independent colonies examined. In 4 patients, all 10 isolates were susceptible, but in the fifth patient 2 isolates were highly resistant (MIC >256 μg/mL) because *erm*(C) was present. Immediately after completing treatment, 4 of 5 patients displayed high-level clarithromycin-resistant (MIC >256 μg/mL) isolates (Table 1). The other isolates from this time point were either resistant with lower MIC values (16–96 μg/mL) or susceptible. Highly resistant isolates could be detected 1 year after treatment in 4 patients and 4 years after treatment in 3 patients. All highly resistant isolates harbored *erm*(C), as determined by PCR. In the controls, who did not receive any antimicrobial treatment, no selection of resistant staphylococci was detected. However, in control 4, 1 highly resistant isolate was detected at the first time point. In the same control, 2 of 10 isolates were highly resistant at the second time point, but no resistance was detected at the 1- and 4-year follow-ups. In control patient 5, all isolates were susceptible, except 5 resistant isolates detected at the 4-year follow up (Table 2).

Isolates obtained from patients 1 and 2, chosen to investigate the clonality of resistance, were genotyped by pulsed-field gel electrophoresis (PFGE). Before treatment, each patient carried 5 different *S. epidermidis* strains among the 10 colonies tested. In patient 1, no highly resistant isolates were detected before treatment. However, immediately after treatment, 2 of 10 isolates were highly resistant, both defined as strain H. Based on PFGE patterns, strain H was detected in 8 of 10 isolates 1 year after treatment and in 4 of 10 isolates 4 years after treatment (Table 1). Strain G, which was susceptible to clarithromycin, was present immediately after treatment and 4 years later. Two of the pretreatment strains (B and C) were detected 4 years after treatment.

For patient 2, from whom 2 highly resistant isolates with the same profile (N) were detected before treatment, PFGE showed 2 distinct resistant strains (N and S) to be present immediately after treatment. Clone N was detected in 8 of 10 isolates 1 year after treatment and in 3 of 10 isolates 4 years after treatment. Susceptible strains P and Q, which were present pretreatment, were isolated again 4 years after treatment. Thus, after treatment in both cases,

PFGE analysis showed that highly resistant strains persisted for 4 years, in the absence of further selection pressure, and that both resistant and susceptible strains were present 4 years after treatment (Table 1).

In a similar manner, the isolates from 2 controls were genotyped (Table 2). In control 1, at least 5 different strains were present at the start of the study. After 1 year, the composition had changed, and after 4 years, a new strain predominated in the flora. In control 2, we initially detected 3 different strains. These strains were also represented at each time point and predominated at 4 years. Thus, 1 control showed stable populations, whereas the other showed a dynamic state in the absence of treatment.

Discussion

Since antimicrobial drugs do not distinguish between pathogenic and colonizing bacteria, our indigenous biota is affected every time a drug is given (3,15). Resistance development in staphylococci that normally colonize the skin has previously been observed after antimicrobial prophylaxis or treatment (16–18). Depending on mechanism, resistance can be selected de novo, exist in the pretreatment biota, or be acquired, especially in hospital environments.

In this study of the effect of a 1-week course of clarithromycin on indigenous *S. epidermidis* populations, we show that macrolide-resistant *S. epidermidis* strains are selected during therapy and that, without further selection, resistant clones can persist for up to 4 years. This finding is important for several reasons. First, although *S. epidermidis* belongs to the normal cutaneous microbiota, it may be a pathogen, especially in hospitalized patients (8); stably resistant populations increase the risk for treatment failure. Second, resistance in the normal microbiota might contribute to increased resistance in higher-grade pathogens by interspecies genetic transfer. Since the population size of the normal microbiota is large, multiple and different resistant variants can develop, which increases the risk for spread to populations of pathogens. Persisting populations of resistant microbiota further enhance transfer risk, especially if the selecting agent is used for treatment. Third, antimicrobial drugs may affect the stability of residential populations.

Whether a resistant population persists is mostly determined by the fitness and transmission costs of resistance (19,20). Most resistance involves a cost (21–24), but resistance may occur without detectable cost (25). If most resistance is costly for bacteria, resistant populations should decline once the selective antimicrobial pressure is removed. However, mutations may arise that compensate for the fitness cost, restoring the bacteria's fitness without reversion of the resistant phenotype. This phenomenon, compensatory evolution, is considered to be relevant to stabilizing resistant populations (26). Other important mechanisms that could stabilize resistant populations are no-cost resistance mutations (25) and genetic linkage with adjacent genes. Despite substantially decreased sulfonamide use in the United Kingdom from 1991 to 1999,

Table 1. Characteristics of *Staphylococcus epidermidis* isolated from patients*†

Patient	Pretreatment‡			Posttreatment‡			1 year posttreatment‡			4 years posttreatment‡		
	Isolate	MIC	DNA	Isolate	MIC	DNA	Isolate	MIC	DNA	Isolate	MIC	DNA
1	1A:1	0.094	A	1B:1	0.094	F	1C:1	>256	H	1D:1	>256	H
	1A:2	0.094	B	1B:2	0.094	F	1C:2	>256	H	1D:2	0.094	M
	1A:3	0.125	B	1B:3	0.125	G	1C:3	32	K	1D:3	0.094	M
	1A:4	0.125	B	1B:4	0.094	F	1C:4	>256	H	1D:4	0.094	B
	1A:5	0.125	C	1B:5	>256	H	1C:5	>256	H	1D:5	>256	H
	1A:6	0.094	B	1B:6	0.094	J	1C:6	>256	H	1D:6	0.094	C
	1A:7	0.094	D	1B:7	0.094	F	1C:7	>256	H	1D:7	0.094	M
	1A:8	0.094	D	1B:8	0.125	B	1C:8	>256	L	1D:8	0.094	G
	1A:9	0.125	B	1B:9	>256	H	1C:9	>256	H	1D:9	>256	H
	1A:10	0.064	E	1B:10	0.094	G	1C:10	>256	H	1D:10	>256	H
2	2A:1	>256	N	2B:1	>256	S	2C:1	>256	N	2D:1	0.094	P
	2A:2	0.125	O	2B:2	>256	N	2C:2	>256	N	2D:2	0.094	T
	2A:3	0.125	O	2B:3	>256	N	2C:3	>256	N	2D:3	>256	N
	2A:4	>256	N	2B:4	>256	S	2C:4	>256	N	2D:4	>256	N
	2A:5	0.125	O	2B:5	>256	S	2C:5	>256	N	2D:5	0.125	O
	2A:6	0.094	P	2B:6	>256	N	2C:6	0.094	T	2D:6	0.125	P
	2A:7	0.19	Q	2B:7	96	S	2C:7	0.125	T	2D:7	0.094	T
	2A:8	0.125	R	2B:8	0.094	T	2C:8	>256	N	2D:8	0.125	P
	2A:9	0.094	P	2B:9	>256	N	2C:9	>256	N	2D:9	>256	N
	2A:10	0.094	P	2B:10	>256	S	2C:10	>256	N	2D:10	0.094	P

*Isolates from only 2 patients were DNA fingerprinted (shown); for MIC results for isolates from patients 3, 4, and 5, see the full version of this table online at http://www.cdc.gov/ncidod/EID/vol11no09/05-0124.htm#table1
†For each patient and time point, 10 independent isolations of *S. epidermidis* were performed.
‡Clarithromycin MIC measured by Etest. DNA fingerprinting by pulsed-field gel electrophoresis; different patterns are indicated as letters.

Table 2. Characteristics of *Staphylococcus epidermidis* isolated from controls*†

Control	Pretreatment‡			Posttreatment‡			1 year posttreatment‡			4 years posttreatment‡		
	Isolate	MIC	DNA	Isolate	MIC	DNA	Isolate	MIC	DNA	Isolate	MIC	DNA
1	1A:1	0.064	–	1B:1	0.064	–	1C:1	0.094	FF	1D:1	0.064	JJ
	1A:2	0.064	AA	1B:2	0.064	BB	1C:2	0.094	GG	1D:2	0.047	JJ
	1A:3	0.064	–	1B:3	0.094	–	1C:3	0.047	GG	1D:3	0.064	JJ
	1A:4	0.064	BB	1B:4	0.064	FF	1C:4	0.064	HH	1D:4	0.064	JJ
	1A:5	0.064	CC	1B:5	0.047	BB	1C:5	0.064	HH	1D:5	0.094	JJ
	1A:6	0.064	DD	1B:6	0.047	FF	1C:6	0.032	HH	1D:6	0.064	JJ
	1A:7	0.047	–	1B:7	0.064	–	1C:7	0.032	HH	1D:7	0.064	JJ
	1A:8	0.064	–	1B:8	0.064	FF	1C:8	0.047	HH	1D:8	0.064	JJ
	1A:9	0.047	EE	1B:9	0.064	FF	1C:9	0.047	HH	1D:9	0.064	JJ
	1A:10	0.064	CC	1B:10	0.047	–	1C:10	0.047	HH	1D:10	0.064	JJ
2	2A:1	0.094	KK	2B:1	0.094	KK	2C:1	0.094	LL	2D:1	0.094	KK
	2A:2	0.047	LL	2B:2	0.064	LL	2C:2	0.047	OO	2D:2	0.094	KK
	2A:3	0.094	LL	2B:3	0.064	–	2C:3	0.047	OO	2D:3	0.094	LL
	2A:4	0.047	KK	2B:4	0.064	LL	2C:4	0.047	OO	2D:4	0.094	MM
	2A:5	0.064	KK	2B:5	0.064	LL	2C:5	0.064	LL	2D:5	0.094	LL
	2A:6	0.064	MM	2B:6	0.064	LL	2C:6	0.064	PP	2D:6	0.094	KK
	2A:7	0.064	KK	2B:7	0.064	LL	2C:7	0.047	PP	2D:7	0.094	LL
	2A:8	0.094	LL	2B:8	0.047	KK	2C:8	0.047	PP	2D:8	0.125	KK
	2A:9	0.064	KK	2B:9	0.064	NN	2C:9	0.125	LL	2D:9	0.064	KK
	2A:10	0.094	KK	2B:10	0.032	NN	2C:10	0.047	OO	2D:10	0.094	KK

*Isolates from only 2 controls were DNA fingerprinted (shown); for MIC results for isolates from controls 3, 4, and 5, see the full version of this table online at http://www.cdc.gov/ncidod/EID/vol11no09/05-0124.htm#table2
†Time points are defined with respect to treatment, although the control group received no treatment. For each control and time point, 10 independent isolations of *S. epidermidis* were performed.
‡Clarithromycin MIC measured by Etest. DNA fingerprinting by pulsed-field gel electrophoresis; different patterns are indicated as letters. –, not determined.

Escherichia coli resistance to sulfonamides remained high (39.7% in 1991, 46.0% in 1999) because sulfonamide resistance was linked to other resistance genes that continued to be under selective pressure (27). In poultry, since *vanA* can be co-selected with *erm*(B) in *Enterococcus hirae* isolates (28), vancomycin resistance can be maintained by using macrolides, despite excluding avoparcin from animal feed. Thus, the stability and maintenance of antimicrobial drug resistance depends on the magnitude of selective pressure, compensatory evolution, no-cost associated resistance, and genetic linkage with co-selected resistance genes.

In our study, resistant isolates persisted long after drug treatment was completed. However, a variation in length of persistence between the patients was observed. Whether this variation is related to different costs associated with *erm*(C) carriage or different extents of genetic compensation for an initial cost cannot be concluded from current data. The observed variation in persistence of resistance could further be affected by the degree of recolonization and transient colonization of new strains during the 4-year study period. Although recolonization with *S. epidermidis* is presumably low, it can be enhanced by, for example, nosocomial spread during hospital stays (29). That indigenous *S. epidermidis* populations may naturally change in composition over time was reflected in the control group. According to the PFGE profiles from controls 1 and 2, populations of *S. epidermidis* can either remain stable for 4 years or show a more dynamic state, with new strains appearing over time. A change in the composition of the flora was also observed in control 5, in whom 5 resistant isolates appeared in the susceptible flora after 4 years. Since this patient did not receive any antimicrobial drugs during the study period, this finding is likely due to recolonization or transient colonization of a strain from the environment. However, most importantly, although a few resistant isolates were detected among the controls, no selection of resistant *S. epidermidis* occurred over the 4-year study period, as was observed in the treated patients.

In conclusion, antimicrobial drug treatment affects our indigenous microbiota and can give rise to long-term colonization with resistant populations. Our results show that as part of a combination therapy, a 7-day course of clarithromycin resulted in macrolide-resistant *S. epidermidis* that persisted up to 4 years without any further selection. In total, these observations suggest that selection of resistance in our microbiota after short antimicrobial drug courses may not be a rare phenomenon. However, the extent, to which other antimicrobial treatment regimens select for resistant *S. epidermidis* remains to be investigated.

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Simulated Anthrax Attacks and Syndromic Surveillance

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We measured sensitivity and timeliness of a syndromic surveillance system to detect bioterrorism events. A hypothetical anthrax release was modeled by using zip code population data, mall customer surveys, and membership information from HealthPartners Medical Group, which covers 9% of a metropolitan area population in Minnesota. For each infection level, 1,000 releases were simulated. Timing of increases in use of medical care was based on data from the Sverdlovsk, Russia, anthrax release. Cases from the simulated outbreak were added to actual respiratory visits recorded for those dates in HealthPartners Medical Group data. Analysis was done by using the space-time scan statistic. We evaluated the proportion of attacks detected at different attack rates and timeliness to detection. Timeliness and completeness of detection of events varied by rate of infection. First detection of events ranged from days 3 to 6. Similar modeling may be possible with other surveillance systems and should be a part of their evaluation.

Numerous syndromic surveillance systems are in place to detect potential bioterrorism events, and all of them have common components: a nonspecific indicator of disease available in near real time for a definable population, a means of generating the expected counts for each day of the year (accounting for day of week and seasonal variability), a detection algorithm, a defined threshold for action, and a system to investigate a signal. Assessing the sensitivity and timeliness of these systems has been difficult. Because of the lack of real events, modeling of hypothetical events is necessary to assess the performance of these systems. Although existing systems can be assessed by using naturally occurring illness events such as the beginning of the influenza season each year or gastrointestinal outbreaks, this assessment provides little information as to how well these systems will detect the release of a bioterrorism agent such as anthrax. Buehler et al. (1), Sosin and de Thomasis (2), and Reingold (3) have called for additional assessment of syndromic surveillance systems.

The existing literature on the ability of syndromic surveillance systems is sparse. Mandl et al. used a purely temporal approach with real background data and simulated spiked outbreaks (4). They described a variety of ways to design the spiked data, including specifically using data from the Russian anthrax release in 1979 (5). They describe 4 steps in detection: grouping data into syndromes; the modeling stage, in which historic data are studied to understand temporal trends; the detection stage, in which predictions based on the modeling are compared with observed data and deviations of data from expectations are used to set off alarms; and the threshold stage, in which the health department determines if the outbreak is worth investigation. Buckeridge et al. describe a complex model to produce a realistic space-time simulation of spiked outbreaks of anthrax superimposed on real background data (6). Their model of a simulated event has 4 stages: agent dispersal, infection, disease and behavior, and data source. Kulldorff et al. produced a testing data set with simulated space-time outbreaks and simulated background data (7). Within these data sets, positive predictive value, sensitivity, and specificity can be evaluated for a variety of testing systems that analyze both purely temporal and temporal-spatial aspects of outbreaks.

None of these efforts has used a functioning system to analyze how effective it would be at detecting a biological attack. This article presents the first attempt to evaluate the performance of an operational syndromic surveillance

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system to detect a bioterrorism attack in a quantitative and rigorous manner and provides a useful construct for other systems to follow. A comparison of our methods with those used in previous bioterrorism surveillance assessments is shown in Table 1.

Methods

This syndromic surveillance system is part of the National Syndromic Surveillance System (8,9). This current investigation is based on existing historic use data contributed by the HealthPartners Medical Group (HPMG). We assumed that anthrax spores were released into one of the air intakes of the Mall of America in Bloomington, Minnesota, and then dispersed by the ventilation system, which provided a uniform exposure throughout the mall. Modeled visits of patients with respiratory symptoms were produced by using 3 factors: demographic data from the mall, demographic data on HPMG patients, and data on time to symptom onset from the anthrax outbreak in Sverdlovsk (5). Historic data were used to add the number of respiratory cases that would be expected under ordinary conditions. Finally, the detection sensitivity and timeliness of the system were analyzed.

Syndromic Surveillance Data

HPMG provides medical care in 20 clinics to ≈250,000 patients, or ≈9% of the total population in the Minneapolis-St. Paul, Minnesota metropolitan area. It uses an electronic medical record that captures in real time nearly all physician visits and makes them accessible at the end of each day. Each evening, the data system is queried to obtain all visits for respiratory symptoms for that day. Respiratory symptoms were used to build the model for this simulation. One year of this dataset, from July 1, 2003, through June 30, 2004, was used to test this project. We had 2.5 years of earlier data, which allowed us to establish the number of visits that would ordinarily be expected.

Anthrax Simulation Model

The number and geographic distribution of patients with anthrax were modeled by using visitor data from the mall, the US Census bureau (http://www.census.gov/geo/www/gazetteer/places2k.html), and demographic data from HPMG. We purposely chose to model the first 3 stages of infection of Buckeridge et al. (6) (agent dispersion, infection, and disease and behavior) as a simple rate of visits for respiratory illness because those data were available from the Sverdlovsk outbreak, and they produced a simpler model. Keeping the model simple is initially important to allow the basic relationships between the variables to be understood. The rate of physician visits for respiratory symptoms ranged from 4% to 100% of the visitors to the mall that day. The specific rates for which models were run were as follows: 4%, 8%, 12%, 16%, 20%, 40%, 60%, and 100%.

The Sverdlovsk outbreak generated no physician visits on day 1; the number of visits increased until day 9 and then decreased. We created a cumulative distribution of the probability of a visit for respiratory symptoms each day from day 0 to day 30. We did not run the simulation beyond day 30 because detecting an outbreak with our system would not be beneficial at that point. To prevent a continuous signal pattern, we introduced variation by using a Poisson distribution consistent with the cumulative distribution. This distribution simulates the natural variation that would be expected in such scenarios.

We used the following approach. First, we created a cumulative distribution of the respiratory visits expected each day from 1 through 30. For example, the cumulative distribution was 0.0 for day 1, 0.01 (0.00 + 0.01) for day 2, 0.03 (0.00 + 0.01 + 0.02) for day 3, etc. Second, we assigned a random number from 0.0 to 1.0 from a uniform distribution as each randomly created day for the number of infections. Third, if the random number generated was between the minimum cumulative range for a day and the maximum cumulative range for a day, we then produced a new visit from the infection. All calculations were rounded down to the nearest integer. The effect is shown in Table 2, which shows how many visits occurred during the simulations from 1 zip code on day 6. Of the 1,000 simulations, no visits occur 123 times on day 6. Four times, however, 8 visits occur. Most of the time 1, 2, or 3 visits occur on day 6.

Each of the 1,000 simulations at the given infection rate was randomly assigned (with replacement) to an attack date. The additional cases were added to the historic data based on the date randomly chosen for each iteration, which created 1,000 new files.

The expected number of visits added for a specific release can be expressed as

$$\sum_{i=1}^{n} \frac{m_i \cdot h_i \cdot d}{pop_i}$$

where n = the number of zip codes in the outbreak, m_i = number of mall visitors in zip code i, h_i = number of

Table 1. Methods used in bioterrorism surveillance assessments

Study (Reference)	Epidemic data	Background data	Analysis	Surveillance system
Mandl et al. (4)	Simulated	Real	Temporal	Nonfunctional
Buckridge et al. (6)	Detailed simulation	None	Spatial-temporal	None
Kulldorff et al. (7)	Simulated	Simulated	Spatial-temporal	Nonfunctional
Nordin et al. (this study)	Simulated	Real	Spatial-temporal	Functioning system

Table 2. Visit distribution of 1,000 simulations at a 40% infection rate for day 6 from infection for zip code 55125 (St. Paul, Minnesota)

No. visits on day 6	Frequency
0	123
1	258
2	274
3	191
4	97
5	41
6	5
7	7
8	4

HealthPartners patients in zip code *i*, *d* = infection rate of the simulation, and pop_i = population of zip code *i*. The actual number of added cases was random and followed Poisson distribution centered on the expected count.

This equation allows us to vary the infection rate of the attack and the number of patients from the zip code. With a limited number of simulations, this variation allows us to assess the effect of an attack with a variety of infection rates and rates of HPMG's penetration in the community and the zip code.

Release Detection Method

The Poisson-based prospective space-time scan statistic was used to detect releases (10). This method uses a large number of overlapping cylinders in which the height of the cylinder represents time and the circular base represents space in such a way that all zip code areas whose centroid (the population-weighted geographic center of the area) is within the circle are included in the cylinder. Each cylinder represents a candidate area and duration for a true disease outbreak, and the method adjusts for the multiple testing inherent in the many cylinders evaluated. We evaluated all cylinders for which the circle center was identical to the centroid of one of the zip code areas; for each circle center the maximum radius of the circle was set so that ≤50% of the at-risk population was contained in the zip codes included within the circle, and the height of the cylinder was set to be ≤3 days. Purely temporal cylinders, including all zip code areas and either 1, 2, or 3 days, were also evaluated. The method needs expected counts for each day and zip code, and these were determined on the basis of historical data from January 1, 2001, to June 30, 2003, by using a generalized linear mixed modeling approach that accounts for natural seasonal and weekly variation in the data (11). Analyses were performed by using the freely available SaTScan software (www.satscan.org).

A signal is detected when the number of episodes of respiratory illness is substantially greater than expected. The rarity of an outbreak signal is measured as a recurrence interval, which is defined as the expected number of days of surveillance needed for a signal of at least the observed magnitude to occur, in the absence of any true outbreaks, and it is the inverse of the nominal p value from the space-time scan statistic. Thus, the larger the recurrence interval, the more unusual the outbreak signal. We present results that use recurrence intervals of 3 months (p = 0.011) and 2 years (p = 0.0013). At the 3-month recurrence interval level, ≈4 positive signals will occur per year by chance, whereas at the 2-year level, only 1 positive signal expected by chance will occur every 2 years.

For each simulated attack, the spiked data were analyzed for each of the 10 days after the attack. We then report the proportion of all attacks that generated an outbreak signal on or before each of days 2 to 10.

Results

Timeliness and completeness of detection of events varied by rate of infection and by percentage of population covered by HPMG. Initial models were done at the current 9% population coverage. At a 40% infection rate and a recurrence interval of 3 months (p = 0.011), the first events (outbreaks) were detected on day 2, one fourth by day 6, three fourths by day 7, and all 1,000 by day 8. With higher percentages of infections, all events were detected earlier. At an infection rate <40%, not all events were detected. At a 20% infection rate, 845 of 1,000 events were detected at a 2-year recurrence interval, and 926 of 1,000 events were detected at a 3-month recurrence interval (both peaking at day 8). The number of events detected decreases proportionately as the infection rate decreases from 20% to 4%. At a 4% infection rate little is detected; 7 events are detected at a 2-year recurrence interval, and 57 events are detected at a 3-month recurrence interval.

At a 16% infection rate, more events were detected during the summer than during the winter, with an intermediate number of events detected in the fall and spring (Table 3). The day of release also affected the number of events detected. When the number of cases peaked on Saturday or Sunday, more events were detected (Table 4). The number of days until detection peaked increased as the rate of infection decreased.

At infection rates >16%, the relationship of detection to season was weaker because all events were detected. However, events are detected more rapidly in the summer when respiratory conditions are less common. The relationship of the day of the week with release is more

Table 3. Number of releases detected by season at a 16% infection rate

Season of release	No. releases	No. detected (%)
Winter	248	131 (52.8)
Spring	276	204 (73.9)
Summer	189	165 (87.3)
Fall	287	196 (68.3)

Table 4. Number of releases detected by day of week at a 16% infection rate*

Day of release	No. releases	No. detected (%)
Sunday	146	146 (100.0)
Monday	132	64 (48.5)
Tuesday	142	50 (35.2)
Wednesday	142	63 (44.3)
Thursday	156	100 (64.1)
Friday	143	134 (93.7)
Saturday	139	139 (100.0)

*Most outbreaks at this level are detected 7 or 8 days later. Thus, for outbreaks starting on Saturday or Sunday, more are detected during the next weekend.

complex. As the infection rates increase to >40%, the number of days until all events are detected gets smaller, and the highest rate of detection occurs closest to the time of release. Events were best detected when the highest rates of visits occurred on the weekend.

The percentage of the population in the area covered by the surveillance system directly affects the smallest size of outbreaks that can be reliably detected and the timeliness of detection of those outbreaks. We also computed system characteristics if 36% of the population (4 times as much) were covered by the surveillance system. In this situation, at a recurrence interval of 3 months ($p = 0.011$) and an infection rate of 50%, all events were detected by day 3 and most by day 2. At a 10% infection rate, the first events were detected on day 2, one fourth by day 6, three fourths by day 7, and all 1,000 events by day 8.

The extremes of sensitivity and timeliness are shown in Figures 1 and 2. The first extreme, with a high threshold and 9% of the population, shows the lowest sensitivity, while the second extreme, with a low threshold and 36% of the population, shows high sensitivity. Most configurations would fall somewhere between these 2 extremes.

Discussion

This study has several limitations. Simplifying assumptions was crucial to the construction of this simulation and are both its strength and weakness. We assumed that most patients would have respiratory symptoms. The first 3 stages of the model of Buckeridge et al. (6) are merged. The distribution of intervals from exposure to initial observance of disease we used was based on incubation periods determined from the Sverdlovsk outbreak in Russia (2).

The inadvertent release of anthrax spores from a military microbiology facility in Sverdlovsk, Russia, in 1979 is the largest documented epidemic of inhalational anthrax and clearly demonstrates the potential for *Bacillus anthracis* to be used as a weapon. In Sverdlovsk, the spores were likely released on a single day. The incubation period of anthrax in this outbreak ranged from 2 to 3 days to slightly more than 6 weeks; the modal incubation period was 9–10 days. Other investigators have used the Sverdlovsk data to compute a median incubation period of 11 days (12).

Some patients will likely have initial symptoms only a few days after the exposure, and their conditions diagnosed a few days later. Thus, a suspicious clinician may detect the first case of anthrax before the surveillance system sounds an alarm and public health determines it is an anthrax release. However, even if the first alarm is sounded by a clinician, these additional data will help define what is happening and plan a response. This simulation exercise could be applied to a surveillance system in any metropolitan area where gathering place is different enough from residence so that exposed persons would live far from each other.

The sensitivity of such a system in detecting small releases of anthrax depends on the proportion of the population covered by the system. The greater the proportion of the population covered by such a system, the more sensitive it is. According to this model, a system that includes 36% of the population in the area would detect most events in which >5% of mall shoppers were affected.

Outdoor releases, similar to the outbreak in Sverdlovsk, have been modeled previously. These models produce marked geographic clustering, with some spread from persons who pass through the area. In a large regional shopping center that draws people from large areas, detection is more difficult. Because infected persons live far from each other, a larger number of cases were needed to detect the outbreak in our model than in earlier models of outdoor releases.

The relationship between the days of the week and detection of events is complex. Fewer patients visit the clinic on weekends since only 4 urgent-care clinics are open instead of the usual 20. As the rate of infection increases, the maximum number of events detected occurs more quickly. When the maximum number of events

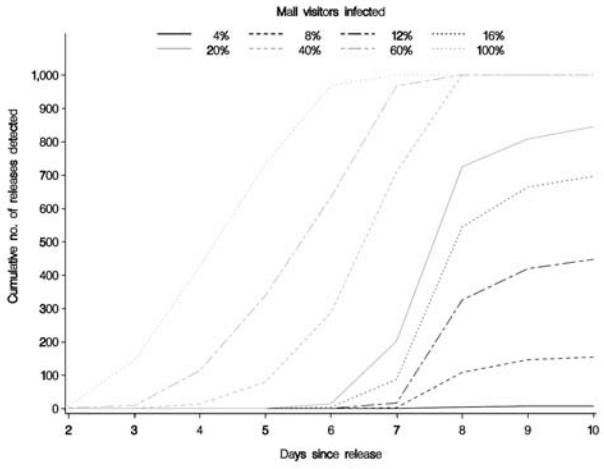

Figure 1. Cumulative number of releases detected in a recurrence interval of 2 years ($p = 0.0013$) with 9% of the population covered.

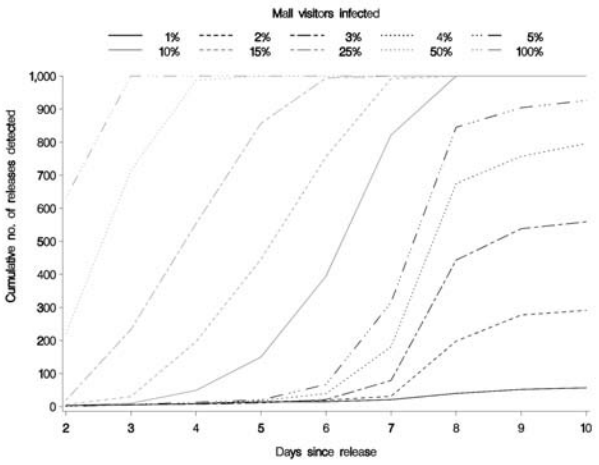

Figure 2. Cumulative number of releases detected in a recurrence interval of 3 months (p = 0.011) with 36% of the population covered.

detected occurs on weekends, the system is more sensitive. Thus, as the rate of infection increases, the day of the week for the release with the most sensitivity shifts closer to the weekend.

Simulation modeling is necessary to test and prepare syndromic surveillance systems. Although more complex simulation modeling can be done, it requires more assumptions and may be more sensitive to error because of the additional risk of false assumptions.

Conclusions

This article reports the evaluation of an operational bioterrorism surveillance system. This analysis allows an understanding of limitations of the system and characteristics unique to the region the surveillance system is monitoring. The HPMG bioterrorism surveillance system, which receives data for ≈9% of the population in the area, can detect an anthrax release in the Mall of America most of the time if 20% of the persons at the mall at the time of release are infected and all of the time at a 40% infection rate. The time to detection gets progressively shorter as the infection rate increases >40%. Modeling with 36% population coverage showed that such a system would be capable of detecting a release at a 5% infection rate most of the time and at a 10% infection rate all the time. Similar modeling may be possible with other surveillance systems and should be used as a part of their evaluation.

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West Nile Virus–infected Mosquitoes, Louisiana, 2002

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Human cases of West Nile virus (WNV) disease appeared in St. Tammany and Tangipahoa Parishes in southeastern Louisiana in June 2002. Cases peaked during July, then rapidly declined. We conducted mosquito collections from August 3 to August 15 at residences of patients with confirmed and suspected WNV disease to estimate species composition, relative abundance, and WNV infection rates. A total of 31,215 mosquitoes representing 25 species were collected by using primarily gravid traps and CO_2-baited light traps. Mosquitoes containing WNV RNA were obtained from 5 of 11 confirmed case sites and from 1 of 3 sites with non-WNV disease. WNV RNA was detected in 9 mosquito pools, including 7 *Culex quinquefasciatus*, 1 *Cx. salinarius*, and 1 *Coquillettidia perturbans*. Mosquito infection rates among sites ranged from 0.8/1,000 to 10.9/1,000. Results suggest that *Cx. quinquefasciatus* was the primary epizootic/epidemic vector, with other species possibly playing a secondary role.

Since the first appearance of West Nile virus (WNV) (family *Flaviviridae*: genus *Flavivirus*) in the Western Hemisphere in 1999 (1), the virus has spread rapidly south and west from its initial focus in the New York City metropolitan area. By the end of 2001, WNV-infected mosquitoes, birds, horses, or humans had been reported from 27 states, and human cases of WNV disease occurred as far south as southern Florida and as far west as Arkansas and Louisiana (2,3).

In the northeastern United States, the primary epizootic/epidemic vector of WNV is *Culex pipiens*, a species that feeds primarily on birds (4–6). Other potentially important vector species, based on frequency of isolations of WNV or laboratory vector competence studies, include *Cx. restuans* and *Cx. salinarius* (7,8). WNV has been isolated from an additional 57 species, but their status as vectors is unknown (Centers for Disease Control and Prevention [CDC], http://www.cdc.gov/ncidod/dvbid/westnile/MosquitoSpecies.htm). In the southern United States, WNV was isolated from *Cx. quinquefasciatus*, *Cx. salinarius*, and *Cx. nigripalpus* in Florida and Georgia (9), *Cx. nigripalpus* in northern Florida (10), and from *Anopheles atropos*, *Deinocerites cancer*, and *Aedes taeniorhynchus* in the Florida Keys (11). However, the role these species play in epidemics of WNV disease in the southern states has not been determined. *Ae. albopictus* is common in urban, suburban, and rural residential settings throughout the southern states and is a competent laboratory vector of WNV (12,13). Although the virus has been isolated from *Ae. albopictus* in the Northeast (14), this species' importance in transmission of WNV to humans is unknown.

During May and June 2002, WNV infection was identified in chickens, horses, dead wild birds, and in pools of *Cx. quinquefasciatus* mosquitoes from St. Tammany Parish, on the north shore of Lake Pontchartrain in southeastern Louisiana (15). Human cases of WNV neuroinvasive disease began to appear in late June, and 27 cases were reported by the end of July. Intense local WNV transmission was indicated by the St. Tammany Parish Mosquito Abatement District's surveillance program, which detected WNV immunoglobulin (Ig)M antibody in 17% of their sentinel chickens and WNV antigen from 11 mosquito pools by the end of July (15). The human cases tended to cluster in 2 areas of St. Tammany Parish, Slidell and the Covington-Mandeville area. In neighboring Tangipahoa Parish, human cases were also being reported, with most clustering in the Hammond-Pontchatula area (Louisiana Department of Health and Hospitals, unpub. data).

The recognition of a growing outbreak of WNV disease in humans provided an opportunity to describe the transmission dynamics of WNV in locally occurring mosquitoes during epidemic transmission and to compare

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these dynamics to patterns seen in the northeastern states (4–6). Accordingly, we conducted an entomologic survey in St. Tammany and Tangipahoa Parishes during August 2002. The specific aims of the survey were to document species composition, relative abundance, and WNV infection rates in mosquitoes at residences of patients with confirmed cases and at residences of patients with suspected cases of WNV fever, the most likely locations where transmission to humans occurred. We were particularly interested in attempting to ascertain the importance of *Cx. quinquefasciatus* and *Ae. albopictus* as vectors of WNV in this epidemic.

Materials and Methods

Study Sites and Specimen Collection

Mosquitoes were collected in St. Tammany and Tangipahoa Parishes from August 3 to August 15, 2002. Two study sites were selected in each parish (denoted as St. Tammany A and B, and Tangipahoa A and B). These sites were located at or near residences of patients with confirmed cases of WNV neuroinvasive disease. As suspected cases of WNV fever (persons reporting as outpatients with undifferentiated febrile illness with headache) were identified, collections were made at the residences of these patients.

Mosquitoes were collected primarily by using CDC miniature light traps baited with dry ice to collect host-seeking females, Reiter gravid traps (16) to collect females seeking a location to deposit eggs, and ovitraps to collect eggs from container-breeding mosquitoes. Both light and gravid traps at the 4 initial study sites were operated for 24 h/day in an attempt to maximize the collection of *Ae. albopictus*, a daytime feeder. Some additional collections were made by using Fay-Prince traps and duplex cone traps and by aspirating resting adult mosquitoes from the outside of residences or other structures. Collections were transferred to 2.0-mL cryovials and frozen on dry ice until returned to the CDC laboratory in Fort Collins, Colorado, where they were stored at –80°C. Mosquito eggs collected in ovitraps were hatched in the insectary, reared to adulthood, held for 48 h at 27°C and 80% relative humidity, then identified and processed for virus testing as described below.

Mosquito Processing and Testing

Mosquitoes were identified to species on a refrigerated chill table. Pools of ≤50 specimens sorted by species and collection site and date were triturated in 1.75 mL of diluent by using a Mixer Mill apparatus (Qiagen Inc., Valencia, CA, USA) and centrifuged (17). Supernatants from the mosquito suspensions were tested for the presence of WNV RNA by TaqMan reverse transcription–polymerase chain reaction (RT-PCR), and positive pools were retested by using a different primer set to confirm the presence of WNV RNA (18). Mosquito infection rates were determined by calculating the maximum likelihood estimate (MLE) with 95% confidence intervals (19).

Results

Mosquito Collections

Collections were made at 14 sites, 12 in St. Tammany Parish and 2 in Tangipahoa Parish. Residences of WNV neuroinvasive disease or fever case-patients are denoted by upper case letters. Non-case-patient residences are denoted by italicized lower case letters. Eight St. Tammany sites (A, C, D, E, F, *g*, I, J) were in or near the city of Slidell in the southeast corner of the parish, St. Tammany site B was located in Abita Springs, east of Covington, and 3 sites (K, *l*, *m*) were in Pearl River in the east-central region of the parish. The 2 Tangipahoa parish sites (A, B) were on the northwest and northern outskirts of Ponchatoula.

Trapping effort at each site and elapsed time between onset of illness and mosquito collection are shown in Table 1. Although traps were run for 24 h/day at some sites, only mosquitoes collected overnight are used to calculate mosquitoes per trap night. The earliest date of onset was June 21, and the latest date of onset was August 4. Mosquito collection dates ranged from 8 to 50 days after onset of illness. Trapping effort per site ranged from 2 to 60 trap nights for light trap collections, and from 2 to 59 trap nights for gravid trap collections. No notable changes in the weather occurred during the collection period that might bias comparisons of mosquito abundance.

A total of 31,215 mosquitoes were collected during the trapping period of August 3 to August 15 (Table 2). *Cx. erraticus* was the most commonly collected species, accounting for 28% of the total collected. *Cx. quinquefasciatus*, *Ae. albopictus*, *Coquillettidia perturbans*, and *Cx. salinarius* were other commonly collected species. Ovitraps yielded 335 *Ae. albopictus* and 778 *Ae. triseriatus/hendersoni* reared to adults. Aspirator collections yielded 658 mosquitoes of 16 species, of which 474 were *Ae. albopictus*. Cone traps collected 33 mosquitoes (9 species) and Fay-Prince traps yielded 214 mosquitoes (15 species). Mosquitoes were sorted into 2,471 pools for processing and virus testing.

Relative population densities (light trap or gravid trap counts per trap night) of the species in which we detected WNV RNA, and of *Ae. albopictus*, were calculated for case and non-case sites (Table 3). For most species, light trap counts per night greatly exceeded gravid trap counts. For *Cx. quinquefasciatus*, however, gravid trap counts were 7–58 times greater than were light trap collection

Table 1. Trapping effort at West Nile virus (WNV) case-patient and non–case-patient residences, August 3–15, 2002

Parish	Site*	Case onset date	Collection dates	No. trap nights†		Other methods‡
				Light	Gravid	
St. Tammany	A	Jul 11	Aug 3–6	55	59	
	B	Jul 13	Aug 3–6	59	51	
	C	Jul 24	Aug 12–15	12	12	A
	D	Jul 29	Aug 12–15	8	8	A
	E	Aug 2	Aug 12–15	11	16	4 F, 4 D, A
	F	Aug 4	Aug 12–15	19	21	
	I	Jul 28	Aug 12–15	12	12	A
	J	Aug 4	Aug 12–15	3	3	
	K	Jul 29	Aug 13–15	6	6	
	g	Not given	Aug 13–15	9	15	3 F
	l	Not given	Aug 14–15	2	2	
	m	Not given	Aug 14–15	4	4	
Tangipahoa	A	Jul 11	Aug 7–10	54	59	
	B	Jun 21	Aug 7–10	60	48	

*Capital letters denote confirmed WNV neuroinvasive disease and WNV fever case sites; italicized lower case letters denote non-WNV case sites.
†Only night collections used for trap night calculations.
‡A, aspiration outside buildings; F, Fay-Prince trap; D, duplex cone. Numbers denote trap nights; aspiration times not recorded.

counts. Neither gravid traps nor light traps collected large numbers of *Ae. albopictus*. Light trap counts per trap night for *Ae. albopictus* were approximately the same as gravid trap counts except at site *l* where 35.5 mosquitoes were collected per gravid trap night compared to 4.5 per light trap night.

No relationship was shown between the population densities of the species examined and whether the site was a case-patient or non–case-patient residence, except for *Cx. quinquefasciatus*, for which much higher densities were found at sites of non-case-patients. *Cx. quinquefasciatus* gravid trap counts per trap night ranged from 0.4 to 44.1 for confirmed WNV disease case-patient residence sites, and 59.6 to 142.8 for non–case-patient sites ($p<0.001$, Wilcoxon rank sum test).

WNV Detection

WNV RNA was detected in 9 mosquito pools by TaqMan RT-PCR (Table 4). Five viral RNA positive pools were from St. Tammany Parish and 4 were from Tangipahoa. Seven of the positive pools contained *Cx. quinquefasciatus*; 4 of these were from St. Tammany Parish, and 3 were from Tangipahoa. The other 2 positive pools consisted of a pool of *Cx. salinarius* from St. Tammany and a pool of *Cq. perturbans* from Tangipahoa. All of the WNV-positive *Cx. quinquefasciatus* were

Table 2. Mosquito species collected in St. Tammany and Tangipahoa Parishes, Louisiana, August 3–15, 2002

Species	No. of pools	No. of mosquitoes			
		Light traps	Gravid traps	Other methods*	Total (%)
Culex erraticus	310	8,319	411	3	8,733 (27)
Cx. quinquefasciatus	311	539	6,326	98	6,963 (22)
Aedes albopictus	321	1,007	1,457	860	3,324 (11)
Coquillettidia perturbans	107	2,159	114	0	2,273 (7)
Cx. salinarius	144	1,809	155	49	2,013 (7)
Culex species	171	389	1,318	37	1,744 (6)
Ae. triseriatus/hendersoni†	159	198	86	782	1,066 (3)
Psorophora ferox	117	909	27	37	973 (3)
Ps. howardii	112	680	2	4	686 (2)
Uranotaenia sapphirina	59	631	48	0	679 (2)
Ae. vexans	83	500	18	24	542 (2)
Ae. infirmatus	90	465	7	17	489 (2)
Ae. atlanticus/tormentor†	78	371	28	37	436 (1)
Ae. taeniorhynchus	41	231	12	46	289 (<1)
Aedes species	59	198	15	17	230 (<1)
Ps. columbiae	54	188	4	4	196 (<1)
Anopheles crucians complex	66	184	4	3	191 (<1)
10 other species	193	317	65	6	388 (1)
Total	2,471	19,094	10,097	2,024	31,215 (100)

*Other methods: mechanical aspirator, duplex cone trap, Fay-Prince trap, oviposition trap.
†Not identified to species.

Table 3. Population densities of selected mosquito species at West Nile virus (WNV) case-patient and non–case-patient residences*

	No. of mosquitoes collected per trap night (LT/GT)†			
	Culex quinquefasciatus	*Cx. salinarius*	*Aedes albopictus*	*Coquillettidia perturbans*
St. Tammany				
A	1.7/21.7	1.6/0.05	1.8/1.2	0.2/0
B	0.5/3.7	1.5/0.1	0.9/1.7	0/0
C	0.3/17.4	2.6/0	1.5/1.0	0/0
D	0.6/18.1	1.0/0	1.6/2.8	0/0
E	3.7/44.1	5.4/0.06	2.2/3.0	0/0
F	1.0/19.4	5.0/0	2.8/2.1	0/0
I	1.3/15.7	3.2/0	2.4/2.9	0/0
J	2.3/39.3	6.7/0.3	2.3/0.3	0/0
K	1.5/12.2	1.0/0	9.8/11.2	0.2/0
g	4.4/59.6	6.7/0.07	4.1/3.6	0/0
l	6.5/105.0	0.5/0	4.5/35.5	0.5/0
m	7.0/142.8	0/0	0.8/0.3	0/0
Tangipahoa				
A	0.02/0.4	6.4/1.0	0.7/1.9	17.7/1.6
B	3.2/15.1	8.0/1.2	5.2/8.5	16.2/0.3

*Capital letters denote confirmed WNV neuroinvasive disease and WNV fever case sites; italicized lower case letters denote non-WNV case sites.
†LT, light trap; GT, gravid trap; only night collections used for trap night calculations.

collected in gravid traps, while the positive *Cx. salinarius* and *Cq. perturbans* were collected in light traps. No virus was detected in mosquitoes collected by the other methods. WNV infection rates ranged from 0.81/1,000 to 10.91/1,000 by MLE (Table 4). The highest infection rate was seen in *Cx. salinarius* and the lowest in *Cq. perturbans*. Infection rates in *Cx. quinquefasciatus* were similar among sites (2.31/1,000–5.64/1,000).

No relationship was found between the relative densities of mosquitoes collected and the finding of WNV-infected mosquitoes (Tables 3 and 4). Three infected pools of *Cx. quinquefasciatus* were collected from Tangipahoa site B**,** with 15.1 mosquitoes per gravid trap night, whereas no infected pools were collected from St. Tammany site *m*, which had the highest *Cx. quinquefasciatus* count per gravid trap night (142.8). Likewise, the only WNV-infected *Cx. salinarius* pool was from St. Tammany site B, which had 1.6 mosquitoes per light trap night, 1 of the lower density sites for that species. Eight other sites had higher light trap counts but no WNV-positive mosquitoes were detected. *Cq. perturbans* was found in high densities at only Tangipahoa sites A and B, and the densities at these sites were similar at 17.7 and 16.2 per light trap night, respectively. Infected *Cq. perturbans* were found only at Tangipahoa site A.

Detection of WNV-infected mosquitoes was not influenced by elapsed time between dates of onset of illness (a surrogate for date of infection) and mosquito collection dates. We obtained 3 isolates from Tangipahoa site B, where the date of onset was 47–50 days before mosquito collection (Tables 1 and 4).

Discussion

The results of our survey indicate that the natural history of WNV in the southern United States is similar to that seen in the northern states, where *Cx.* mosquitoes, especially *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*, are thought to be the species primarily involved in enzootic, epizootic, and epidemic transmission (3–6). Seven of 9 (78%) WNV-infected mosquito pools were *Cx. quinquefasciatus*. Both *Cx. pipiens* and *Cx. quinquefasciatus* are primarily ornithophilic, although some studies indicate that *Cx. quinquefasciatus* feeds more readily on mammals (20–22). One of the 2 other positive pools was of *Cx. salinarius*, which feeds primarily on mammals (20–22). WNV has been isolated frequently from this species (5,6,23), and

Table 4. Estimated mosquito pool West Nile virus (WNV) infection rates per 1,000 mosquitoes and 95% confidence intervals (CIs)*

Parish	Site†	Sampling period	Trap type‡	WNV+ pools	Species	No. mosquitoes tested	Infection rate: MLE (95% CI)
St. Tammany	B	Aug 3–6	Light	1	*Cx. salinarius*	92	10.91 (5.46–21.83)
	E	Aug 12–15	Gravid	2	*Cx. quinquefasciatus*	829	2.61 (1.31–5.22)
	F	Aug 12–15	Gravid	1	*Cx. quinquefasciatus*	427	2.31 (1.16–4.62)
	l	Aug 14–15	Gravid	1	*Cx. quinquefasciatus*	223	5.64 (2.82–11.28)
Tangipahoa	A	Aug 7–10	Light	1	*Cq. perturbans*	1,223	0.81 (0.41–1.62)
	B	Aug 7–10	Gravid	3	*Cx. quinquefasciatus*	922	3.41 (1.71–6.82)

*Calculated by using a bias-corrected maximum likelihood estimate (MLE).
†Capital letters denote confirmed WNV neuroinvasive disease and WNV fever case sites; italicized lower case letters denote non-WNV case sites.
‡Light denotes CO_2-baited CDC miniature light trap; gravid denotes Reiter gravid trap.

laboratory studies indicate that it is a competent vector (8). *Cx. salinarius* has been associated with an outbreak of human WNV illness in New York City (6) and appears likely to be important in transmitting WNV to humans and domestic mammals in the southern United States as well. The other positive pool was of *Cq. perturbans*. WNV isolates previously have been obtained from this species, but it is an inefficient vector in the laboratory (8).

Eight mosquito pools containing WNV RNA were collected at 5 (45%) of 11 confirmed WNV case-patient residences, while the remaining pool was from 1 (33%) of 3 non–case-patient sites. This finding suggests that many, perhaps most, human infections are acquired near their residences.

Although substantial numbers of *Ae. albopictus* were tested, no virus was detected in this competent laboratory vector of WNV. This finding was perhaps due to the blood-feeding habits of this species. Two studies of engorged specimens wild caught in the continental United States found that 1% and 17% of blood meals were taken from birds (24,25). The remaining meals were from a variety of mammals, including humans. In our study area, relatively few blood meals may have been taken from birds, thus reducing the exposure of host-seeking *Ae. albopictus* to the high-titered levels of WNV viremia seen in many species of birds. Little data have been published on WNV viremia levels in mammals, but in horses, dogs, and cats, viremia levels are transient, of low titers, or both (12,26). If this condition is also the case for other mammalian species, then most blood meals taken by *Ae. albopictus* from WNV-infected hosts would be below the threshold titer necessary to initiate infection.

In our study, gravid traps were clearly preferable to light traps as an effective surveillance tool for detecting WNV RNA in mosquitoes. All the positive *Cx. quinquefasciatus* pools and 91% of total *Cx. quinquefasciatus* were from gravid traps. The other 2 WNV-positive pools were from mosquitoes collected in light traps. Gravid traps were a more effective means of collecting *Ae. albopictus* than were light traps. Unlike *Cx. quinquefasciatus*, most female *Ae. albopictus* collected in gravid traps were not gravid, and numerous males were also collected. *Ae. albopictus* were also readily collected by aspiration and ovitrapping.

Although active transmission of WNV was still occurring at the time of our collection efforts during the first half of August, most human patients had dates of onset between late June and late July. Thus, the relative numbers and species composition we observed may not have been representative of the situation when most human infections were occurring. Mosquito control activities intensified in St. Tammany Parish in response to the high level of WNV activity (15). Mosquito surveillance by the parish showed large reductions in total mosquito counts and in *Cx. quinquefasciatus* counts in CDC light traps and in New Jersey light traps from May to August. Eleven WNV antigen-positive mosquito pools were detected, all in June and July. Ten of these positive pools were of *Cx. quinquefasciatus*, and 1 was of *Cx. salinarius*, similar to our findings in August. Notably, the number of sentinel chickens developing WNV IgM antibody peaked during the third week of July, declined during early August, then rose again during late August (15). This finding suggests that exposure of sentinel chickens to infected mosquitoes was ongoing, and perhaps increasing, during the period of our study. Serologic conversions in sentinel chickens continued to be detected into November. Serologic studies of wild birds caught in mist nets in St. Tammany Parish were conducted in August, and again in October (27). These data indicated that enzootic WNV transmission continued to occur in the parish, although likely at a reduced level, after human cases were no longer being reported. Long-term studies are needed to monitor the transmission dynamics of WNV in mosquito populations during epidemic and nonepidemic years.

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Legionellosis from *Legionella pneumophila* Serogroup 13

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We describe 4 cases of *Legionella pneumophila* serogroup 13–associated pneumonia. These cases originate from a broad geographic range that includes Scotland, Australia, and New Zealand. *L. pneumophila* serogroup 13 pneumonia has a clinically diverse spectrum that ranges from relatively mild, community-acquired pneumonia to potentially fatal severe pneumonia with multisystem organ failure. All cases were confirmed by culture and direct fluorescent antibody staining or indirect immunofluorescent antibody tests. Proven or putative sources of *L. pneumophila* serogroup 13 infections in 2 patients included a contaminated whirlpool spa filter and river water. An environmental source was not found in the remaining 2 cases; environmental cultures yielded only other *L. pneumophila* serogroups or nonpneumophila *Legionella* species. We describe the clinical and laboratory features of *L. pneumophila* serogroup 13 infections. *L. pneumophila* serogroup 13 pneumonia is rarely reported, but it may be an underrecognized pathogenic serogroup of *L. pneumophila*.

Legionella species are relatively common causes of pneumonia (1). *Legionella pneumophila* is the most common pathogenic species, with 15 serogroups described. *L. pneumophila* serogroup 1 accounts for most culture-confirmed cases of legionellosis; non–serogroup 1 *L. pneumophila* causes only ≈7% of legionellosis cases (2). Legionellae are widely distributed in the environment, including in lakes, rivers, creeks (1,3), and artificial aquatic habitats such as potable-water supplies (1,3); amebae are the natural hosts in the environment (4).

L. pneumophila serogroup 13 was first described as a new pathogenic serogroup of *L. pneumophila* in 1987 (5), but a detailed clinical account of these cases was not included in the initial report. *L. pneumophila* serogroup 13 is rarely reported in humans; it accounted for 2 (0.4%) of 508 isolates from a recent international survey of culture-confirmed legionellosis (2). Furthermore, a recent European study of 1,335 unrelated clinical isolates yielded only 2 isolates of *L. pneumophila* serogroup 13 (6). We are unaware of published reports of *L. pneumophila* serogroup 13 infections in which the epidemiology and the clinical spectrum are outlined. We therefore report 4 cases of *L. pneumophila* serogroup 13 infections to describe in detail these aspects of this uncommon human pathogen.

The Cases

Case 1

A 27-year-old woman was admitted to an intensive care unit in Glasgow, Scotland, after she had nearly drowned in estuarine water. On admission, her vital signs were as follows: core body temperature 32.8°C, pulse 92 beats per min, blood pressure 90/65 mm Hg, respiratory rate 28 breaths per min, and Glasgow Coma Scale score 13/15. Arterial blood gas analysis on 100% oxygen showed partial arterial oxygen pressure (PaO_2) 46 mm Hg (reference range [RR] >90 mm Hg), $PaCO_2$ 48 mm Hg (RR 25–35 mm Hg), and bicarbonate 16.2 mmol/L (RR 24–30 mmol/L). Her blood biochemistry showed hyponatremia and hypokalemia. Chest radiograph showed bilateral consolidation. She was intubated, given ventilatory assistance, and actively rewarmed; inotropes were administered, and empiric intravenous cefotaxime and metronidazole were given. With the patient's further clinical deterioration, intravenous vancomycin replaced metronidazole on day 6.

On day 7, high-dose methylprednisolone was administered for worsening respiratory function and chest

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radiographic evidence of acute respiratory distress syndrome. Given the patient's poor clinical response, on day 14, intravenous clarithromycin and gentamicin were given, with intravenous ciprofloxacin added 24 hours later. Acute renal failure required hemofiltration, and respiratory function deteriorated further on day 15, despite prone ventilation and nitric oxide therapy. On day 18, she died of refractory hypoxemia and multisystem organ failure. A tracheal aspirate collected on day 14 plus postmortem lung tissue samples subsequently yielded colonies of a *Legionella* species on buffered-charcoal yeast extract (BCYE) agar (Oxoid Ltd., Basingstoke, England). *L. pneumophila* serogroup 13 infection was diagnosed by *mip* gene sequencing of the isolate, which demonstrated 99% homology with the type strain of *L. pneumophila* serogroup 13 (GenBank accession no. AF022327). Seroconversion was demonstrated retrospectively by indirect immunofluorescent antibody (IFA) against monoclonal *L. pneumophila* serogroup 13 antisera, with a titer rise from <1:32 on admission to 1:512 on day 12. Domestic or nosocomial sources of legionellosis were not sought. Nevertheless, several months later, estuarine water samples were taken near the site of immersion; cultures were negative for *Legionella* spp.

Case 2

A 51-year-old man who was undergoing induction chemotherapy for acute myeloid leukemia at Royal Perth Hospital, Australia, was initially given regular and extended periods of home leave in the first week after chemotherapy. However, on day 12 neutropenic typhlitis developed, and a right hemicolectomy was performed. This procedure was followed the next day by the onset of dyspnea, hypoxemia, nonproductive cough, and persistent fever. Chest radiograph showed left lower lobe consolidation, and meropenem with teicoplanin was administered empirically. He initially remained profoundly neutropenic (neutrophil count $<0.1 \times 10^9$/L [RR 2.0–7.5 $\times 10^9$/L]). Two days later his hypoxemia and chest radiograph results had worsened, and trimethoprim-sulfamethoxazole, amphotericin B, and roxithromycin were administered. On day 18, he had extensive consolidation that involved most of both lung fields, and his neutrophil count had increased to 2.46×10^9/L.

Bronchoscopic samples collected on day 19 were positive for *Legionella* species antigen by direct immunofluorescent monoclonal antibody stain (DFA) (Genetic Systems, Seattle, WA, USA), but results of his *Legionella* urinary antigen test (Binax Now, Binax, Portland, ME, USA) were negative. Four days later, *Legionella* species were isolated on BCYE agar (Oxoid Ltd.) and confirmed by DFA (Legionella Poly-ID Test Kit, Remel, Lenexa, KS, USA) and also by *L. pneumophila* serogroup 2-14 (LP-2-14) antisera (MarDx Diagnostics, Scotch Plains, NJ, USA). Environmental samples were taken of both hospital and home water. All isolates were then referred to the Australian Legionella Reference Laboratory, Institute of Medical and Veterinary Science, Adelaide, Australia, for typing. Typing confirmed that the clinical isolate was *L. pneumophila* serogroup 13, both by monoclonal antisera (MarDx Diagnostics) and *mip* gene sequencing. Hospital water samples yielded *L. pneumophila* serogroup 10 from both a hand basin cold-water outlet in the patient's room and a cold-water drinking fountain on an adjacent ward. Restriction fragment length polymorphism (RFLP) and pulsed-field gel electrophoresis (PFGE) typing confirmed that the clinical and environmental isolates were genotypically distinct. The patient was then given intravenous erythromycin followed by oral ciprofloxacin for 3 weeks; subsequently, he made a slow but complete recovery. Testing the patient's home potable water supply yielded only *L. feeleii*.

Case 3

A 48-year-old, previously healthy man was admitted to the hospital in Christchurch, New Zealand, with a 6-day history of increasing dyspnea and a 4-day history of watery diarrhea. He also complained of a dry cough, myalgia, loss of appetite, and poor fluid intake. He appeared flushed and unwell and had dry mucous membranes. His vital signs were the following: temperature 39ºC, pulse rate 103 beats per min, blood pressure 154/83 mm Hg, respiratory rate 22 breaths per min, and oxygen saturation 94% on room air. His leukocyte count was 6.1×10^9/L (RR 4.0–10×10^9/L), and his serum sodium and potassium levels were 130 mmol/L (RR 136–146 mmol/L) and 3.3 mmol/L (RR 3.5–5.0 mmol/L), respectively. Liver biochemistry was abnormal. His C-reactive protein (CRP) was >220 mg/L (RR<10 mg/L). Chest radiographs showed pneumonia with segmental consolidation of the lingula and left lower lobe.

The patient's temperature continued to increase to 39.4ºC and returned to normal only after 8 days, despite prompt initiation of intravenous amoxicillin and clarithromycin. Liver biochemistry initially deteriorated, peaking on day 6, thereafter slowly returning to normal when the patient was discharged 11 days after admission.

After 6 days of culture on BCYE agar (Oxoid Ltd.), *Legionella* spp. were isolated from sputum collected on the day after admission. The isolate was typed by Environmental Science and Research Limited (ESR), Communicable Diseases Group Laboratory Services, Porirua, Wellington, New Zealand. A strong positive reaction was seen to polyvalent antisera for *L. pneumophila* serogroups 1–14 (Monoclonal Technologies Inc., Alpharetta, GA, USA). Monoclonal antisera (Monoclonal

Technologies Inc.) gave a strong positive reaction to *Legionella* strain 82A3105 (CDC 1425-CA-H; ATCC 43736), which identified the isolate as *L. pneumophila* serogroup 13 (David Harte, pers. comm.).

Public health service investigation of the patient's home yielded *L. pneumophila* serogroup 13 from the filter of an outdoor whirlpool spa. The patient recalled cleaning this filter several days before falling ill. One month later the patient had made a good recovery, although he was easily fatigued.

Case 4

A 56-year-old man was admitted to the hospital in Christchurch, New Zealand, with a 1-week illness characterized by myalgia, nausea, sweats, and chills but no respiratory symptoms. He was a nonsmoker with negligible alcohol intake; however, his past history included aortic dissection requiring aortic valve replacement.

On admission he was afebrile, blood pressure was 112/66 mm Hg, and his respiratory rate was 16 breaths per min. His chest was clear to auscultation, but his oxygen saturation was 91% on room air, and chest radiograph showed patchy bibasal consolidation. Leukocyte count and serum sodium level were both normal, but his CRP was elevated to 176 mg/L (RR<10 mg/L). Liver biochemistry was slightly abnormal.

He was treated empirically for possible prosthetic valve endocarditis with intravenous penicillin and gentamicin. On the day after admission, he had productive cough and sharp, left-sided chest pains; a temperature of 39ºC ensued. Examination showed left basal dullness with bibasal crackles and left basal bronchial breathing. However, he improved rapidly without specific antimicrobial therapy for legionellosis and was discharged 5 days later.

A sputum sample collected on day 2 yielded *Legionella* species after 9 days of incubation on BYCE agar. With the abovementioned methods, ESR typed the isolate as *L. pneumophila* serogroup 13. Extensive investigations by the local public health services yielded *L. micdadei* from spa whirlpool water where the patient regularly swam.

Discussion

The patients we report had typical signs and symptoms of *Legionella* pneumonia, including headache, anorexia, dry cough, and fever, often with hyponatremia and abnormal results of liver function tests (7). Patients had hypoxemia, and disease often involved multiple lobes or both lungs on chest radiograph (7). However, these patients generally had minimal preexisting illnesses, apart from the patient with acute myeloid leukemia who was undergoing chemotherapy. The outcome for 3 of our patients was positive, including the patient with an underlying hematologic malignancy and neutropenia; legionellosis is often associated with a markedly elevated death rate in these cases (3,7,8). The patient who died was severely ill after nearly drowning and had aspiration pneumonitis. Moreover, specific therapy that was effective against legionellosis was not started until 14 days after the putative infection; delay in administering appropriate therapy is known to adversely affect outcome (7,9).

L. pneumophila is responsible for ≈90% of infections caused by members of the family Legionellaceae (1,3,7). *L. pneumophila* serogroups 1, 3, 4, and 6 cause most human infections (1,3,7). An international survey found that of 15 serogroups of *L. pneumophila*, 79% of all culture- or urine antigen–confirmed infections were caused by *L. pneumophila* serogroup 1 (8). Legionellae are fastidious organisms that are not readily recovered from routine diagnostic media; indeed, an American College of Pathologists' survey indicates that 32% of clinical microbiology laboratories could not grow a pure culture of *L. pneumophila* (10). All of our isolates grew only on specialized media. Failure to diagnose legionellosis in many hospital microbiology laboratories is generally due to a limited availability of specialized media and expertise in the culture of legionellae.

In the United States, from 1980–1998, clinicians have increasingly relied on urinary antigen tests to diagnose legionellosis. Use of these methods has led to an increase in *L. pneumophila* serogroup 1 diagnoses from 0% to 69%, with a corresponding decreased frequency of serogroups other than 1 from 38% to 4% (11). Urinary antigen tests do not reliably diagnose non–serogroup 1 *L. pneumophila* infections (12,13), as illustrated by Benson et al., who found sensitivities of 35% (Binax EIA) and 46% (Biotest-EIA), respectively. However, no isolates of *L. pneumophila* serogroup 13 were included in their evaluation (12), although as illustrated by 2 of our cases, urinary antigen assays will not likely diagnose this specific serogroup in most cases. Furthermore, in the United States, surveillance systems have found that diagnosis of legionellosis by culture, DFA, and serologic testing (IFA) decreased significantly from 1980 to 1998 (11). Nevertheless, in case 2, the DFA of bronchoalveolar lavage fluid was positive to polyvalent antisera. Also in case 1, IFA serology showed seroconversion to *L. pneumophila* serogroup 13–specific antigen. However, serologic diagnosis of *Legionella* infection has only been fully validated for *L. pneumophila* serogroup 1 (14). Thus, the increasing reliance on nonculture-based tests as the sole methods of diagnosing legionellosis is a cause for concern, given the variable utility of these assays.

In most cases, *L. pneumophila* pneumonia is attributed to inhaling contaminated aerosols produced by cooling towers, showers, and nebulizers (1,3,7). Aspiration is also a possible mechanism of transmission (15,16). The source

and reservoir of *L. pneumophila* are generally not identified in sporadic legionellosis (17,18). However, in case 3, the infection was linked epidemiologically and microbiologically with a contaminated spa whirlpool filter. Although legionellosis is well described in association with spa whirlpools (19,20), we are unaware of any previously reported cases of *L. pneumophila* serogroup 13 infections linked to spa whirlpools. Additionally, aspirating water and nearly drowning has a rare but well-recognized association with *L. pneumophila* pneumonia (21–23), although we are unaware of any previously reported cases of *L. pneumophila* serogroup 13 pneumonia after a person's nearly drowning. For the remaining 2 cases, despite extensive environmental sampling, a source or reservoir of infection was not established. In case 2, whether the infection was nosocomial is unclear, given that the patient had had extended periods of home leave. Despite isolation of *L. micdadei* from a spa whirlpool where patient 4 regularly swam, investigations failed to find *L. pneumophila* serogroup 13. Serologic diagnosis of *L. pneumophila* serogroup 13 pneumonia has been occasionally reported from New Zealand and Australia, although clinical data from these cases are unreported. Recovery of environmental isolates mainly from soil and water is also reported in this region (24–26).

Serotyping, serogrouping, typing, and subtyping legionellae are technically challenging. Both phenotypic and genotypic analyses of *L. pneumophila* are required to reliably epidemiologically link patient and environmental isolates. Phenotypic or genotypic studies in higher reference laboratories were performed on our isolates, thereby reliably confirming their identity as *L. pneumophila* serogroup 13. Epidemiologic studies to identify possible sources of legionellosis require careful investigation, including sensitive and discriminatory subtyping techniques to identify similarities and differences between possibly related strains. Methods reported include various panels of monoclonal antibodies, plasmid analysis, RFLPs, ribotyping, macrorestriction enzyme digestion followed by PFGE, and *mip* gene sequencing (27). Recently, Fry et al. have suggested that amplified fragment length polymorphism typing may be the best method for investigating the epidemiology of travel-related legionellosis (28). Newer typing techniques for legionellosis include multilocus sequencing typing and DNA chip technologies (29).

In summary, we describe for the first time in detail the clinical and laboratory features of *L. pneumophila* serogroup 13 infections. *L. pneumophila* serogroup 13 is a rare but perhaps underrecognized pathogenic *L. pneumophila* serogroup. Although the organism was first reported in the United States (5), its global distribution is highlighted here. We found that this organism produces a broad spectrum of clinical disease, from relatively mild disease to severe, potentially fatal pneumonia. Finally, this report emphasizes that culture for *Legionella* species remains important if the prevalence and incidence of legionellosis are to be reliably and fully appreciated.

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Malaria Attributable to the HIV-1 Epidemic, Sub-Saharan Africa

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We assessed the impact of HIV-1 on malaria in the sub-Saharan African population. Relative risks for malaria in HIV-infected persons, derived from literature review, were applied to the HIV-infected population in each country, by age group, stratum of CD4 cell count, and urban versus rural residence. Distributions of CD4 counts among HIV-infected persons were modeled assuming a linear decline in CD4 after seroconversion. Averaged across 41 countries, the impact of HIV-1 was limited (although quantitatively uncertain) because of the different geographic distributions and contrasting age patterns of the 2 diseases. However, in Botswana, Zimbabwe, Swaziland, South Africa, and Namibia, the incidence of clinical malaria increased by ≤28% (95% confidence interval [CI] 14%–47%) and death increased by ≤114% (95% CI 37%–188%). These effects were due to high HIV-1 prevalence in rural areas and the locally unstable nature of malaria transmission that results in a high proportion of adult cases.

HIV-1 infection increases the risk and severity of malaria (1,2). In African settings of both high- and low-intensity (epidemic, unstable, or strongly seasonal) malaria transmission, increases in the severity and case fatality of malaria have been observed in all age groups (3–8). In areas of high-intensity transmission, HIV-1 also increases the incidence of clinical malaria among adults (9).

The population-level impact depends on HIV prevalence, the age distribution of both infections (which for malaria is determined by the transmission intensity), and their geographic overlap. Local distributions of CD4 cell counts and clinical stages of HIV-infected patients are also important because the effects of HIV multiply with increasing immunosuppression (9,10).

The HIV-1 epidemic in Africa has matured over the past 25 years and may now be reaching a peak (11). From the 1980s to the 1990s, malaria death and disease increased, especially among children in rural, malaria-endemic parts of East and West Africa (12), and in populations at risk for unstable malaria in South Africa (13–15). Chloroquine resistance (16), breakdown of vector-control operations (17), and HIV-1 may all have contributed to these trends (3,4,13). We assessed the number of additional malaria cases and deaths caused by HIV-1 in sub-Saharan Africa by calculating the impact of HIV-1 separately for urban and rural populations, areas of high- and low-intensity malaria transmission, and different age groups in each country.

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Methods

Malaria Incidence in the Absence of HIV-1

In the absence of reliable data from routine health information systems, the incidence of uncomplicated and severe clinical malaria episodes, collectively referred to here as "malaria incidence," was calculated from estimates of incidence rates (Table 1) and national populations at risk for malaria in 2004 (Table 2), by using the 1998 map of climatic suitability for malaria transmission (18). The climate suitability index was used to indicate high- (≥0.75) or low-intensity transmission (>0 and <0.75) (19). For high-intensity transmission areas, average incidence of malarial fevers in the absence of HIV was estimated at 1.4 per person per year among children <5 years of age, 0.59 per person per year in those 5–14 years of age, and 0.11 per person per year in those ≥15 years of age (19). In areas of low transmission, incidence among those <5 years of age was estimated at 0.182 per person per year (19). Because immunity against clinical malaria increases slowly with age in areas of low-intensity transmission, the incidence rate among 5- to 14-year-olds and those ≥15 years were considered to be the same as that in young children and half that rate, respectively (19).

Table 1. HIV-1, malaria incidence and death rates. and their interactions*

Parameter	Assumption
Malaria transmission intensity	Index >0 and <0.75 denotes low-intensity transmission and ≥0.75 denotes high-intensity transmission, except for southern Africa, where index >0.75 denotes unstable transmission (18,19)
Overall malaria incidence	Middle Africa, high-transmission areas: 1.4 per person per year in children <5 y, 0.59 per person per year at 5–14 y, 0.11 per person per year at ≥15 y (19) Middle Africa, low-transmission areas: 0.182 per person per year in children <15 y, 0.091 per person per year at ≥15 y (19) Southern Africa: 0.0294 per person per year as all-age average in areas with (unstable) malaria transmission; divided as twice the rate at ≥15 y compared to ≤14 y (19)
Relative malaria incidence urban/rural	0.50 (20)
Malaria deaths	High-transmission areas: 0.8% of incident cases in children <5 y, 0.3% at ≥5 y; Low-transmission and unstable transmission areas: 0.8% of incident cases in all age groups (21).
Effect of HIV-1 on incidence of clinical malaria	≥5 years in areas with high-intensity malaria transmission, and all age groups in areas with low-intensity or unstable malaria transmission: CD4 ≥500/μL RR = 1.2 CD4 200–499/μL RR = 3.0 CD4 <200/μL RR = 5.0† <5 years in high-transmission areas: no effect
Effect of HIV-1 on malaria case fatality rate	All malaria transmission intensities and age groups: CD4 ≥500/μL RR = 2.0 CD4 200–499/μL RR = 4.0 CD4 <200/μL RR = 10‡
Survival after HIV-1 infection	Median 9 years, following a Weibull curve with shape parameter 2.28 (22)
CD4 decline over the course of HIV-1 infection	Linear from 825/μL at seroconversion to 20/μL at death of AIDS (23–26)

*RR = relative risk associated with HIV-1 infection; y = years of age.
†From (9,10). Earlier studies did not consistently show these effects, but these were cross-sectional and/or hospital-based (1). Effects of HIV-1 in these studies may have been obscured by a lack of adjustment for prestudy treatment with antimalarial drugs (which might be more common in HIV-1 patients with recurrent fevers [27]) and by their inherent dependence on the relative survival of HIV-infected and HIV-uninfected participants, given the increased case fatality of malaria among HIV-infected patients (6). At the specified CD4-stratum-specific relative risks, the relative risk averaged over all HIV-infected people would be 2.1 in Madagascar and 2.5 in all other countries (see Methods, CD4 distributions among HIV-infected people).
‡At these CD4-stratum-specific relative risks, the relative risk averaged over all HIV-infected people would be 3.4 in Madagascar and 4.1 in all other countries (see Methods, CD4 distributions among HIV-infected people).

In Botswana, Namibia, South Africa, Swaziland, and Zimbabwe, only areas with a climate suitability index ≥0.75 on the Malaria Risk in Africa map were considered to have transmission of unstable nature because vector control has been successful in some places (19). The average incidence in malarious areas in these countries has been estimated as 0.0294 per person per year (19). Age-specific incidence rates from clinic data have only been reported from Zimbabwe. We, therefore, applied the age distribution from areas of low transmission in Central Africa (see above and Table 1), which gave proportions of cases among children <5 years of age from 16% to 22%, in agreement with the 21% among reported cases in Zimbabwe.

We assumed that the urban-rural ratio in malaria incidence rates was 0.50, a conservative estimate based on 3- to 24-fold lower entomologic infection rates in periurban and urban areas compared to rural areas (20) and the approximately linear increase in infection rates with increasing entomologic infection rates up to a certain saturation point (29). Proportions of urban persons were assumed to be the same in areas of high, low, and unstable transmission, because the Malaria Risk in Africa risk classification (18) did not incorporate small-scale variation due to urban environment. Urban and rural populations were based on countries' definitions (30), without standardization between countries.

Effects of HIV-1 on Malaria Incidence

The best evidence for associations between HIV-1 and clinical malaria comes from 2 longitudinal studies in Uganda, where malaria transmission is of high intensity. A community-based study in rural Masaka found odds ratios of clinical malaria among HIV-positive adults compared to HIV-negative adults, of 1.2, 3.4, and 6.0 for CD4 counts ≥500/μL, 200–499/μL, and <200/μL, respectively ($p = 0.0002$) (9). As in earlier studies, malaria incidence was defined as any acute fever concurrent with malaria parasitemia, without excluding alternative causes of the fever through further laboratory tests. Because malaria infection may occur without symptoms, in particular among adults in settings of high-intensity transmission, and because HIV-infected people often have acute nonmalarial fevers (10), the effect of HIV may have been overestimated.

The second study in Uganda, on HIV-positive persons only, excluded alternative causes of acute febrile illness, such as bacteremia (10). Malaria incidence for CD4 counts ≥500/μL, 200–499/μL, and <200/μL was 57, 93, and 140

Table 2. Estimated HIV-1 impact on malaria cases and deaths, sub-Saharan Africa, 2004*

		Population at risk for malaria, by intensity of transmission, %		HIV-1 prevalence in adults, %		Malaria incidence/ 1,000 py‡		Malaria deaths/ 1,000 py‡	
Country†	Urban, %	Low or unstable	High	National§	Urban-rural ratio¶		Increase due to HIV, %		Increase due to HIV, %
Angola	34	53	46	3.9	1.6#	291	1.2	1.8	4.2
Benin	42	0	100	1.9	1.8	434	0.4	2.4	1.4
Botswana	49	13	0	37.3	1.0	3.5	28.0	0.028	114.4
Burkina Faso	17	0	100	4.2	3.1	538	0.8	3.1	2.9
Burundi	9	64	21	6.0	3.6	209	2.4	1.4	8.6
Cameroon	49	24	74	6.9	1.7	317	1.9	1.8	6.5
Central African Republic	41	0	100	13.5	1.2	419	3.2	2.3	11.3
Chad	24	14	86	4.8	1.3	451	1.1	2.6	4.1
Congo	65	0	100	4.9	1.6#	380	1.1	2.1	3.8
Cote d'Ivoire	44	0	100	7.0	2.1	402	1.6	2.2	5.2
Democratic Republic of the Congo	30	10	85	4.2	1.6	423	0.9	2.4	3.3
Equatorial Guinea	48	2	97	11.6	1.6#	401	2.5	2.3	8.50
Eritrea	19	83	16	2.5	1.6#	197	1.3	1.4	4.2
Ethiopia	16	50	14	4.4	4.8	142	1.7	1.0	5.9
Gabon	81	0	96	8.1	1.9	287	1.9	1.5	6.0
Gambia	31	0	100	1.2	0.7	429	0.3	2.3	1.0
Ghana	36	2	98	3.1	1.2	401	0.8	2.1	2.6
Guinea	28	1	99	3.2	1.6#	468	0.7	2.6	2.2
Guinea-Bissau	32	0	100	3.8	1.6#	480	0.7	2.7	2.7
Kenya	33	57	21	6.7	1.8	164	2.9	1.1	10.4
Liberia	45	0	100	5.9	1.6#	437	1.1	2.5	4.0
Madagascar	30	36	60	1.7	0.7	327	0.4	1.9	1.3
Malawi	15	22	77	14.2	1.6#	435	3.5	2.6	13.3
Mali	30	10	90	1.9	1.5	464	0.4	2.7	1.5
Mauritania	58	59	41	0.6	1.6#	221	0.2	1.4	0.72
Mozambique	32	4	96	12.2	1.2	437	2.8	2.4	10.3
Namibia	31	8	0	21.3	1.3	2.3	14.5	0.018	52.4
Niger	21	11	89	1.2	3.2	496	0.2	2.9	0.8
Nigeria	44	1	99	5.4	1.1	420	1.2	2.3	4.3
Rwanda	6	60	7	5.1	3.6**	129	2.6	0.9	8.9
Senegal	47	3	97	0.8	1.1	395	0.2	2.2	0.65
Sierra Leone	37	0	100	1.8	1.6#	440	0.4	2.5	1.4
Somalia	28	96	3	0.7	1.6#	148	0.4	1.1	1.3
South Africa	57	15	0	21.5	1.3	3.5	17.0	0.028	62.1
Sudan	36	42	56	2.3	1.6#	281	0.7	1.7	2.5
Swaziland	26	69	0	38.8	1.6#	21	26.1	0.17	107.0
Tanzania	32	21	75	8.8	2.7	372	2.0	2.1	7.4
Togo	33	0	100	4.1	2.6	445	0.9	2.5	3.0
Uganda	14	20	73	4.1	1.9	437	1.1	2.6	4.1
Zambia	40	16	83	16.5	2.1	396	3.6	2.3	14.0
Zimbabwe	35	54	0	24.6	1.2	16	16.7	0.128	61.9
Average††	34	23	67	6.9	2.0	320	1.3	1.8	4.9
Median	33	14	85	4.8	1.6	396	1.2	2.2	4.2

*Malaria incidence and deaths denote estimates in the absence of HIV, all age groups combined. Abbreviations: /1,000py = per 1,000 person-years.
†Excluded from analysis were the following small countries: Lesotho and the islands The Seychelles, Reunion, Comoros and Mauritius, which are at negligible malaria risk, and Sao Tome & Principe and Cape Verde, which may be subject to stable malaria transmission but whose transmission intensity has not been precisely characterized in the Malaria Risk in Africa model.
‡Calculated by using country-specific age distributions.
§Estimates by UNAIDS/World Health Organization (WHO) for end of 2003 (28).
¶From estimates by UNAIDS/WHO for end of 2003 or from national household surveys (11). Same ratios assumed for children as for adults.
#In the absence of reliable urban/rural data, we applied the median urban/rural ratio across countries with urban and rural data.
**In the absence of specific urban and rural data, the estimate for neighboring Burundi, which was judged to be similar in HIV epidemiology, was applied.
††Weighted according to population size, except for the increases in malaria incidence and deaths due to HIV-1, which are weighted according to the absolute number of malaria cases and deaths in each country.

per 1,000 person-years, respectively, and when confined to fever episodes with high parasitemia, 22, 53, and 90 per 1,000 person-years (10). We, therefore, assume that HIV-1 increases malaria incidence in adults by 1.2, 3, and 5 times for the above CD4 categories.

No community-level data are available for children, but 4 hospital-based cohorts in settings of high malaria transmission were studied in the late 1980s (6,8,27,31). In a birth cohort in Kinshasa, HIV-1 infection at any stage and clinically diagnosed AIDS increased malaria incidence by 1.2- and 2-fold, respectively, but the increases were not significant (8). A birth cohort in Blantyre, Malawi, found no HIV-related increase in the incidence of parasitemia (31). In Kampala, perinatally HIV-infected children with AIDS experienced notably fewer malaria episodes than HIV-uninfected children (6), which the authors attributed to the increased use of chloroquine before hospitalization among the HIV-infected children. In contrast, among children in Kinshasa from 1986 to 1988 who had received blood transfusions, those who were HIV-infected experienced 1.4 times more clinical malaria than those who were not (27). The effect of HIV-1 on malaria incidence is likely less apparent in children than in adults in high-transmission areas because young children have a high incidence of symptomatic malaria anyway (19). However, the observations may be confounded by prehospital use of antimalarial drugs or the shorter follow-up times and younger ages of HIV-infected children. We assumed that HIV-1 does not increase malaria incidence in children <5 years of age in high-transmission areas. Few data from areas of low-level and unstable malaria transmission in Africa exist and we assumed that HIV-1 increases malaria incidence equally in adults and children by the risk ratios specified above for adults in high-transmission areas.

The observed effect of HIV-1 on the incidence of clinical malaria may in part be the result of an increased incidence of recrudesences after failing antimalarial treatment because HIV-1 lowers the efficacy of antimalarial treatment (32,33). No published studies report specifically on the effect of HIV on malaria recrudescence. If such an effect exists, however, it will have been accounted for under the assumed overall effect of HIV on clinical malaria, since none of the studies from which we derived this assumed overall effect (6,8–10,27,31) adjusted malaria incidence rates observed in HIV-positive and HIV-negative participants for differences between these groups in treatment failure.

Malaria Mortality and Effect of HIV

Malaria mortality was derived from malaria incidence, assuming fixed case-fatality rates. In high-transmission areas, 0.3% of malaria episodes were assumed to be fatal in adults and 0.8% in children (21). In areas of low-level transmission, we assumed a fatality rate of 0.8% for all ages (21).

Studies on adults in areas of high malaria transmission showed that HIV-1 infection increased case fatality among hospitalized persons with severe malaria by 1.6- to 2.5-fold (7,34,35), while the incidence of severe malaria, a precursor of fatal episodes, increased by 2.7-fold (36). In children exposed to high transmission in Kinshasa, HIV-1 increased the rate of hospitalization for malaria by 6-fold and malaria case fatality by 9.8-fold, although these effects were not significant; among HIV-infected persons who did not meet clinical criteria for AIDS, rates of hospitalization or death due to malaria did not increase (8). In Kampala, perinatally infected children were hospitalized for malaria 2.8 times more often than HIV-uninfected children ($p = 0.001$) (6).

In areas of low intensity and unstable malaria transmission, malaria diagnosis is less problematic because of less acquired immunity. Hospital-based studies in Zimbabwe in 1999 and Kwa-Zulu Natal, South Africa, in 2000, documented 6.9- and 8.8-fold increases in malaria case fatality among HIV-infected adults relative to HIV-negative patients (4,5). In Soweto, South Africa, a significant 1.7-fold increase in the rate of severe malaria was observed (37). For children living in areas of unstable malaria transmission, a hospital-based study in Kwa-Zulu Natal found a 2.7-fold increase in severe malaria ($p = 0.05$) and a 3.6-fold increase in fatality among severe cases ($p = 0.1$) associated with HIV-1 (3).

These data collectively suggest that HIV-1 increases malaria deaths by increasing the proportion of severe cases, case fatality among them, and the failure rate of antimalarial treatment (32,33). We conservatively assumed that the malaria death rate is increased by 4 times, taking into account the problem of attributing fevers to malaria. Lacking further evidence, we applied this increase to all age groups and malaria transmission intensities. One study found that the effect of HIV-1 was greater for CD4 <200/μL (37), and we assumed that malaria death rate increases with falling CD4 counts in the same way as malaria incidence, giving relative death risks among HIV-1–positive participants relative to HIV-1–negative ones of 2, 4, and 10 for CD4 ≥500 cells/μL, 200–499/μL, and <200/μL.

HIV-1 Prevalence

Estimates of national HIV-1 prevalence among adults (>15 years of age), children <5 years of age, and children 5–14 years of age are available from the Joint United Nations Programme on HIV/AIDS (UNAIDS) for 2003 (28). To evaluate impact separately for urban and rural areas, which differ in malaria transmission intensity (20),

urban-to-rural ratios in HIV-1 prevalence were estimated from national household surveys or antenatal clinic surveillance data (UNAIDS and [11]).

CD4 Distributions among HIV-infected Persons

The distribution of CD4 counts among HIV-infected persons follows from the pattern of CD4 decline after initial infection and the trend in HIV-1 prevalence over preceding years (online Appendix, available at http://www.cdc.gov/ncidod/EID/vol11no09/05-0337_app.htm). Data from a variety of populations not receiving antiretroviral therapy suggest that CD4 decline is approximately linear after infection with HIV-1 (26,38). In African patients, CD4 declines from ≈825/μL, the median value in HIV-uninfected adults (23–26,39), to a mean of 20/μL at death of AIDS (40).

CD4 distributions among HIV-infected adults in countries with different HIV-1 epidemics were determined by exploring 4 different epidemic patterns: 1) Uganda, where adult prevalence fell from a peak of ≈13% in the early 1990s to an estimated 4.1% (2.8%–6.6%) in 2003; 2) Ghana, where adult prevalence was relatively stable in recent years at 3.1% (1.9%–5.0%) in 2003; 3) South Africa, where the epidemic started only in the 1990s but reached an estimated prevalence of 21.5% (18.5%–24.9%] in 2003; and 4) Madagascar, where adult prevalence has risen rapidly in recent years to an estimated 1.7% (0.8%–2.7%) in 2003 (Figure 1) (28)]. In all 4 countries, most HIV-1 patients had CD4 ≥500/μL at the start of the epidemic (Figure 2). In Uganda, the prevalence of CD4 <500/μL rose rapidly until 1996, 6 years after the peak in HIV-1 prevalence. In the other epidemics, the prevalence of low CD4 rose more slowly, following their later stabilization. At means of 430 to 660/μL for 1996, the modeled CD4 counts are consistent with empiric data on HIV-infected African adults, which found means of 400 to 630/μL (23,24,26) and medians of 325 to 660/μL (9,23,24,26,39). Also, the modeled CD4 counts for South Africa from 2000 to 2005 (Figure 2) were in agreement with the observed distribution of 50%, 40%, and 10% with CD4 ≥500/μL, 200–499/μL, and <200/μL, respectively, in Soweta in 2002 (39).

Despite their different epidemic curves, the modeled CD4 distributions among HIV-1 patients were fairly similar for Uganda, Ghana, and South Africa in 2004: 44%–45% have CD4 ≥500/μL, 36%–41% have CD4 200–499/μL, and 15%–19% have CD4 <200/μL. We assume similar CD4 distributions for all other countries in which HIV prevalence has also stabilized. In Madagascar only, where HIV prevalence still increases rapidly, CD4 counts are notably higher (Figure 2). We therefore applied 2 distributions: in Madagascar, 60% of HIV-infected adults had CD4 ≥500/μL, 30% had CD4 200–499/μL, and 10% CD4 <200/μL; for other countries, assumed proportions were 44%, 39%, and 17%, respectively.

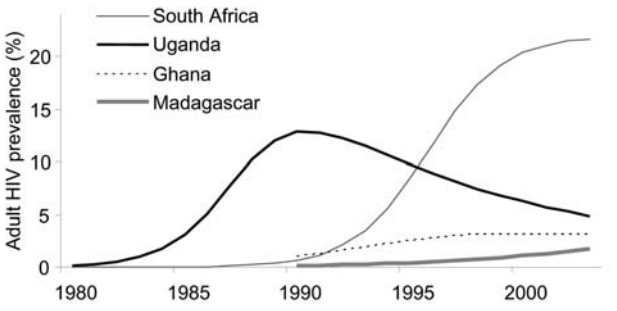

Figure 1. Modeled time trends in HIV-1 prevalence (adults 15-49 years), based on UNAIDS estimates from sentinel surveillance data in antenatal clinics (28).

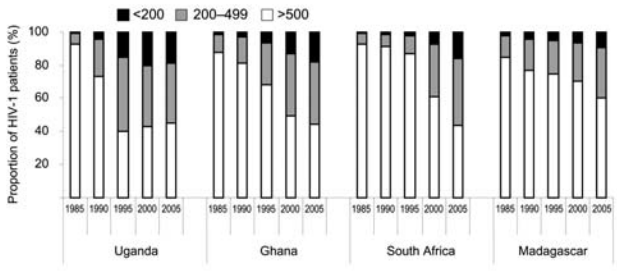

Figure 2. Modeled time trends in CD4 count distributions (per microliter) among HIV-infected adults in selected African countries. Madagascar: example of a rising HIV-1 epidemic at low grade; Ghana: example of a stable epidemic at low grade; Uganda: example of a high-grade epidemic that has declined and leveled off; South Africa: example of a high-grade epidemic that recently started leveling off.

Results

HIV-1 increased malaria incidence by 0.20% to 28% across countries (Table 2). The largest increases were in Botswana, South Africa, Swaziland, Zimbabwe, and Namibia, where HIV-1 prevalence is highest, especially in rural areas, and malaria transmission most unstable. For any given HIV-1 prevalence, HIV-1 impact was greatest in countries with low-intensity or unstable malaria transmission, where relatively more malaria occurs in adults. For example, impact was greater in Burundi than in Liberia, where malaria transmission is higher, despite an HIV prevalence of ≈6% in both countries. Outside southern Africa, impact was relatively high in the Central African Republic, with a comparatively high HIV-1 prevalence rurally, and Kenya, with a high proportion of low-intensity malaria transmission.

Across 41 countries, HIV-1 increased malaria incidence by 1.3%. This relatively small impact is explained, first, by the different geographic distributions of the 2 diseases.

Malaria incidence rates are highest in West and Central Africa, where HIV-1 prevalence is comparatively low (Table 2). HIV-1 is most prevalent in southern Africa, where malaria transmission is rarely stable and comparatively well controlled. Second, within countries, the impact of HIV-1 was further limited because HIV-1 is more prevalent in cities (median urban/rural ratio 1.6, Table 2), whereas malaria is more prevalent rurally (assumed urban/rural ratio 0.5). Third, HIV-1 mainly affects adults; whereas in countries of high malaria transmission, malaria has the greatest impact on young children.

HIV-1 increased malaria deaths by 0.65% to 114% across countries (Table 2). As for malaria incidence, the largest increases were in southern Africa (Figure 3), and the ranking among countries was very similar to the ranking by impact on malaria incidence. On a continental scale, HIV-1 increased malaria deaths by 4.9%. The impact on malaria deaths was greater than that on malaria incidence for 2 reasons. First, in the individual patient, HIV-1 increases death risk more than incidence (Table 1). Second, the impact of HIV-1 on death was compounded by its impact on death rates.

Against our baseline of ≈228 million malaria cases and ≈1.3 million malaria deaths among all ages in sub-Saharan Africa, these increases would correspond to an additional 3 million malaria cases and 65,000 malaria deaths annually due to HIV-1, or to ≈3% of the estimated 2.3 million HIV/AIDS deaths in sub-Saharan Africa in 2004 (28). However, the assumed baseline malaria incidence and death rates were cruder than we would have liked and do not take into account the impact of malaria control in the different countries. We, therefore, focus on relative increases in malaria due to HIV.

Alternative Scenarios

To assess the sensitivity of results to assumptions made, we recalculated the impact of HIV-1 for several alternative scenarios (Table 3). Estimated impact increased or decreased considerably with larger or smaller assumed relative risks at the individual level. Most critical, however, were the assumed age patterns in malaria incidence and case fatality. The smaller the decline with age in malaria incidence and fatality rates, the greater the impact of HIV-1 would be (≤12.5% increase in malaria deaths and 4.4% increase in malaria incidence, Table 3) because of the concentration of HIV-1 in adults.

Estimates were relatively insensitive to whether the effect of HIV-1 on malaria incidence applied also to children in high-transmission areas, as a recent study in Uganda suggested (41). Alternative assumptions concerning the range over which CD4 counts decline during HIV infection also made little difference. Abandoning the assumption that malaria occurs more frequently in rural than in urban areas resulted in only a slight increase in HIV-1 impact because the countries with highest HIV-1 prevalence and Nigeria, which has most malaria cases, had similar HIV-1 prevalence in cities and rural areas (Table 2). Finally, estimated impacts were moderately sensitive to uncertainties in national HIV prevalence for adults but not to uncertainties in HIV prevalence for children.

Combining the ranges of estimates from these scenarios into 1 multivariate analysis, with the Monte Carlo technique and assuming triangular distributions for all parameters, overall 95% confidence intervals (CIs) on the continentwide estimates would be 0.6%–7.9% (best estimate ≈1.3%) for clinical malaria incidence, and 3.1%–17.1% (best estimate ≈4.9%) for malaria deaths. For Botswana, the country with the largest estimated HIV impact, 95% CI would be 14%–47% (best estimate ≈28%) for malaria incidence and 37%–188% (best estimate 114%) for malaria deaths.

Discussion

Across 41 countries in sub-Saharan Africa, the HIV-1 epidemic may have increased the incidence of clinical malaria by 1.3% (95% CI 0.6%–7.9%) and malaria deaths by 4.9% (95% CI 3.1%–17.1%) in 2004. Continentwide impact was limited by the different geographic distributions of the 2 diseases and their different age patterns.

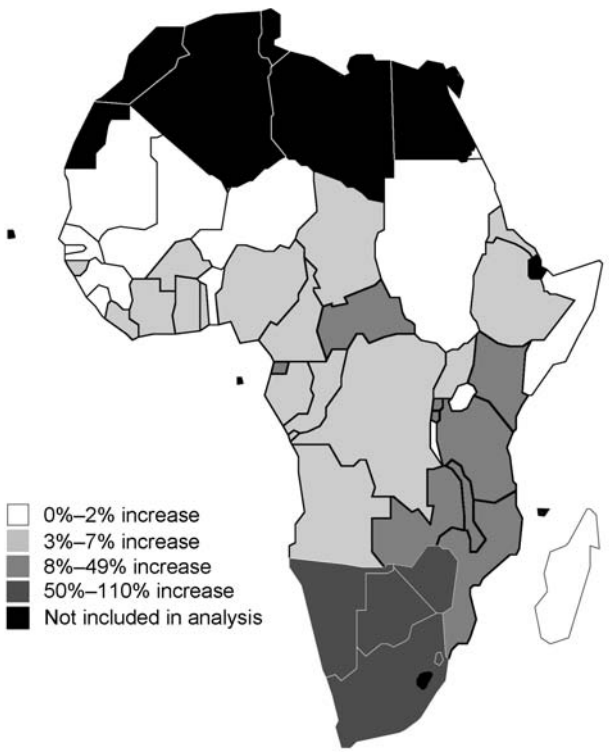

Figure 3. Estimated proportional increases in malaria deaths due to HIV-1 in sub-Saharan African countries in 2004, for all ages combined.

For southern Africa, estimated proportional increases were ≤28% (95% CI 14%–47%) for malaria incidence and ≤114% (95% CI 37%–188%) for malaria deaths. An impact of HIV-1 of this magnitude may have contributed to observed increases of malaria in the 1990s in areas of unstable transmission, including Kwa-Zulu Natal (13,14) and northern Zambia (15). Outside southern Africa, however, HIV-1 is unlikely to be a major contributor to rises in malaria, and where this appears to be so, a more plausible explanation may be overdiagnosis of fevers as malaria in

Table 3. Univariate sensitivity analyses of HIV-1 impact on malaria incidence and deaths, sub-Saharan Africa, 2004*

Scenario	% increase in malaria incidence due to HIV (minimum and maximum)†	% increase in malaria deaths due to HIV (minimum and maximum)†
Default scenario: see Tables 1 and 2.	1.3 (0.20–28)†	4.9 (0.65–114)†
Weaker effect of HIV-1 on malaria incidence: RR = 1.0 at CD4 ≥500/μL, RR = 2.0 at CD4 200–499/μL, and RR = 4.0 at CD4 <200/μL.	0.8 (0.11–16)	4.4 (0.60–90)
Stronger effect of HIV-1 on malaria incidence: RR = 1.5 at CD4 ≥500/μL, RR = 4.0 at CD4 200–499/μL, and RR = 8.0 at CD4 <200/μL.	2.2 (0.33–47)	5.7 (0.73–153)
Weaker effect of HIV-1 on malaria mortality (all groups): RR = 1.5 at CD4 ≥500/μL, RR = 2.0 at CD4 200–499/μL, and RR = 4.0 at CD4 <200/μL.	n.a.	2.4 (0.30–59)‡
Weaker effect of HIV-1 on mortality in children <5 y in areas of high malaria transmission specifically, analogous to the comparatively weak effect of HIV-1 on incidence in this group: RR = 1.5 at CD4 ≥500/μL, RR = 2.0 at CD4 200–499/μL, and RR = 5.0 at CD4 <200/μL.	n.a.	3.7 (0.45–114)
Stronger effect of HIV-1 on malaria mortality (all groups): RR = 3.0 at CD4 ≥500/μL, RR = 6.0 at CD4 200–499/μL, RR = 12.0 at CD4 <200/μL.	n.a.	6.9 (0.92–157)§
Stronger decrease with age in malaria incidence: RRs compared to <5 y of 0.30 for 5–14y and 0.05 for ≥15 y in high malaria transmission areas, and 0.60 for 5–14 y and 0.10 for ≥15 y for areas of low and unstable malaria transmission including southern African countries.	1.0 (0.13–15)	4.0 (0.45– 59)
No decrease with age in malaria incidence at any malaria transmission intensity.	4.4 (0.54–37)	12.5 (1.5–153)
Stronger decrease with age in malaria CFR: 1.2% in <5 y at all malaria transmission intensities, 0.8% in ≥5 y at low and unstable transmission, 0.15% in ≥5 y at high transmission.	n.a.	4.0 (0.53–107)
No decrease with age in malaria CFR at any transmission intensity.	n.a.	5.7 (0.77–114)
HIV-1 increases malaria incidence also in children <5 y in areas of high malaria transmission.	2.0 (0.26–28)	5.9 (0.78–114)
CD4 count decline during HIV-1 infection: 1,000–100/μL¶	1.0 (0.15–21)	3.7 (0.50–82)
CD4 count decline during HIV-1 infection: 700–0/μL#	1.7 (0.24–35)	6.2 (0.81–151)
No urban/rural difference in the malaria incidence rate	1.4 (0.20–28)	5.3 (0.67–114)
Lower HIV prevalence in adults: lower bound country estimates by UNAIDS/WHO**	0.9 (0.08–27)	3.2 (0.28–108)
Higher HIV prevalence in adults: upper bound country estimates by UNAIDS/WHO**	2.2 (0.37–29)	8.0 (1.2–121)
Lower HIV prevalence in children ≤14 y: lower bound country estimates by UNAIDS/WHO††	1.3 (0.20–27)	3.9 (0.47–111)
Higher HIV prevalence in children ≤14 y: upper bound country estimates by UNAIDS/WHO††	1.5 (0.23–29)	6.8 (1.08–119)

*N.A., not applicable; CFR, malaria case-fatality rate; RR, relative risk; UNAIDS, Joint United Nations Programme on HIV/AIDS/WHO.
†Continental total. In none of the scenarios did the ranking of countries in magnitude of HIV impact change appreciably. Across all scenarios, the minimum and maximum increases (in brackets) were always in Senegal or Mauritania, and in Botswana, respectively. An exception was the scenarios of lower HIV prevalence in adults, for which the lowest malaria impacts would be in Sierra Leone and Somalia.
‡The overall relative risk for malaria mortality due to HIV-1 in stable HIV-1 epidemics is now 2.1, i.e., does no longer fit the observed value of ≈4 (see Methods, Malaria mortality and Effect of HIV).
§The overall relative risk for malaria mortality due to HIV-1 in stable HIV-1 epidemics is now 5.7, i.e., does no longer fit the observed value of ≈4 (see Methods, Malaria death and effect of HIV).
¶As in Western populations (25).
#To allow for a possible initial drop in CD4 immediately upon infection, i.e., still before seroconversion.
**Cross-country median lowerbound estimate of HIV prevalence in adults 2.7%; cross-country median upperbound HIV prevalence estimate 8.8% (compared to default point estimate of 4.8%).
††Cross-country median lowerbound estimate of HIV prevalence in children ≤14 years of 0.2%; cross-country median upperbound HIV prevalence estimate 1.1% (compared to default point estimate of 0.5%).

HIV-1 patients. Such over-diagnosis may occur unintentionally in settings where malaria is diagnosed without parasitologic confirmation because of the increased frequency of acute fevers in HIV-1 patients (10). Intentional misdiagnosis could also occur, if doctors are reluctant to diagnose illness as HIV-related for fear of social stigma.

These estimates have several limitations. First, the magnitude of effects of HIV-1 on malaria incidence and death risk in individual patients is critical (Table 3) but uncertain because of diagnostic problems in settings of high malaria transmission and a lack of population-based data from areas of low intensity and unstable transmission.

Second, results are sensitive to age patterns in malaria (Table 3), which are not well known. The sharp contrast in estimated impact of HIV-1 between the 5 southern African countries and the remainder of Africa depends on the assumption that malaria declines more slowly with age in South Africa, where all malaria is assumed to be unstable. In practice, the shift from unstable to stable malaria transmission, i.e., from clinical effects in all age groups to a predominance in young children, is more gradual; thus, effects of HIV on malaria in Zimbabwe and Zambia, for example, may be more similar than we estimated. The estimation method developed here could, nevertheless, be applied to more refined age-specific estimates of malaria incidence and death.

Finally, subnational heterogeneity in malaria or HIV, apart from urban/rural differences, was not considered, and this fact may have biased the estimation for countries where either or both diseases are heterogeneously distributed, such as Kenya, Ethiopia, Tanzania, and South Africa (42). For example, in South Africa, both malaria and HIV-1 are concentrated in Kwa-Zulu Natal, so that their interaction may be greater than our estimate.

The impact of HIV-1 that we have estimated only pertains to malaria cases and deaths and does not include effects on anemia or adverse birth outcomes attributable to concurrent malaria and HIV-1 in pregnant women. In areas of high-intensity transmission, such as in Kenya and Malawi, the latter effects might be more important than malaria cases and deaths per se. Also, our analysis did not cover the effect of HIV-1 on demand for antimalarial drugs. In most of rural Africa, antimalarial drugs are presumptively prescribed to treat any fever without an obvious nonmalarial cause. Recurrent fevers in HIV-1 patients may, therefore, cause considerable overuse of antimalarial drugs, increasing not only costs but also the risk for drug resistance. The HIV-1 epidemic thus underlines the need to improve capacity for laboratory diagnosis of febrile disease in Africa.

To limit the impact of HIV-1 on malaria, HIV-infected persons, in addition to young children and pregnant women, may form a target group for provision of insecticide-treated mosquito nets (2). In areas of low intensity and unstable transmission, HIV may be a reason for intensifying or resuming indoor residual spraying to control malaria vectors. For HIV-infected persons who are prone to treatment failure with conventional antimalarial drugs (27,32,33,43), effective combination therapy is of utmost importance.

Highly active antiretroviral combination therapy has great potential to reduce HIV-related malaria (44). Cotrimoxazole prophylaxis, recommended for adults and children living with HIV in Africa (45), is also effective in reducing clinical malaria, independent of baseline CD4 (41,46,47). Combined HIV and malaria interventions might best be delivered at peripheral health centers, including antenatal clinics (2).

HIV-1 appears to have increased the impact of malaria disease and death in South Africa compared to the 1980s, although data do not allow a precise quantification of this effect. In areas of high HIV and low-intensity or unstable malaria, continued vigilance and intensified malaria control are indicated. In HIV-infected adults, pregnant women, and children, malaria is among the simplest opportunistic infections to prevent and treat.

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Molecular Epidemiology of SARS-associated Coronavirus, Beijing

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Single nucleotide variations (SNVs) at 5 loci (17564, 21721, 22222, 23823, and 27827) were used to define the molecular epidemiologic characteristics of severe acute respiratory syndrome–associated coronavirus (SARS-CoV) from Beijing patients. Five fragments targeted at the SNV loci were amplified directly from clinical samples by using reverse transcription–polymerase chain reaction (RT-PCR), before sequencing the amplified products. Analyses of 45 sequences obtained from 29 patients showed that the GGCTC motif dominated among samples collected from March to early April 2003; the TGTTT motif predominanted afterwards. The switch from GGCTC to TGTTT was observed among patients belonging to the same cluster, which ruled out the possibility of the coincidental superposition of 2 epidemics running in parallel in Beijing. The Beijing isolates underwent the same change pattern reported from Guangdong Province. The same series of mutations occurring in separate geographic locations and at different times suggests a dominant process of viral adaptation to the host.

Severe acute respiratory syndrome (SARS) is a new infectious disease that spread worldwide in early 2003, affecting >30 countries, with >8,098 cases and 774 deaths reported (1). Beijing, People's Republic of China, experienced the largest SARS outbreak in the world, with 2,523 cases and 181 deaths by June 12, 2003 (2,3). The epidemic occurred in 2 phases. The first phase began on March 5, 2003, and was caused by a patient who had been infected in Guangzhou and was involved in a superspreader event (SSE) in Beijing hospitals. Most patients in this period proved to be directly or indirectly linked with the index patient by traditional epidemiologic investigations. Molecular epidemiology, based on genome sequencing of the early isolates, also provided evidence that Beijing infections were closely related to those from the Guangdong epidemic (4). The second phase was marked by widespread transmission in healthcare facilities and communities, with incidence peaking in late April, followed by a dramatic decline in occurrence during the first week of May. The last probable case was noted on May 29, 2003 (5). During this phase, many case-patients had no apparent contact with SARS patients.

After the sequencing of the whole genome (6–9) information on viral strains from different geographic and temporal origins became available in GenBank. Comparative sequence analyses identified 5 loci, sequence variants of which segregated together as specific genotypic patterns, which could be used to define epidemic phases (10). All or some of the 5 loci were included in previous molecular epidemiologic studies (4,11–13), making them important genetic signatures to differentiate lineage-specific and temporal-specific patterns. In this study, we investigated the genetic variations of SARS-CoV in Beijing based on the 5-locus signature. Also, by sequence comparison among patients from 1 case cluster and different samples from 1 patient, the adaptable mutation of the virus in the host was further explored.

Methods

Participants

Study participants were recruited from 2 hospitals designated for SARS patients in Beijing. All of them fit the World Health Organization (WHO) case definition for probable SARS, i.e., temperature ≥38ºC, cough or shortness of breath, new pulmonary infiltrates on chest radiograph, and a history of exposure to a SARS patient or of

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living in an area of on-going SARS transmission (14). After informed consent was obtained, epidemiologic and clinical data were collected from the participants by using a standard data collection form with interview and medical record review. The information obtained included the following items: age, sex, occupation, medical history, time and nature of exposure, symptoms and physical findings, laboratory tests at admission to hospital, and outcomes on discharge or transfer. Patients also provided clinical specimens (sputum and stool) for SARS-CoV detection by RT-PCR assay with specific primers (COR1, COR2) recommended by WHO. Only the patients with positive RT-PCR results were included in the study.

Laboratory Methods

Specimens were analyzed by using RT-PCR techniques. Briefly, total RNA was extracted by using the QIAamp virus RNA mini kit (Qiagen, Hilden, Germany) as instructed by the manufacturer. RNA was used to synthesize cDNA with the SuperScript II RNase H^- reverse transcriptase system (Invitrogen, Carlsbad, CA, USA). Five sets of primers were used in nested PCR to amplify the fragments covering the 5-locus genetic signatures (17564, 21721, 22222, 23823, and 27827) (Table 1). Then, with the purified PCR products as templates and the second round primers as sequencing primers, the fragments were sequenced in ABI Prism 377 DNA sequencer (Applied Biosystems Inc, Foster City, CA, USA). Each PCR fragment was directly sequenced from both inward and outward directions, in duplicate.

All the original base data were processed for base calling, assembly, and editing by the SegMan II sequences analysis software of DNA Star package (DNASTAR, Madison, WI, USA). The comparisons with other sequences available from public database (GenBank) were made by using the default parameter of ClustalW (http://www.ebi.ac.uk/clustalw/). Single nucleotide variations (SNVs) were indicated, and the deduced amino acid changes were described.

Results

A total of 160 samples (81 stools and 79 sputum samples) from 62 patients with positive results by RT-PCR were included this study. Of these, 45 samples (36 sputum samples and 9 stools) from 29 patients (17 men and 12 women, with a median age of 32 years) yielded amplicons for the 5 targeted loci (Table 2). The patients came from 2 SARS-designated hospitals in Beijing, with disease onset ranging from March to May, 2003. Four patients had serious conditions during hospitalization, including pulmonary aggravation requiring oxygen ventilation or transfer to an intensive care unit. No patient died.

The sequences of the 45 positive specimens were compared with SARS-CoV genome sequences available from the public database (GenBank). The sequence variants in 5 loci (17564, 21721, 22222, 23823, and 27827) defined 3 kinds of motifs: GGCTC, TGTTT, and GATTC (Table 2). In addition, 4 new SNVs were identified at nucleotides 17620, 22077, 22589, and 27749 in >1 patient. These variations appeared independently in several isolates, which indicates that they are not RT-PCR artifacts. None of them had been previously reported, with 3 nucleotide substitutions leading to amino acid changes (Table 3).

Twelve patients in this study belonged to a cluster. They derived from an SSE indirectly linked with the earliest SARS patients in Beijing. The first 2 patients of this cluster, who became ill on March 10 and 21, respectively, harbored the GGCTC motif. The remaining patients, who became ill from March 31 to May 4, showed the TGTTT motif. Among patients outside of the cluster, 5 of 6 patients with onset date before April had the GGCTC motif, while the TGTTT motif became predominant later (9 of 11 patients until May 12). A new motif, GATTC, was found in 2 patients outside the cluster. In addition, no intrapatient variation was observed in the 5 amplicons from specimens collected at different times or from different sources (sputum or stools).

The possible role of genetic mutations in patients' prognosis was also investigated. The presence of nucleotide substitution was compared between 2 groups of patients: 1

Table 1. Primers used for nested polymerase chain reaction and sequencing

Position	Amplification region*		Primer sets (starting from 5′)
17564	17440–18281	Forward	ACGTCTATATTGGCGATCCTTGTGCAGACTTATGAAAACAATA
		Reverse	GTTTTGCATTAACTCTGGTGGTTAGTACCCACAGCATCTCTAGT
21721	21585–22304	Forward	GATGATGTTCAAGCTCCTAATTACCTTAACAGAGCATTTGAGTTCAG
		Reverse	CAACATACTTCATCTATGAGGGGTGTACCATTTTCATCATACTTGAG
22222	22177–22874	Forward	AGATGTAGTTCGTGATCTACCTTCTTAATGGCCAATAACAATTAAGA
		Reverse	CAAATTTTAGAGCCATTCTTACAGGGAGAAAGGCACATTAGATATGTC
23823	23455–24263	Forward	CGACACTTCTTATGAGTGCGATGCAGTTGATGTTGTTGTAAG
		Reverse	GCATTTGTGCTAGTTACCATACAGTGATGTTGTTGTAAGTGATTCTTG
27827	27449–28270	Forward	CCATCAGGAACATACGAGGGACCACTATTGGTGTTGATTG
		Reverse	TAGCACACACTTTGCTTTTGCAGTATTATTGGGTAAACCTTGG

*The nucleotide position was given with TOR2 as the reference strain (accession no. NC004718).

Table 2. Epidemiologic and phylogenetic data on 29 severe acute respiratory syndrome patients, Beijing, 2003*

Patient no. (sex, age [y])	Onset date†	Sampling date,† clinical sample	5-loci genotype	Other variant loci
1‡ (M, 25)	3/10	4/28, Sp	GGCTC	22589
2‡§ (F, 48)	3/21	4/28, Sp	GGCTC	22589, 27749
3 (M, 19)	3/31	4/28, Sp; 5/5, Sp	GGCTC	22589
4‡ (F, 34)	3/31	5/5, St; 4/28, Sp	TGTTT	17620, 22589
5‡§ (M, 21)	3/31	4/28, Sp; 5/5, Sp	TGTTT	22589
6‡ (F, 34)	4/2	4/28, Sp	TGTTT	22077
7 (F, 27)	4/2	4/28, Sp	GGCTC	22589
8‡ (M, 31)	4/3	4/28, Sp	TGTTT	22077, 22589, 27749
9‡ (M, 20)	4/5	5/5, Sp; 4/28, Sp	TGTTT	17620, 22077, 22589
10 (F, 23)	4/8	5/22, St; 5/15, Sp	GGCTC	22589
11§ (M, 47)	4/8	5/5-Sp; 5/5, St	GGCTC	22589
12 (M, 73)	4/9	4/28, Sp; 4/28, St	TGTTT	22589, 27749
13 (M, 54)	4/9	4/28, Sp; 4/28, St	GGCTC	22589, 27749
14‡ (F, 21)	4/11	4/28, Sp; 4/28, St; 5/5, Sp	TGTTT	
15§ (M, 61)	4/12	5/22, Sp	GATTC	22589
16‡ (F, 25)	4/15	5/5, Sp	TGTTT	17620, 22589, 27749
17 (M, 25)	4/17	5/5, Sp	TGTTT	22077, 22589, 27749
18 (F, 20)	4/18	4/28, Sp; 4/28, St	TGTTT	22589, 27749
19‡ (F, 25)	4/20	5/5, Sp; 5/5, St	TGTTT	22077, 22589,
20 (F, 34)	4/21	4/28, Sp; 5/5, Sp	TGTTT	22077
21 (M, 33)	4/23	5/5, Sp; 5/5 St; 4/28-Sp	TGTTT	17620, 22589
22 (M, 28)	4/24	5/5, Sp	TGTTT	22589, 27749
23 (M, 61)	5/1	5/15, Sp	GATTC	22589
24‡ (F, 31)	5/1	5/5, Sp	TGTTT	22077, 2589, 7749
25 (M, 25)	5/2	5/7, Sp	TGTTT	22077
26‡ (M, 25)	5/4	5/22, Sp	TGTTT	22077
27 (M, 19)	5/6	5/22, Sp	TGTTT	22589, 27749
28 (F, 28)	5/7	5/22, Sp	TGTTT	17620, 22589, 27749
29 (M, 22)	5/12	4/28, Sp; 5/5, Sp	TGTTT	22589, 27749

*F, female; M, male; Sp, sputum; St, stool.
†All dates are in 2003.
‡Patients were from the same cluster.
§Patients with adverse clinical outcome.

with good prognosis (absence of pulmonary aggravation; n = 25) and 1 with adverse outcome (pulmonary aggravation 8–12 days after onset of symptoms requiring oxygen ventilation or transfer to ICU; n = 4). No mutation was found associated with disease severity (Table 2).

Discussion

During the 2003 SARS epidemic, conventional epidemiologic investigation, aided by viral sequencing analysis, identified viral genetic signatures that are linked to geographic and temporal clusters of infection (4,10–12, 15–18). Findings of these studies are summarized in the Figure, connecting the worldwide epidemic to a transmission event in hotel M in Hong Kong in late February 2003.

Beijing had experienced the SARS epidemic from March to June; however, only a few Beijing strains from the early epidemic have been analyzed in previous studies. Our study is the first to provide phylogenetic information on Beijing strains from the early and middle epidemic, as well as the late epidemic, by using the 5-locus motif of previous studies. The series of mutations in the 5-locus motif observed in Beijing followed the same path as isolates in Guangdong Province and the worldwide epidemic, i.e., the early introduction of GACTC motif was followed by transition to a GGCTC motif, before switching to a stable TGTTT motif. The observation of the same series of mutations occurring in 2 separate locations at different times suggests a dominant process of viral adaptation to the host.

Table 3. Characterization of nucleotide (nt) substitutions in 29 severe acute respiratory syndrome patients, Beijing, China*

ORF or protein	Position†	nt substitution	aa change	No. patients
ORF 1b	17620	C→T	Leu→Ser	5
S protein	22077	G→T	Phe→Tyr	9
S protein	22589	C→T	Noncoding region	24
ORF 9	27749	G→A	Lys→Glu	12

*SARS, severe acute respiratory syndrome; ORF, open reading frame; aa, amino acid.
†The nt positions are numbered with TOR2 as reference strain (accession no. NC_004718).

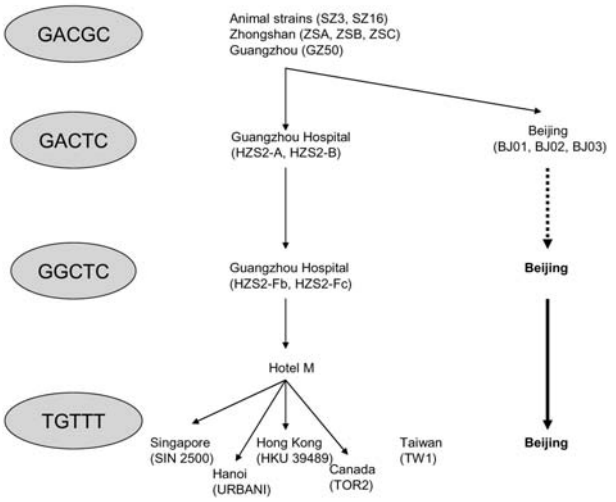

Figure. Epidemiologic and phylogenetic links between patients of different worldwide SARS outbreaks (4,10,11,12). New information that concerns the Beijing epidemic is represented in **boldface**. Epidemiologic links that are still speculative are in dotted lines.

Moreover, this finding can expand our understanding of SARS-CoV response to selection pressures in humans, since early Beijing isolates (BJ01, BJ02, and BJ 03), which are traceable to Guangdong, underwent an independent selection process and would not be subject to the same sampling bias caused by superspreading events in Hong Kong isolates. The GGCTC→TGTTT switch was observed among patients belonging to the same cluster in this study, which rules out the possibility of the coincidental superposition of 2 epidemics (GGCTC and TGTTT) coexisting in Beijing.

The mutations involved in the GGCTC→TGTTT switch are responsible for amino acid changes in a nonstructural protein (17564, region Orf1b) in S protein (21721 and 22222) and in a noncoding region (27827, X3). We were not able to identify a correlation between these changes and the clinical status of patients. We did not find sequence variations in specimens obtained from the same patients either collected at different times or among different specimen types, which suggests that within-individual variations are rare in the partial genome of this study, although the phenomenon was described in a previous study (15). A new motif, GATTC, which represents a new transitional motif between GACTC and TGTTT, was described on 2 occasions in patients who were not part of the cluster. Similarly, 4 new SNVs were identified at nucleotides 17620, 22077, 22589, 27749.

In summary, this study confirms the evolution of SARS-CoV strains towards a TGTTT motif in positions 17564, 21721, 22222, 23823, and 27827 in Beijing, as was observed in Guangdong province before the hotel M outbreak in Hong Kong. Whether this motif is associated with higher transmission or virulence remains to be elucidated.

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Malaria in Kenya's Western Highlands

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Records from tea estates in the Kericho district in Kenya show that malaria reemerged in the 1980s. Renewed epidemic activity coincided with the emergence of chloroquine-resistant *Plasmodium falciparum* malaria and may have been triggered by the failure of antimalarial drugs. Meteorologic changes, population movements, degradation of health services, and changes in *Anopheles* vector populations are possible contributing factors. The highland malaria epidemics of the 1940s were stopped largely by sporontocidal drugs, and combination chemotherapy has recently limited transmission. Antimalarial drugs can limit the pool of gametocytes available to infect mosquitoes during the brief transmission season.

Epidemic malaria is a term applied to describe *Plasmodium falciparum* transmission characteristics of the highlands of East Africa and the Horn of Africa. These areas are fringe regions between stable and unstable transmission, which are affected to some degree by annual variations in rainfall but primarily by low ambient temperature. Such areas are often densely populated and of economic and political importance because of their agricultural potential. Much attention has been given to the increasing frequency and clinical costs associated with epidemics among highland populations in Africa (1–3). We examine historical and contemporary data to define the long-term epidemiologic transition of malaria in 1 district of the western highlands of Kenya. We use these data in support of our hypothesis that drug resistance is a key element in highland malaria epidemics in East Africa, and we examine how past control might guide future efforts to reduce the clinical impact of epidemics.

Geography and Land Use

The Kericho district is located on the western side of the Great Rift Valley in a highland area near Lake Victoria (elevation 1,600–3,000 m above sea level) (Figure 1). The soils are deep and well drained. Because of adequate and reliable rainfall, the district can produce a surplus of crops (forestry and horticultural products, pyrethrum, cereals, fruit trees, tea). Tea is grown on self-contained farms, called estates, that usually have ≈1,000 workers tending ≤1 square mile of tea bushes. Two tea plantations (Figure 2), each consisting of 18 estates, employed 18,000–18,500 workers in 1998. Employees reside at the estate with 3 to 4 dependents each (4).

Migration and Mobility

Arrival of the railroad in the early 20th century and improvement of roads facilitated movement from the Kenyan coast and lake districts to the highlands, which were thought free of malaria. European settlement began

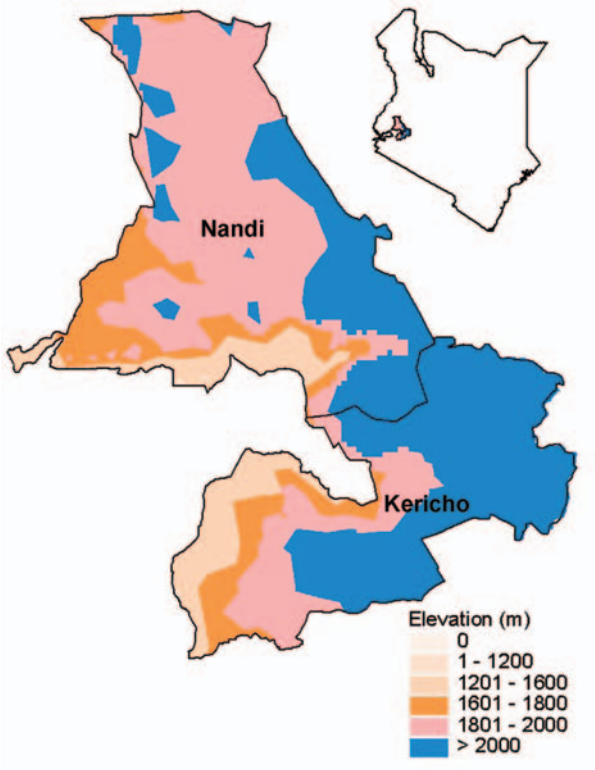

Figure 1. Map of Kenya showing Nandi and Kericho districts.

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Figure 2. Kericho, Kenya, tea plantation in 1998.

before World War I as an extension of other farming areas in the Rift Valley. A large proportion of the tea estate labor force were recruited from the Lake Victoria region (elevation <1,000 m) or from Kisii (elevation 1,500 m) in Nyanza province. The Lake Victoria region is an area of holoendemic malaria whose inhabitants often have asymptomatic infections (5). Tea pickers are given 1 month leave per year to return to their families in their natal village. Over time, dependents of the tea pickers have come to live on the estates. Travel back and forth from the highland estates to the Lake Victoria lowlands is common (4).

Climate

Variations in climate affect the distribution and abundance of malaria vectors. The effects of temperature on the transmission cycle are manifold, but its specific effect on sporogonic duration and mosquito survival is critical. When the temperature is <18°C, transmission is unlikely because few adult mosquitoes (0.28%) survive the 56 days required for sporogony at that temperature and mosquito abundance is limited by long larval duration. At 22°C, sporogony is completed in <3 weeks, and mosquito survival is sufficiently high (15%) for the transmission cycle to be completed. The potential number of infective mosquitoes reaches a peak at 30.6°C, after which it decreases rapidly. The relationship between mosquito abundance and rainfall is complex and best studied when temperature is not limiting. *Anopheles gambiae* s.l. breed more prolifically in temporary and turbid water bodies, while in permanent bodies predation is an important limitation. Rainfall is a good indicator of vectors, their survival, and the potential for malaria transmission. Long-term, complete meteorologic data are available from the Tea Research Foundation. Analysis of the meteorologic relationships of malaria in Kericho has been reported (6–8).

Health Service Provision and Drug Resistance

The public health sector in sub-Saharan Africa has been overwhelmed by AIDS, lack of money, and other problems. In Kenya, population increases of 3% to 4% per year, an inflationary economy in the face of fixed health service wages, and administrative failures have depleted public health services (9). However, this situation does not apply to the tea plantations, where private agricultural companies maintain a healthy, productive workforce (3). Whatever the cause of recent increases in malaria in the western highlands of Kenya, the tea plantation health systems have continued identifying, counting, and treating malaria infections among their workers. Since 2000, worker residences have been sprayed with insecticide every 4 months, primarily for pest control. This measure is unlikely to have differentially influenced the malaria data because both plantations were using the same regimen.

Chloroquine was used to treat uncomplicated malaria infections in Kenya from the 1950s until the national drug policy was changed in 1998 (10). Chloroquine resistance in Kenya was first documented in 1979 in a tourist who had visited Kenya. After resistance became widespread (11), chloroquine continued to be used as the first-line antimalarial drug for another 19 years. A 1985 survey indicated that parasites from the adjacent Nandi district were still sensitive to chloroquine (12), whereas by 1996 chloroquine was unable to clear 50% of clinical infections in children by day 7 (13).

Sources of Clinical Data since 1900

We used 3 principal sources of data. The first source was reports in the Kenya national archives. The second was local and international journal reports of malaria epidemics in the region. The third was hospital data from 2 adjacent tea plantations directly accessed to obtain the temporal and secular patterns of malaria in Kericho. Inpatient data have been located in admission registers of the hospital at tea plantation 1 from 1965 to 2004 (3,14). The second tea plantation hospital is adjacent to plantation 1 and since 1970 has

maintained a weekly infectious disease notification system that identifies all blood smear–positive malaria cases. Although the system includes inpatients, it consists mostly of outpatients. Although the 2 hospitals are adjacent, the populations served are separated according to the company of employment. Combined, these reports, data, and anecdotes build a qualitative and quantitative picture of Kericho epidemics during the 20th century.

Epidemics

1918–1919 and 1928

During the 19th century, local malaria transmission was nonexistent or negligible (15). Increasing trade and transport led to major population movements from 1906 onwards. Movement of people associated with the opening of civil and military posts probably introduced malaria into the highlands. During World War I, soldiers from Kericho were recruited to fight against the German forces in Tanganyika. With troop demobilization and resettlement in 1918 and 1919, a malaria epidemic followed the influenza pandemic (15). Further development in this region, including the completion of the Ugandan railway from the Mau escarpment to the malaria-endemic Lake Victoria region, increased movement of people and parasites.

In 1928, an epidemic in Kericho district that involved 1,727 hospital case-patients occurred (15). Epidemics were also reported in 1931, 1932, 1934, 1937, and 1940 (5). Malaria was much more severe in highland workers than in their presumably functionally immune counterparts from Lake Victoria (16). Health authorities used mass drug administration for epidemic control, dispensing 57,600 ten-grain (600 mg) doses of quinine in 1 month (16).

1939–1948 and Introduction of Control

Epidemic malaria became a major infection on the tea estates during World War II (17) because soldiers returning from Ethiopia through the malarious coastal areas were encamped along the adjacent railway (18). Epidemics appeared to be caused by the large pool of parasitemic soldiers who infected the local mosquitoes during the brief mid-year period suitable for malaria transmission at high altitudes and occurred mostly within the military camp, the township of Kericho, and the tea estates, while sparing most "native reserve areas" (18). Removal of the military camp at the end of the war did not stop the now indigenous malaria transmission (17).

Given apparently fragile malaria transmission in the agriculturally important western Kenyan highlands, interventions in Kenya were often first tried in Kericho. Proguanil was a safe chemoprophylactic agent that had just been developed as the result of an emergency wartime project of the British and Australian armies. The medical officer for plantation 1, D. Strangeways-Dixon, used mass drug administration of proguanil, as the British army had been doing in war areas (Figure 3) (17). In March 1948 (before the epidemic in the rest of the district), prophylactic proguanil was given twice a week (a single 100-mg tablet) to all plantation 1 employees and their dependents. This strategy decreased malaria incidence in June and July 1948 (0.5 and 2.0 cases per 1,000 population, respectively, compared with nearly 10 times that number the previous year). Two rounds of house spraying with DDT in 1949 (March and June) complemented the prophylactic measures. During this period, malaria was virtually controlled. Proguanil did not eliminate epidemics, however, and in 1952 the prolonged rainy season shifted the epidemic period from July to January, and malaria returned before the start of proguanil prophylaxis. Reinstitution of proguanil resulted in epidemic control, probably by blocking further transmission.

Intermission, 1960–1980

Malaria in the Kericho district was largely imported by persons traveling back from Lake Victoria. For the years in which data were provided in the medical officer's reports (1966, 1967, 1970, 1975, and 1976), 95 deaths occurred among 5,686 malaria patients admitted to Kericho district hospital (case-fatality rate 1.7%). Recent studies on the Kenyan coast showed an overall case-fatality rate of 3.5%, varying greatly depending on the clinical syndrome that caused hospitalization (19). At the Kericho tea estates, malaria ceased to be a major problem from 1960 to 1980, which roughly coincides with the period during which chloroquine was fully effective against *P. falciparum* malaria.

Reemergence of Seasonal Epidemics

In Kericho, annual mid-year malaria epidemics began in 1990 at plantation 1, although epidemic peaks were evident in 1981 at plantation 2 (Figure 4). Increasing malaria

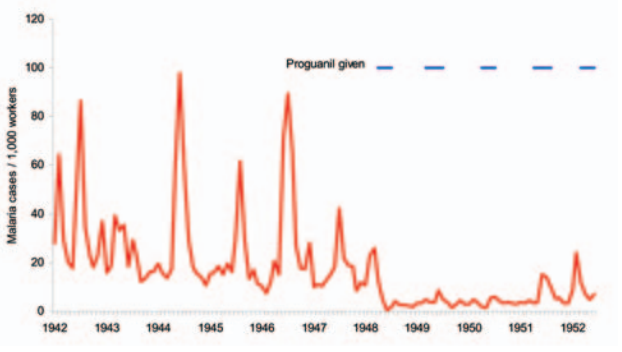

Figure 3. Monthly malaria cases on the Kericho tea estates, Kenya, 1942–1952, showing mass administration of proguanil. Data were obtained from Strangeways-Dixon (17) and hospital records.

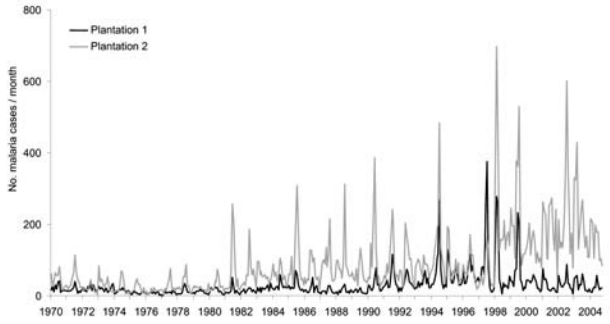

Figure 4. Monthly malaria incidence at 2 adjacent tea plantations in Kericho, Kenya, 1970–2004. Plantation 1 data are from inpatient admission registers, and plantation 2 data are from weekly malaria slide reports that include outpatients.

incidence was not related to overall warmer temperatures but still depended on the annual pattern seen in the 1940s in which malaria would increase after the rains in March through April and decrease after the onset of cool weather in July (8,9). Malaria admissions at plantation 1 increased accordingly, from 5% of all admissions in 1970 to 47% in 1998 (Figure 5). However, before this increase, other changes were noted such as increase in the percentage of malaria inpatients of highland (>1,500 m) origin in 1981 and decrease in the adult-to-child ratio of inpatients in the mid-1980s (Figure 5) (20). A high adult-to-child ratio of malaria indicates a less immune population in which adults become symptomatic when infected with malaria, which is unusual in areas of high transmission (20). The case-fatality rate for malaria admissions at plantation 1 from 1965 to 1972 was 1.3%. The rate increased to 6% from 1990 to 1998, despite good medical and nursing inpatient care (14). On the Kericho tea estates, most malaria deaths are the result of severe anemia in young children, not cerebral malaria (21). In other areas of Kenya, chloroquine resistance increased case-fatality rates, and this trend could be reversed by using more effective antimalarial drugs (22).

Malaria incidence on the 2 adjacent tea plantations generally followed each other closely, despite separate worker populations and medical systems. A striking divergence, however, was noted in 2002, when heavy rains followed 2 years of low malaria incidence at both plantations because of drought (Figure 6) (23). This difference was confirmed when malaria incidence between outpatient malaria patient visits at plantation 1 (4) and outpatient visits at the Kericho district hospital were compared (24). Absence of increased malaria at plantation 1 in 2002, while epidemic conditions existed in both adjacent plantation 2 and the surrounding district, is difficult to explain on the basis of weather, human migration, medical access, or vector population changes (24). One change that coincided with the abatement of malaria at plantation 1 was a switch in first-line malaria treatment for outpatients from chloroquine to sulfadoxine-pyrimethamine (SP) in 1999 (Figure 7). A prospective malaria surveillance project involving febrile outpatients on plantation 1 showed that after the substitution of SP for chloroquine, *P. falciparum* gametocyte rates decreased in young children from 5% (1998) to <1% (2002), while remaining unchanged in adults (<1%) (4). This result occurred in the absence of any change in asexual parasitemia (≈50% of febrile pediatric outpatients were positive) over the same period. Although no simultaneous surveillance of malaria parasites occurred at plantation 2, which switched to SP in mid-2000, the appearance of epidemic malaria in the 1980s, as well as continued seasonal malaria on plantation 2 after 2000, suggests that other factors contributed to local transmission.

At least 3 chemotherapeutic explanations exist for progressive decrease in gametocytes on plantation 1 without a significant effect on the percentage of febrile children with asexual parasites. 1) Chloroquine-resistant *P. falciparum* strains produce more gametocytes and infect more mosquitoes than chloroquine-sensitive strains, which may explain why chloroquine resistance has spread rapidly across Africa (25). The removal of chloroquine drug pressure on plantation 1 in 1999 likely reduced the number of infected mosquitoes. 2) Combination chemotherapy with pyrimethamine increased from <20% of all treatment courses on plantation 1 in 1998 to 85% of all treatment courses by 2001. Pyrimethamine blocks the development of parasites in the mosquito (26). Even increased numbers of gametocytes produced after SP treatment are transmitted poorly through the mosquito because of the antifolate action of pyrimethamine (27,28). The sporontocidal effect of the pyrimethamine component of SP is separate from its ability to clear parasites from the blood. Pyrimethamine

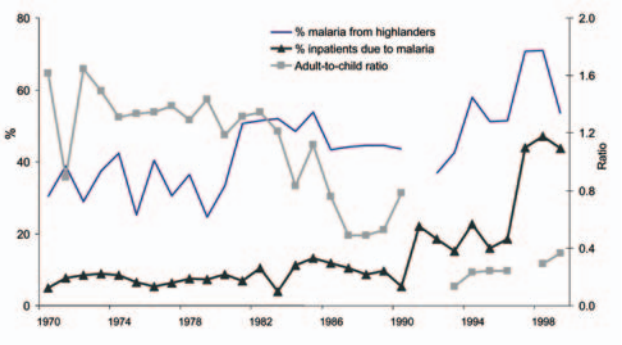

Figure 5. Annual malaria inpatient characteristics from tea plantation 1 in Kericho, Kenya, 1970–1999. Percentage of malaria patients compared with percentage of all hospital admissions, percentage of malaria inpatients of highland (>1,500 m) family origin, and ratio of adults to children (<15 years of age) of all malaria inpatients are shown annually as collected from the same admission registers. Gaps indicate missing data.

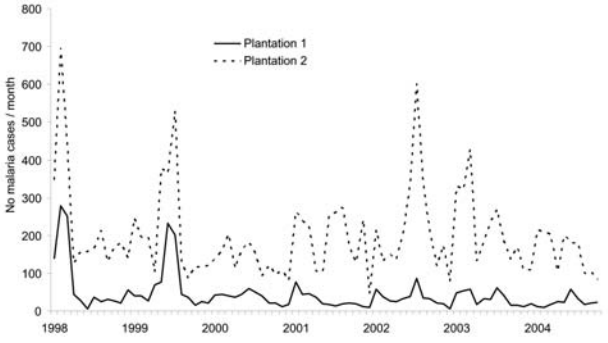

Figure 6. Monthly malaria incidence at 2 adjacent tea plantations in Kericho, Kenya, 1998–2004. Shown are the same data in Figure 4 in an expanded scale. See section on sources of clinical data since 1900 to distinguish outpatient and inpatient composition.

had been used successfully as part of mass drug administration to block transmission in the adjacent Nandi district during the 1950s (29). 3) The use of artemisinin-containing combinations increased on plantation 1 from 3% of all treatment courses in 2001 to 9% in 2002, whereas artemisinin combinations represented <2% of all antimalarial drugs purchased at plantation 2 in 2002. From 2000 to 2002, 52% of hospitalized malaria patients at plantation 1 received an artemisinin compound compared with 36% at plantation 2, but this percentage represented a relatively small number of total malaria cases. Artemisinin compounds killed gametocytes and blocked transmission in Thailand (30) and The Gambia (31). Increased use of artemisinin combinations primarily for sick children on plantation 1 would have limited the pool of gametocytes available to infect mosquitoes. The combined effect of discontinued chloroquine and increased use of SP and artemisinin combinations resulted in too few infective mosquitoes to start an epidemic during the brief highland transmission season.

Combining the Evidence

Reemergence of mid-year malaria epidemics in the western Kenyan highlands has progressed to annual incident peaks, which suggests that this area is now one of seasonal rather than epidemic malaria (9,24). Reemergence is the proper term because mid-year malaria increases were common during World War II, and Kericho has not been free of malaria for over 60 years (Figure 3). What has changed to cause malaria to revert to its earlier pattern after a long quiescence? Possible factors are changes in climate variability, population movements, decrease in quality of health services, changes in mosquito vectors, and antimalarial drug resistance.

Doubts exist as to the plausibility of climate change as proximate cause of epidemic malaria because global warming cannot explain the World War II epidemics. Dramatic increases in malaria during the 1990s are not mirrored by prospectively collected climate data from Kericho (7). No warming trend or increase in temperature records that extend back nearly a century was observed at several points in East Africa (8). Extensive comparison of temperature, rainfall, and malaria records in Kericho after 1965 has not indicated any convincing multiple-year link between either rainfall or temperature and malaria (32). Furthermore, continent-wide trends in malaria transmission suitability during the 20th century do not show the Kenyan highlands as an area of substantial change (33,34).

Malaria epidemics during World War II were probably the result of population movement; stationing of soldiers in Kericho after military operations in malarious areas started the epidemic. This epidemic was presumably the result of the focal concentration of imported human gametocyte carriers who infected mosquitoes during the brief mid-year period when local transmission was possible. Extensive travel between holoendemic Lake Victoria and Kericho has occurred at least since World War II. Prospective data suggest that persons returning from the malaria-endemic lowlands transport malaria parasites up the mountain to Kericho as asymptomatic or symptomatic infections (4). Population movements alone cannot explain the lack of substantial malaria from 1960 to 1980. Although population movements are important in introducing malaria into a malaria-free area, the highlands of western Kenya have not been free of malaria infections since World War I.

In the last 25 years, Africa has seen a decrease in the quality and quantity of public health services as result of rapidly expanding population, decrease in purchasing power of African currencies, severe institutional or organizational problems, and increase in HIV infection. Yet Kericho tea plantations have maintained high-quality

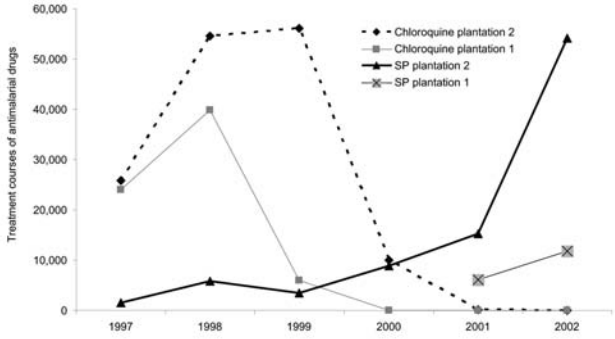

Figure 7. Annual antimalarial drug purchases recorded in the respective tea plantation hospital pharmacy records, Kericho, Kenya, 1997–2002, showing the discontinuation of chloroquine as sulfadoxine-pyrimethamine (SP) became first-line therapy. Records of SP purchases at plantation 1 prior to 2001 were not available.

health services to a defined population (3). Whatever the problems of the African district hospital, the interest of the tea companies in maintaining healthy workers eliminates decrease in healthcare services as an explanation of highland malaria epidemics.

Three entomologic surveys from 1946 to 1948 established that *An. gambiae* is the principal vector in Kericho with *An. funestus* playing a minor role (35). Investigations from 1998 to 2000 to characterize the mosquito vectors on the Kericho tea estates confirm the findings of Garnham obtained >50 years ago (18), which indicated that small numbers of *An. gambiae* in houses are malaria vectors (R. Dunton, pers. comm.). Unpublished studies conducted by Garnham on the same tea estates in the 1940s indicated that residual insecticide spraying of house walls could be very effective in controlling malaria. Modern vector control measures have not had any apparent differential effect on malaria in the 2 plantations, but their potential has not been fully explored. Similarly, long-lasting insecticide-impregnated bed nets have not been systematically implemented. Therefore, any major shift in species composition of the malaria vectors is unlikely to have contributed to malaria reemergence.

Drug resistance, specifically chloroquine resistance, may be key in the increase of highland malaria. The period without major malaria epidemics in the Kenyan highlands extends from chloroquine introduction until chloroquine resistance became widespread. Just as the parasites from nearby military camps caused the epidemics of World War II, the inability of chloroquine to eliminate parasites recreated a similar situation during the 1990s in the resident population. The increase in death rates of hospitalized malaria patients since 1990 also indicates chloroquine resistance and has been observed in other parts of Africa (36). Population increases that contribute to a decrease in access to health care can explain some highland malaria epidemiology, but this did not occur in Kericho, where number of workers and resident dependents has been stable (20). Drug resistance to chloroquine remains the leading explanation for the reemergence of highland malaria in the Kericho tea plantations and has been implicated in nearby areas (37). The absence of an epidemic on plantation 1 after use of more effective antimalarial drugs is additional evidence that the prime factor influencing highland malaria in Kericho is antimalarial drug resistance. Drug resistance is not a universal explanation for epidemic malaria in the highlands of East Africa, especially given the highly controlled nature of the tea estates, which is atypical of rural Africa. Kabale, Uganda (38), and Nandi, Kenya (39), are reminders that malaria is a focal disease susceptible to many factors that vary by specific location and season.

The Future

Increasing chloroquine failure rates directly influence clinical outcomes and affect public health consequences in areas of marginal malaria transmission, such as Kericho. Small changes in the number of persons carrying infective gametocytes can initiate malaria epidemics through increased mosquito transmission. SP lowers transmission pressure by curing more infections and decreasing the infectivity of surviving gametocytes by the sporontocidal action of pyrimethamine (26–28). However, SP fails to cure uncomplicated malaria in East Africa and can only be viewed as an interim replacement for chloroquine (40). Appropriate case management of uncomplicated malaria is still a valuable preventive measure for malaria epidemics. The key point is to cure enough infections so that the remaining parasites cannot rapidly expand during seasonal transmission. Therefore, the choice of first-line malaria chemotherapy is crucial to cure those treated, as well as allow the maximum number of persons to be treated. Epidemic-prone areas of marginal transmission are good places to examine the public health consequences of treatment options because a high proportion of infections progress to symptomatic malaria. When multidrug-resistant malaria was encountered on the Thailand–Burma border, the impending epidemic was aborted by use of artemisinin combination therapy (ACT), which cured patients and blocked transmission by killing gametocytes (30). The most promising African example of ACT is from KwaZulu-Natal, where malaria transmission on the South Africa–Mozambique border was substantially blocked by sequentially replacing chloroquine with SP and then with ACT supplemented with residual insecticide spraying (41). Effective antimalarial drugs are not just good for sick African children, they can also help decrease transmission pressure in areas of seasonal transmission and stop the increasingly steep spiral of drug resistance that has now left sub-Saharan Africa without readily available treatment for uncomplicated malaria. Effective drug combinations are urgently needed not only to prevent African children from dying of malaria, but also to prevent highland malaria epidemics.

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Protective Behavior and West Nile Virus Risk

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We conducted a cross-sectional, household survey in Oakville, Ontario, where an outbreak of West Nile virus (WNV) in 2002 led to an unprecedented number of cases of meningitis and encephalitis. Practicing ≥2 personal protective behavior traits reduced the risk for WNV infection by half.

Little is known about risk factors for infection with West Nile virus (WNV). Data about the effect of personal protective behavior traits recommended by public health agencies, such as wearing long sleeves and long pants, using mosquito repellent, and avoidance of mosquito areas, are sparse (1).

A household-based seroprevalence survey in Oakville, Ontario, where a large outbreak of WNV occurred in the summer of 2002, allowed us to assess modifiable risk factors for WNV infection. Oakville is located in Halton, a region that had the highest reported incidence of clinical WNV infection in Ontario in the 2002 season. Sixty cases (58 confirmed and 2 probable) occurred in a population of almost 400,000, with onset during the months of August and September 2002 (Figure 1). A peak in dead crow sightings in Halton (600 per week) occurred 5 weeks before the peak in human cases. Within this region, most cases occurred in south Oakville, in the L6L and L6K forward sortation areas (FSAs, i.e., the first 3 digits of the postal code) (Figure 2). We hypothesized that personal protective and source-reduction behavior would be associated with reduced risk for WNV infection.

The Study

The survey was conducted from March to April 2003. Households in the L6L and L6K FSAs of south Oakville were selected with random digit dialing. Within households, a randomly selected household member ≥18 years of age was invited to participate. Given that pediatric neuroinvasive disease is rare, children were excluded (2). The 2001 census population of these areas that was ≥18 years of age was 30,467.

After verbal consent was obtained, respondents were administered a standardized telephone survey. Survey data were collected for respondents who resided in the study area from July 1 to September 30, 2002. Single serum samples were collected from March 23 to June 5, 2003 (specimen collection was interrupted from March 29 to April 16 because of severe acute respiratory syndrome), from persons who had completed the survey. Respondents were unaware of their serologic status at the time of the telephone interview, which reduced the possibility for recall bias. Samples were collected and stored at –70°C until they were tested. Each sample was tested with Centers for Disease Control and Prevention WNV enzyme immunoassay immunoglobulin (Ig) G. Reactive samples were forwarded to Health Canada's National Viral Zoonotic Laboratory in Winnipeg for plaque reduction neutralization tests (PRNT) against West Nile, dengue, and St. Louis encephalitis viruses (3). Since our case definition relied on IgG, a positive result may have been caused by infection before the outbreak. However, the prevalence would have been low and would not likely affect our results; surveillance for WNV in Ontario began in 2000, and no positive clinical specimen was seen until the 2002 outbreak (4). The ethics review board at McMaster University approved the study.

Based on an assumed population of 30,500, for a prevalence as low as 1%, a sample of 1,500 allows for 95% confidence interval (CI) from 0.5% to 1.5%, and for a prevalence as high as 4% the sample allows 95% CI from 3% to 5%. Initially, 1,500 persons completed the survey, but not all consented to provide a blood sample. As a result, an additional 150 persons were surveyed in April of 2003 to achieve the required sample. Of the 1,650 persons surveyed, 1,505 (91%) consented to provide a blood sample. This fraction represented 25% of persons initially contacted about the study. No significant differences were found in demographic characteristics, so the 2 groups were

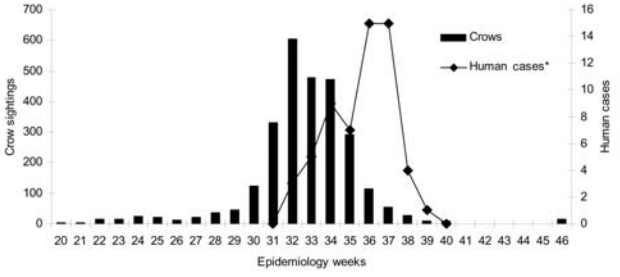

Figure 1. Reported human West Nile virus disease cases and dead crow sightings in Halton Region, May to November 2002, by epidemiology week.

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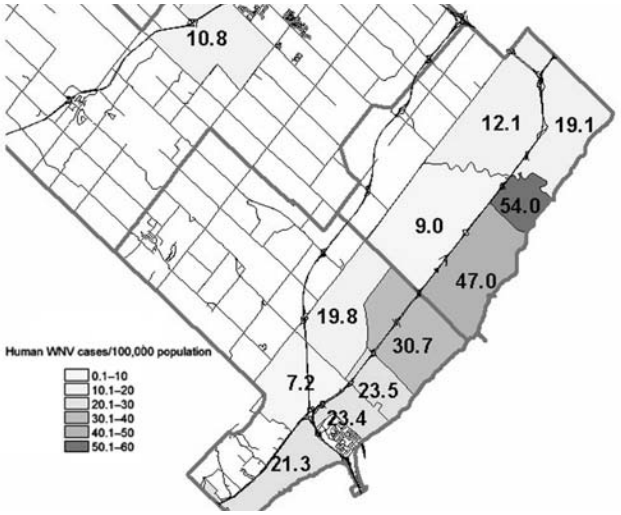

Figure 2. Calculated incidence of human West Nile virus (WNV) cases in south Halton, 2002. The incidence was 47 cases per 100,000 in the L6L forward sortation area (FSA) and 54 cases per 100,000 in the LKL FSA.

pooled for subsequent analysis. Because our sample did not correspond in age to the 2001 population (Table 1), we standardized our sample by using age-specific WNV seroprevalences.

To assess risk factors for WNV infection, we conducted a univariate analysis with chi-square test to assess categorical variables and Student *t* test to assess differences between infected and uninfected persons. Multivariable analysis with logistic regression was performed by using a backwards, stepwise approach, selecting 1 variable from each category to include in the model (indoor exposures, outdoor exposures, personal behavior, source-reduction behavior) if the p value was <0.10.

Forty-six (3.1% [95% CI 2.2%–4.0%]) of the 1,505 persons who provided a blood sample tested positive for WNV IgG, which was confirmed by PRNT. Two (6%) respondents 18–24 years of age, 7 (2%) respondents 25–44 years of age, 26 (4%) respondents 45–64 years of age, and 11 (2%) respondents ≥65 years of age were infected. In addition to the 46 participants, 14 persons were positive for WNV by IgG enzyme-linked immunosorbent assay but were negative by PRNT. Of these, 11 showed evidence of dengue IgG on PRNT confirmatory testing. No positive respondent had evidence of antibodies to St. Louis encephalitis virus on PRNT testing. The overall estimate of 3.1% did not change based on the 2001 census after adjusting for age.

Within the 2 FSAs from which the sample was drawn were 6 patients with encephalitis (all hospitalized), 5 with meningitis (1 hospitalized), and 8 with WNV fever (1 hospitalized). The calculated rate of WNV illnesses was 47 per 100,000 population in the L6L area and 54 per 100,000 in the L6K area (Figure 2). Cases were defined by the attending physician's diagnosis. No cases of meningitis or encephalitis were seen in persons <50 years of age. Five cases of meningitis and 1 case of encephalitis were seen in persons 50–64 years of age; 2 cases of encephalitis were seen among those 65–74 years, and 3 cases of encephalitis were seen in persons ≥75 years. Cases were ascertained by the Halton Region Health Department, which did epidemiologic follow-up on all patients with positive WNV serologic results. If we extrapolate the 2.2%–4.0% range to the entire population of adults in the areas studied (30,467), an estimated 670–1,219 persons were infected with WNV in the L6L and L6K areas in the summer of 2002. The ratio of persons with severe illness (defined as meningitis or encephalitis) to asymptomatic or mild cases is, therefore, 1:85 (95% CI 1:60–1:110).

Results of the univariate analysis to assess modifiable risk factors for infection are shown in Table 2. Having an open deck or unscreened porch, time spent outside at dusk or dawn on a work day, time spent outside at dusk or dawn on a nonwork day, and total time spent outside on a nonwork day were associated with WNV infection. Personal behavior associated with WNV infection included rarely or never avoiding areas where mosquitoes are likely to be a problem, rarely or never avoiding going outdoors, and

Table 1. Age and sex of south Oakville, Ontario, survey respondents compared to 2001 census population

Characteristic	Respondents, n (%) (N = 1,650)	2001 population age ≥18 years, n (%) (N = 30,467)
Sex		
Female	827 (50)	16,015 (53)
Male	823 (50)	14,452 (47)
Age (y)		
18–24	31 (2)	4,045 (13)
25–44	404 (24)	10,740 (34)
45–64	679 (41)	9,465 (30)
≥65	531 (32)	7,510 (24)
Education		
Completed high school	1,519 (92)	27,040 (93)
Did not complete high school	116 (7)	2,085 (7)
No answer	15 (1)	

Table 2. Risk factors for West Nile virus (WNV) infection among household members in south Oakville, Ontario*

Characteristic	No. (%) respondents or mean (SD)		OR (95% CI), p value
	Seropositive (n = 46)	Seronegative (n = 1,459)	
Indoor exposures			
Open deck or unscreened porch on home	40 (87)	1074 (74)	2.36 (0.99–6.9), 0.04
Tears in screens	12 (26)	343 (24)	1.14 (0.55–2.32), 0.69
Mosquitoes in home ≥1×/wk	10 (22)	314 (22)	1.01 (0.46–2.14), 0.98
Outdoor exposures			
Time outside at dusk or dawn on work day (h)	2.7 (1.5)	2.1(1.4)	1.32† (1.09–1.58), 0.004
Total time outside on a work day (h)	6.01 (3.9)	5.0 (3.4)	1.08† (1.00–1.16), 0.066
Time spent outside at dusk or dawn on a nonwork day (h)	3.1 (1.9)	2.2 (1.3)	1.48† (1.23–1.78), 0.001
Time total outside on a nonwork day (h)	8.2 (4.5)	6.7 (3.4)	1.13† (1.04 to 1.22), 0.003
Personal behavior			
Rarely or never avoid areas where mosquitoes are likely to be a problem	30 (65)	685 (47)	2.11 (1.10–4.08), 0.015
Rarely or never avoid going outdoors	43 (93)	1,190 (82)	3.2 (1.01–16.20), 0.041
Rarely or never wear long sleeves or long pants when outdoors	30 (65)	715 (49)	1.94 (1.01–3.76), 0.031
Rarely or never wear mosquito repellent when outdoors ≥30 min	31(67)	944 (65)	1.12 (0.58–2.19), 0.73
Practice ≥2 personal protective behavior traits‡	19 (41)	894 (61)	0.44 (0.23–0.83), 0.005
Source-reduction behavior			
Drain objects that may collect water	13 (28)	457 (31)	0.86 (0.43–1.71), 0.65
Check and clean gutters	29 (63)	1,009 (69)	0.75 (0.40–1.45), 0.36
Use bug lamps/bug zappers	7 (15)	132 (9)	1.80 (0.67–4.17), 0.156
Practice ≥2 source-reduction behavior traits	30 (65)	1,044 (72)	0.74 (0.38–1.43), 0.33

*OR, odds ratio; CI, confidence interval.
†Odds of WNV infection per hour spent outdoors.
‡Avoiding mosquitoes, wearing long sleeves and long pants, using mosquito repellent.

rarely or never wearing long sleeves or long pants when outdoors. However, when ≥2 personal risk reduction behavior traits were followed, the effect was protective.

The following variables were entered in the multivariate model: open deck or unscreened porch, time spent outside at dusk or dawn on a nonwork day, and practicing ≥2 personal protective behavior traits. Time spent outside at dusk or dawn on a nonwork day (adjusted odds ratio [OR] 1.47 per hour, 95% CI 1.22–1.8, p = 0.001) and practicing ≥2 personal protective behavior traits (adjusted OR 0.46, 95% CI 0.25–0.84, p = 0.011) were kept in the final model.

Conclusions

We found in multivariable analysis that respondents who practiced ≥2 personal protective behavior traits (avoidance of exposure to mosquitoes, wearing long sleeves and pants, using mosquito repellent) had ≈50% reduction in risk of infection. We also found that time spent outside at dusk or dawn on a nonwork day was a significant risk factor for WNV infection, which is consistent with findings from a previous report (1). Finding mosquitoes in the home was not associated with WNV infection, as it was in a previous report (5). The seroprevalence in Oakville in 2002 (3%) was within the range of previous reports (1,6,7).

Given the emerging evidence on the long-term sequelae of WNV infection (8–13), preventing WNV infection is a public health priority. This study is the first to provide evidence to support the benefit of personal protective behavior in reducing risk for WNV infection.

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West Nile Virus Detection in Kidney, Cloacal, and Nasopharyngeal Specimens

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We compared kidney tissue samples and cloacal and nasopharyngeal swab samples from field-collected dead crows and blue jays for West Nile virus surveillance. Compared to tissue samples, 35% more swab samples were false negative. Swab samples were usually positive only when the corresponding tissue sample was strongly positive.

Monitoring and surveillance of West Nile virus (WNV) prevalence increasingly depends on the early detection of WNV infection in crows, blue jays, and other members of the avian family *Corvidae* on the basis of reports of dead birds. The virus in these birds usually precedes appearance of WNV in humans and can be an early warning of potential human infection (1–3). After the initial finding of WNV in the United States in 1999, various laboratory techniques have been developed and employed to detect WNV in avian tissue. A WNV-specific RNA assay by TaqMan reverse transcriptase polymerase chain reaction (RT-PCR) has been described (4). This protocol has gained widespread recognition because of its high specificity, sensitivity, and speed. The sensitivity of the TaqMan RT-PCR is equal to, or better than, that of the Vero plaque assay method (5). WNV activity has increased in the United States since 1999; various approaches are being used to fully understand and appropriately respond to the infection (6).

WNV can be detected in a wide variety of bird tissue, such as heart, liver, lung, and spleen. The kidney also provides good specimen material for WNV detection (5). Brain tissue is the most sensitive target organ for detecting WNV with the TaqMan RT-PCR assay. In Ohio, where 31 persons died of WNV in 2002, kidney tissue was removed from >2,500 American crows (*Corvus brachyrhynchos*) and blue jays (*Cynocitta cristata*) to test for WNV. These birds are among the most susceptible species to WNV infection and have high death rates. For field-collected avian samples in Ohio, kidney tissue has been the sample of choice to detect WNV by using RT-PCR, mainly because of the practical ease and convenience of sampling kidney tissue specimens compared to brain tissue specimens.

We examined the suitability of using other specimens that are easier to collect because harvesting tissue invasively is labor intensive, the chance of cross contamination of samples will be minimized, and the possibility of exposing laboratory workers to infection will be reduced. Some researchers hypothesized that cloacal and oral swabs from avian carcasses could replace brain samples, the preferred tissue to test for WNV infection in corvid carcasses (5,7). In this study, we compared the suitability of testing kidney tissue and cloacal and nasopharyngeal swab specimens from field-collected dead birds to detect WNV in crows and blue jays.

The Study

During the 2002 WNV surveillance season, dead crows and blue jays were collected throughout Ohio. The dead birds were wrapped in plastic bags and hand-delivered or shipped in refrigerated containers to the Animal Disease Diagnostic Laboratory of the Ohio Department of Agriculture. In the laboratory, the cloacal and nasopharyngeal areas were swabbed from each bird individually with standard cotton applicators. The swab from each dead bird tissue was put into a separate prelabeled 12 × 75 mm tube containing 0.5 mL BA-1 medium (M-199 salts, 1.0 % bovine serum albumin, 350 mg/L sodium bicarbonate, 100 units/mL penicillin, 100 mg/L streptomycin, and 1.0 mg/L amphotericin in 0.05 mol/L Tris [hydroxymethyl aminomethane], pH 7.6). The kidneys of each of these birds were harvested after evisceration, and specimen samples were put into individual vials. The time of death of the field-collected crows and blue jays could not be ascertained by the collectors but was generally believed to be within 48 hours postmortem. Decomposed carcasses were not accepted for testing. The kidney tissue specimens and cloacal swab and nasopharyngeal swab specimens from the dead birds were stored at –70°C until tested.

To test avian kidneys for WNV with RT-PCR, ≈0.4 g of kidney sample from each bird was homogenized in a prelabeled, snap-cap vial containing 0.5 mL BA-1 medium and 2 ball bearing caliber air gun shot pellets. Vials for processing were centrifuged, and 75 μL of the supernatant from each sample was used for subsequent viral RNA extraction and purification by using the QIAamp Viral RNA minikit according to the manufacturer's recommended protocol (Qiagen, Valencia, CA, USA). The RNA extracts were assayed by a TaqMan RT-PCR with a TaqMan reverse transcriptase-PCR kit (Applied Biosystems, Foster City, CA, USA). For the TaqMan

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assay, primers and probes with the following nucleotide sequences (5′–3′) as previously described by Lanciotti et al. (2000) were used: forward primer CAGACCACGCTACGGCG, reverse primer CTAGGGCCGCGTGGG, and FAM/TAMRA probe TCTGCGGAGAGTGCAGTCTGCGAT. Thermal cycling was performed with the Bio-Rad i-Cycler iQ Real-Time detection system (Bio-Rad Laboratories, Hercules, CA, USA). At the end of the reaction, the amplification plot generated was viewed on a log scale with the system's default threshold. Any sample with a threshold cycle (C_T) of ≤35 was considered to be positive for WNV. This value corresponds to the detection of ≥1–10 PFU in WNV-infected specimens (4).

After the RT-PCR preliminary assays on the kidney samples were conducted, 100 of the avian kidney samples that had tested positive for WNV were randomly selected for further investigation; 61 of the samples were from crows and 39 were from blue jays. The corresponding 100 cloacal and 100 nasopharyngeal swab samples of these positive birds were additionally tested for WNV. In the test, each cloacal or nasopharyngeal swab sample was thoroughly mixed in the BA-1 medium by vortexing. As with the kidney samples, a 75.0-μL aliquot of this medium was used for viral RNA extraction and purification, as well as RT-PCR amplification as described above. Statistical analyses of the results from the tests were performed by using the SPSS for Windows release 10.0.1 (SPSS Inc., Chicago, IL, USA) to analyze the data.

The 100 positive kidney samples had various C_T values (the cycle at which the fluorescence rises appreciably above the background level), which ranged between 15.9 and 35.0. When all of the 100 positive kidney samples were divided into 3 categories of "high" (C_T 15.0–21.9), "medium" (C_T 22.0–28.9), or "low" (C_T 29.0–35.0) positive results based on their threshold cycle values, 57.0%, 27.0%, and 16.0% of the kidney samples fell into these respective positive groups (Table). Because no meaningful difference was seen between the test results of the crows and the blue jays in these categories, the data from both bird species were combined in our analyses. None of the cloacal or nasopharyngeal swab samples were in the high-positive group. Seventy-seven percent of the cloacal and nasopharyngeal swab samples were either in the low-positive or negative categories. The mean C_T value of the kidney samples was ≤0.01 lower than those of the cloacal and nasopharyngeal samples. Contrary to earlier findings (8) in which oropharyngeal swabs were more sensitive than cloacal swabs by using the VecTest antigen-capture assay, we found no appreciable difference in our study between the C_T values of the cloacal and nasopharyngeal swab samples.

A positive correlation was seen between the kidney test results and both cloacal and nasopharyngeal swab samples (R^2 = 0.62 and 0.53, respectively, Figure). The correlation and linear regression analyses indicate that both the cloacal and nasopharyngeal swab samples showed a smaller proportion of the positive specimen than did the kidney testing. Although viral amounts in the samples were not quantified, virus was detected in the cloacal and nasopharyngeal samples, usually only when the viral load in the kidney samples from the birds was high.

Conclusions

The brain is reportedly the most sensitive organ to detect WNV in American crows (5). Other internal organs such as liver, kidney, spleen, heart, and lungs are also useful to detect viruses. However, because these organs can only be obtained through necropsy, easier, but equally sensitive, methods of sample collection are needed. Earlier research results on the value of testing cloacal and nasopharyngeal samples have not been consistent, reflecting differences in sampling methods. WNV was detected in all 20 postmortem brain tissue samples as well as cloacal and oral swabs of crows and blue jays that were experimentally infected with $>10^5$ PFU WNV (7). High viral infections in the bird kidney result in positive swab specimens, however, lower viral amounts may not.

We did not determine if the reduced amount of viral RNA in the swab samples could have been due to inactivation during the 4 months of storage after sampling. Cloacal swabs were less sensitive than oropharyngeal swabs to detect WNV (8). Kidney tissue samples that were ground in BSA-1 solution and stored at –70°C retained their sensitivities in subsequent tests for ≥2 years. Under the field conditions of our study, differences in the time of death, environmental conditions, and stage of virus spread in the bird, may have influenced the results.

The brain, in particular the cerebellum, was a primary target of infection in birds with WNV (9). Several studies have found the testing of avian kidneys to provide suffi-

Table. Percentage of kidney, cloacal, and nasopharyngeal swab samples with high, medium, and low positive or negative RT-PCR results (N = 100)*

Value	High positive	Medium positive	Low positive	Total positive	Total negative
Threshold cycle (C_T) range	15.0–21.9	22.0–28.9	29.0–35.0	$C_T \leq 35.0$	$C_T > 35.0$
Kidney tissues, %	57	27	16	100	0
Cloacal swabs, %	0	23	41	64	36
Nasopharyngeal swabs, %	0	23	43	66	34

*RT-PCR, reverse transcription–polymerase chain reaction.

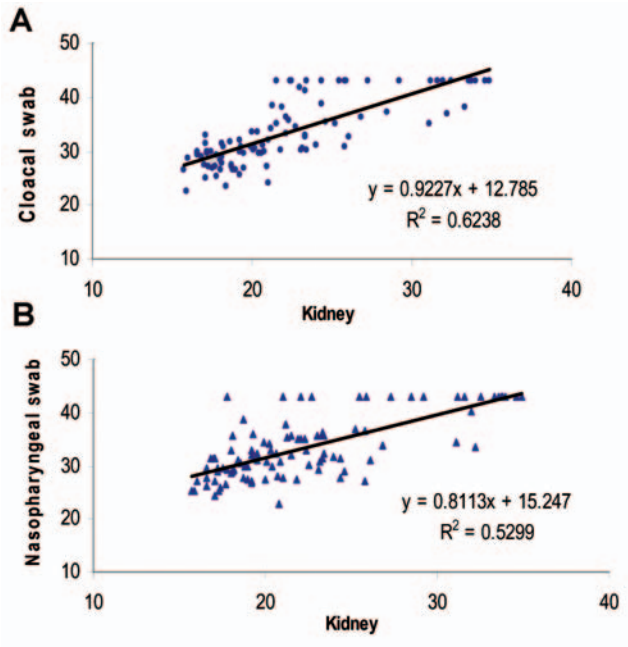

Figure. Linear regression plots of avian kidney C_T values versus A) cloacal and B) nasopharyngeal CT values using the linear regression model Y′i = bX′i + a.

cient information on WNV infectivity in birds (5,8). The sharper focus of this study was to determine testing methods in which it would be easy to extract samples from the birds. However, these findings do provide an insight into expected outcomes if cloacal or nasopharyngeal swab samples, rather than kidney tissue samples, are submitted to test for WNV with TaqMan RT-PCR. The linear regression equations provide a predictive value of determining the C_T value of cloacal and nasopharyngeal swab samples; however, the swab samples usually show positive results only when the viral load in the kidney is high or strongly positive (low or medium C_T values).

When testing for WNV with TaqMan RT-PCR (4), substituting cloacal or nasopharyngeal swab samples for kidney tissue samples would result in ≈35% fewer positive field-collected samples. Testing cloacal or nasopharyngeal swab samples might, however, be useful in situations where underreporting of the positives may not be of concern, when evidence of infection is predominant in a locality, and where the ease of obtaining cloacal or nasopharyngeal swabs makes it an attractive choice to detect WNV in dead crows and blue jays. In practice, we believe that cloacal and nasopharyngeal swabs would be easier to perform in the field. Also, savings are related to lower shipping costs and eliminating necropsy procedures.

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Endemic Tularemia, Sweden, 2003

**Lara Payne,*† Malin Arneborn,†
Anders Tegnell,†‡ and Johan Giesecke†**

Tularemia cases have been reported in Sweden since 1931, but no cyclical patterns can be identified. In 2003, the largest outbreak of tularemia since 1967 occurred, involving 698 cases. Increased reports were received from tularemia-nonendemic areas. Causal factors for an outbreak year and associated geographic distribution are not yet understood.

The ability of *Francisella tularensis*, the bacterial pathogen of tularemia, to infect at low levels and cause a high prevalence of illness and death in humans (1) has led to its inclusion in the ranks of potential bioterrorism agents (2). However, endemic forms of a less virulent subspecies of the agent also exist in parts of the Northern Hemisphere. Bioterrorism concerns further the need for surveillance of tularemia-endemic areas of the world, both to learn more about the disease and to differentiate natural from deliberate outbreaks (3). Sweden has had reported cases of tularemia since 1931, with outbreaks of variable magnitude, but with no cyclical patterns or trends (4). In 2003, 698 cases of tularemia were reported, the highest number since 1967 (Figure 1); this outbreak was larger than those usually observed (100–500 cases). Increased numbers of cases were also reported as acquired outside the identified tularemia-endemic region, similar to the situation in the 2000 outbreak (5). This article describes the epidemiology of cases in 2003.

The Study

Tularemia has been a notifiable disease in Sweden since 1968. Clinicians report diagnoses of tularemia, with or without laboratory evidence, to the county medical officer (CMO) and the Department of Epidemiology, Swedish Institute for Infectious Disease Control (EPI/SMI). Reports from regional hospital microbiology laboratories of positive cases of tularemia are also sent to SMI and CMOs and are matched to clinical reports, on the basis of a unique national personal identifying number. For this analysis, we included all cases reported to SMI and registered in the database between January 1 and December 31, 2003.

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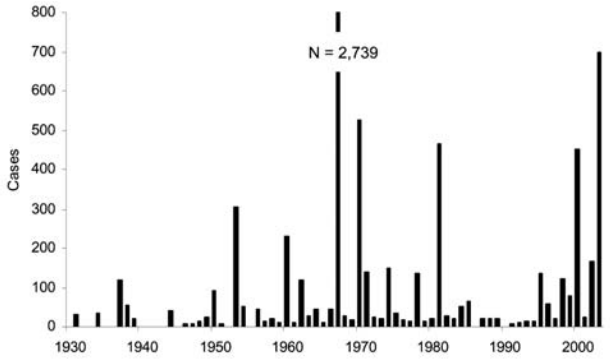

Figure 1. Number of tularemia cases reported in Sweden by year (1930–2003).

Of the 698 cases reported in 2003, 591 were diagnosed in 2003, of which 567 were reported to have been acquired in Sweden (8 cases in Finland, 1 in Turkey, 15 not known). A sharp peak of cases was observed in August, with cases tapering off until December (Table 1). More male patients (322, 57%) were reported than female patients, and the 45- to 64-year age group was the most affected in both sexes (Table 2).

Of 522 cases that had a known route of infection, animal contact or insect bite was most common (n = 475, 91.0%), and 23 cases (4.4%) were reported to have been acquired by inhaling contaminated dust or other material. The remaining reports were of infection acquired by another route (n = 22, 4.2%) or associated with work (n = 2, 0.4%). Possible exposure risks of farm or outdoor work were mentioned in an additional 25 cases, berry or mushroom picking in 5 cases, and outdoor pursuits, such as golf or fishing, in 6 cases. Clinical information about a tularemia case is not systematically collected at the national level, but the oropharyngeal form of tularemia was mentioned in 1 case. This patient had acquired infection in the northern coastal area of Sweden.

Table 1. Tularemia cases acquired and reported in Sweden in 2003 and in 2000, by month of diagnosis

Month	No. 2003 cases (N = 567)	No. 2000 cases (N = 384)
January	0	0
February	1	0
March	1	1
April	2	0
May	0	0
June	1	0
July	51	1
August	300	53
September	164	210
October	39	98
November	7	20
December	1	1

Table 2. Tularemia cases by age group and sex, Sweden, 2003

Age group (y)	No. females (n = 245)	No. males (n = 322)	Total (%) (n = 567)
≤6	8	11	19 (3)
7–17	23	27	50 (9)
18–24	4	10	14 (2)
25–44	58	81	139 (25)
45–64	117	151	268 (47)
65–79	31	36	67 (12)
≥80	4	6	10 (2)

Overall, reports were received concerning infections acquired in 15 of the 21 counties in Sweden (Figure 2). Only 22% of patients in 2003 seemed to have been infected in what is considered the disease-endemic area of central Sweden, and as many as 67% were infected in border or disease-emerging areas (5). The main observations from the largest outbreak of tularemia in Sweden since 1967 were that cases occurred earlier in the year, in greater numbers outside the disease-endemic area in Sweden, affected those 45–64 years of age most frequently, and were mainly acquired through animal contact or insect bites.

We expected that the reporting of tularemia cases for surveillance would be fairly complete because tularemia is a notifiable disease in Sweden, and the public is interested in the infection. An assessment of reporting completeness to SMI identified 98.5% completeness (range 84.6%–99.6%) for tularemia reports (1998–2002) (6). However, mild or even undiagnosed cases are likely to be missed by the reporting system.

The appearance of cases in midsummer is earlier than in the 2000 outbreak (Table 1). Previous outbreaks have usually began in late summer or early autumn (4). Since >90% of cases reported infection through insect bites or animal contact, this seasonal shift may be linked to climatic or ecologic factors in a particular year. Cases are registered in the SMI database by date of reporting and not date of diagnosis. However, median delay between date of sample collection and date of reporting for the period 1998 to 2002 was 11 days (interquartile range 8–19 days) (7). Therefore, the seasonality observed in the epidemic curve of 2003 cases was likely not greatly affected by possible reporting delay.

Conclusions

Responses regarding the route of infection may be biased because many persons in Sweden associate tularemia with mosquito bites. Nonetheless, the seasonal distribution of cases observed would support this as a route of infection. The age and sex distribution did not differ from those of the last large outbreak in 2000 and likely reflect the age and sex distribution of persons working outdoors in farms or gardens in rural areas.

Sporadic tularemia cases outside of the disease-endemic north-central region and northern coastal region have been recorded since 1931 in Sweden. As in the 2000 outbreak, cases were reported from counties that previously had very few reports (4). However, even more reports were received in 2003 than in 2000 of infections acquired outside the tularemia-endemic area (427 [78%] of 544 cases in 2003 vs. 227 [61%] of 370 in 2000, chi-square test = 31.79, $p<0.001$; Figure 2). The geographic distribution of tularemia seems to be changing. Public awareness and

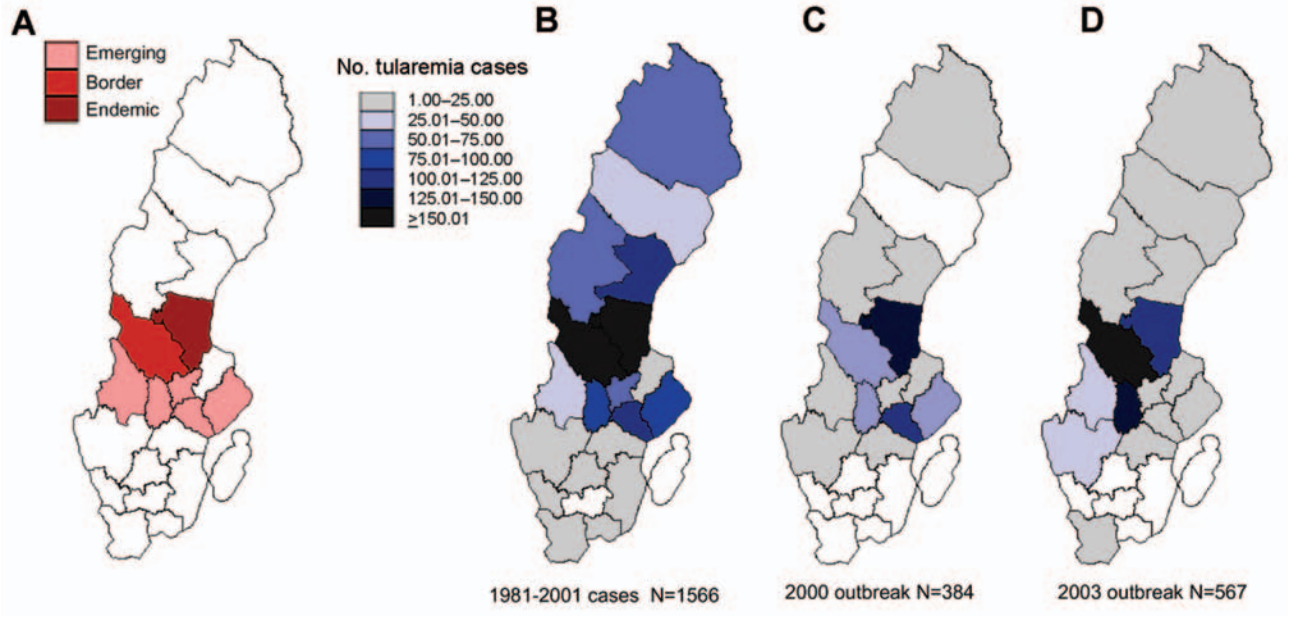

Figure 2. Tularemia cases by county of probable infection, Sweden. A) Areas in analysis by Eliasson et al. B) 1981–2001 cases (N = 1,566). C) 2000 outbreak (N = 384). D) 2003 outbreak (N = 567). White areas indicate no reports.

changes in behavior for seeking medical care could contribute to such a change, but no evidence suggests that these factors differ from those in previous years.

The factors affecting the likelihood of an outbreak year remain unknown, principally because the natural reservoir of infection has yet to be identified (8). After the outbreak in Sweden in 2000, a case-control study found significant associations between acquiring tularemia and being bitten by a mosquito, doing farm work, and owning a cat (5). The role of ticks (9) and mosquitoes as potential vectors for tularemia needs further investigation. Despite the interest and awareness of tularemia in Sweden, much remains to be understood about the dynamics of this infection among reservoir, vector, and human populations.

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Chromobacterium violaceum in Siblings, Brazil

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Chromobacterium violaceum, a saprophyte bacterium found commonly in soil and water in tropical and subtropical climates, is a rare cause of severe, often fatal, human disease. We report 1 confirmed and 2 suspected cases of *C. violaceum* septicemia, with 2 fatalities, in siblings after recreational exposure in northeastern Brazil.

Chromobacterium violaceum is an aerobic, gram-negative bacillus usually found as a saprophyte in soil and water in tropical and subtropical regions (1). Despite ubiquitous distribution, human infection with this organism is rare. Since the first human case was described in Malaysia in 1927 (2), <150 human cases have been reported worldwide, mainly in Asia, the United States, Australia, and Africa (3–6). Only 3 cases have been reported in South America, 1 in Argentina (7) and 2 in Brazil (8,9).

Human infection with this organism results in systemic and severe disease with a high fatality rate (1). *C. violaceum* infection may begin with cellulitis and skin abscesses (10,11), with rapid progression to sepsis and multiple organ abscesses, predominantly in lungs, liver, and spleen (3–5). All previous case reports were of individual, apparently sporadic infections. We report 1 confirmed and 2 suspected cases of systemic *C. violaceum* infection in siblings who shared recreational exposure to stagnant water.

The Study

In May 2004, 3 cases of sepsis syndrome in children from the same family were reported to the State Health Secretariat of Bahia in northeastern Brazil. The 3 patients had contact with soil and stagnant water in a lake in a rural area of Ilheus municipality, during a day of recreational activity. The 3 brothers spent several hours swimming in the lake with other children and adults, including their parents. Sixty persons were in the group.

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Fever, headache, and vomiting developed in patient 1, a previously healthy 14-year-old boy, 2 days after he swam in the lake. He was examined at a local health service; amoxicillin was prescribed and he was sent home. Six days after exposure, he was admitted to a local hospital with fever, dyspnea, and a cervical abscess. The patient's peripheral leukocyte count was 20,000 cells/μL with 5% bands, 78% neutrophils, 14% lymphocytes, 2% eosinophils, and 1% monocytes. Hemoglobin was 11.0 g/dL, aspartate aminotransferase (AST) was 225 U/L, and alanine aminotransferase (ALT) was 120 U/L. Chest radiograph showed diffuse bilateral consolidation, and an abdominal ultrasound showed an enlarged liver. Empiric antimicrobial treatment with oxacillin, ampicillin, and ceftriaxone was initiated. The patient was transferred to the intensive care unit and died of septic shock 36 hours after admission.

Autopsy showed enlargement of lungs, liver, and spleen with many abscessed areas of suppurative necrosis. An extensive bronchopneumonia was also shown. No spleen lymphoid atrophy was observed. Tracheal aspirate culture yielded smooth purple colonies on chocolate agar (Figure 1), identified as *C. violaceum* by the characteristic dark purple pigment and biochemical profile. Antimicrobial drug susceptibility was determined by disk diffusion. The isolate was resistant to cephalothin, ceftazidime, cefoxitin, and ceftriaxone and was sensitive to trimethoprim-sulfamethoxazole, amikacin, gentamicin, chloramphenicol, ciprofloxacin, and meropenem.

Fever and right earache developed in patient 2, a 12-year-old boy, 3 days after he swam in the lake. He was examined at a local health clinic and sent home. After

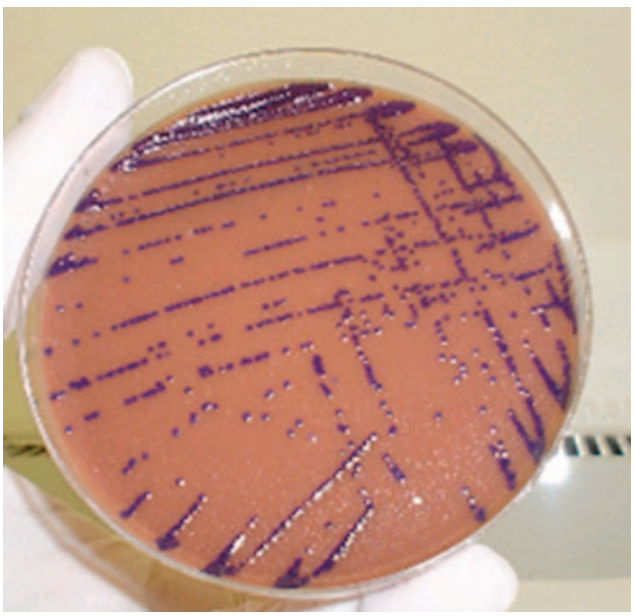

Figure 1. Colonies of *Chromobacterium violaceum* on a chocolate agar plate.

2 days, he was admitted to a hospital with purulent discharge in the right ear, fever, facial cellulitis, and diffuse abdominal pain. Leukocyte count was 1,200 cells/μL with 2% bands, 62% neutrophils, 31% lymphocytes, 1% eosinophils, and 4% monocytes. Hemoglobin was 8.0 g/dL, with a platelet count of 158,000 cells/μL. Chest radiograph showed diffuse bilateral consolidation. Empiric treatment with cephalothin and amikacin was initiated, but the patient's condition worsened quickly, and he died 6 hours after admission. No cultures were obtained and autopsy was not performed; therefore, no samples were available for testing. The patient was considered a suspected case-patient on the basis of signs and symptoms and confirmation of the infection in his sibling.

Vomiting, abdominal pain, and fever developed in patient 3, a 9-year-old boy, 3 days after he swam in the lake. Like his brothers, he was treated at a local health clinic and admitted to a hospital 3 days afterwards. Leukocyte count was 20,500 cells/μL with 4% bands and 82% neutrophils. Hemoglobin was 11.5 g/dL, AST was 115 U/L, and ALT was 26 U/L. Empiric treatment with ceftriaxone, ampicillin, and metronidazole was initiated. After 48 hours, he was transferred to our institution, the Children's Hospital in Salvador, Bahia. On admission, his abdomen was tender and his liver was enlarged; otherwise, the results of the physical examination were normal. Treatment was changed to ceftazidime, oxacillin, and amikacin. Serial blood cultures were negative for bacteria. A chest radiograph showed perihilar consolidations in both lungs. A computed tomographic scan of the abdomen showed multiple, small liver abscesses (Figure 2). Five days after admission, the fever continued in the patient, and cellulitis developed on his left foot and right hand. Antimicrobial therapy was changed to oxacillin plus meropenem. The patient became afebrile after 4 days of meropenem therapy, and symptoms and skin lesions regressed. Studies to rule out underlying immunodeficiency showed no evidence of glucose 6-phosphate dehydrogenase (G6PD) deficiency or HIV infection. The patient received parenteral antimicrobial drug therapy for 6 weeks and an additional 4 weeks of trimethoprim-sulfamethoxazole was prescribed at discharge. He had no symptoms after 3 months of follow-up care and was considered to be a suspected case-patient on the basis of his symptoms and confirmation of the infection in his sibling. Results of the *C. violaceum* culture from case-patient 1 were reported on day 6 of hospitalization.

For microbiologic analysis, samples of water and soil were collected from the lake where the boys had swum. All 6 soil cultures and 4 of 6 water cultures grew *C. violaceum*. Soil and water samples collected near the case-patients' home and neighbors' homes were negative.

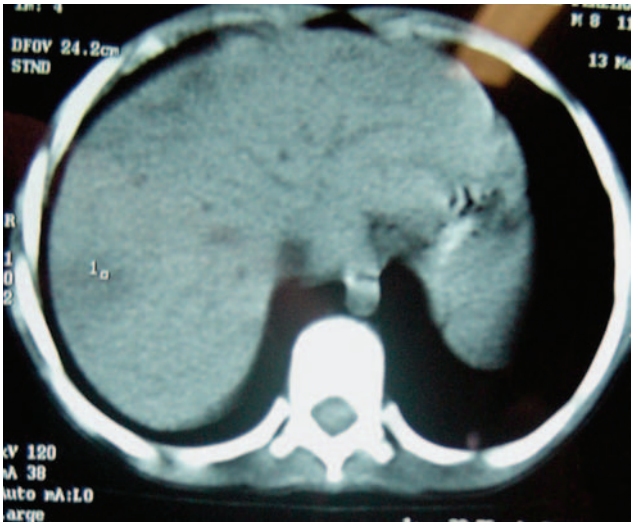

Figure 2. Computed tomographic scan of abdomen of patient 3, showing multiple, small liver abscesses.

Conclusions

In Brazil, *C. violaceum* is abundant in the water and on the borders of the Negro River in the Amazon basin (12); however, this is >1,000 kilometers from the region where the cases occurred. *C. violaceum* infections have been reported at least twice previously in Brazil. In 1984, the organism was cultured from skin abscesses of a young man who had contact with river water in southern Brazil (8). In 2000, it was identified from blood culture in a 30-year-old male farm worker who died of severe septicemia associated with multiple lung and liver abscesses (9). Most reports worldwide have been associated with rural areas (5,8,9) or stagnant water (6).

This report is the first of a cluster of suspected *C. violaceum* infections linked to a common source. Systemic infection caused by *C. violaceum* is rare but severe and is associated with fatality rates ≥60% (1,13). Previous reports of *C. violaceum* sepsis have noted fever, hepatic abscesses, and skin lesions, as observed in this cluster. Facial cellulitis and otitis, as observed in patient 2, have also previously been reported (10). Only our first case was microbiologically confirmed, but the signs and symptoms and common epidemiologic exposure suggest that all 3 patients had *C. violaceum* infection.

Based on the identification of *C. violaceum* in samples from the lake and onset of symptoms 2–3 days after exposure, we believe that the 3 siblings were exposed while swimming and playing on the banks of the lake. One previous report of 2 cases of *C. violaceum* pneumonia implicated aspiration of fresh water in near-drowning victims (6); infection may also have occurred when injured or broken skin is exposed to stagnant water. No cuts or gross abrasions on the skin of the siblings were reported, but

microabrasions may have occurred during the recreational activities.

Why these siblings, 3 of 60 persons exposed to the same environment, were the only ones in whom severe illnesses developed is unclear. We hypothesized an underlying factor or familial predisposition to infection. Previously, underlying defects in host defense, especially of neutrophils, have been hypothesized to predispose to infection: cases have been reported in patients with chronic granulomatous disease (13) and G6PD deficiency (14). However, many case reports describe infections in apparently healthy persons (5). The 1 patient tested in this apparent cluster had no detectable immunodeficiency, and his 2 siblings were apparently previously healthy.

Despite their cost, carbapenems may be an appropriate treatment when *C. violaceum* infection is identified. The recommended antimicrobial treatment for *C. violaceum* infection is not well established; some survivors are treated with ciprofloxacin, carbapenems, chloramphenicol with aminoglycoside, or trimethoprim-sulfamethoxazole. When patient 3 was seen in the late stage of infection, meropenem was prescribed empirically for presumptive melioidosis, an infection with *Burkholderia pseudomallei* that may begin similarly to cases in this cluster (15). Early recognition and aggressive antimicrobial drug therapy can reduce the high mortality rate associated with both *C. violaceum* infection and melioidosis (1,4,15). Physicians in tropical and subtropical regions should consider *C. violaceum* infection as part of the differential diagnosis of sepsis, especially when associated with skin or multiple organ abscesses or with a history of exposure to stagnant water.

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Divergent HIV and Simian Immunodeficiency Virus Surveillance, Zaire

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Recent HIV infection or divergent HIV or simian immunodeficiency virus (SIV) strains may be responsible for Western blot–indeterminate results on 70 serum samples from Zairian hospital employees that were reactive in an enzyme immunoassay. Using universal polymerase chain reaction HIV-1, HIV-2, and SIV primers, we detected 1 (1.4%) HIV-1 sequence. Except for 1 sample, no molecular evidence for unusual HIV- or SIV-like strains in this sampling was found.

HIV-1 and HIV-2 are believed to be the result of cross-species transmission from simian immunodeficiency virus (SIV)–infected chimpanzees and sooty mangabeys, respectively, which represent 2 (SIVcpz and SIVsm) of the 6 major lentiviral phylogenetic lineages (1,2). No evidence exists that SIV strains from the remaining nonhuman primate lineages have infected humans, although many grow in human cells in vitro as do SIVcpz and SIVsm (3). Since humans are exposed to a plethora of primate lentiviruses through blood or body fluids during hunting, butchering, eating bushmeat, and keeping primates as pets (3), the potential exists for zoonotic transmission of diverse primate lentiviruses in many parts of sub-Saharan Africa, including the Congo River basin (4). This potential is supported by the identification of a Cameroonian man whose HIV serologic results were indeterminate but whose serum specimen reacted strongly and exclusively with an SIVmnd V3 loop peptide (5). An even more compelling case for cross-species exposure is the recent finding of a Cameroonian man who may have been exposed to a colobus SIV, as indicated by a strong humoral (*env* IDR and V3) and a weak cellular (*gag*) immune response (6). Although SIV sequences were not identified in either case, the findings suggest that humans are probably exposed to different simian retroviruses that can establish new infections in humans (3).

Nonhuman primates infected with SIV from the currently recognized lineages can harbor antibodies that serologically cross-react with some HIV-1 or HIV-2 antigens (3). In many cases, HIV Western blots (WBs) with indeterminate profiles of SIV-infected monkeys resemble those of HIV enzyme immunoassay (EIA)–positive, WB-indeterminate human sera from Africa. In general, such indeterminate African sera demonstrate a broad range of reactivity to HIV-1 proteins, in contrast to predominant p24 reactivity in WB-indeterminate sera from persons in the United States (7–9). These data suggest that the WB-indeterminate patterns in HIV EIA-reactive sera from persons living in Africa may reflect more than just a recent HIV infection or an infection with a highly divergent HIV-1 strain (10); they may reflect either cross-reactivity with unknown pathogens of African origin or exposure to new HIV- or SIV-like strains.

The Study

We investigated the presence of HIV or SIV in 70 HIV EIA-reactive, WB-indeterminate serum specimens collected in 1984 and 1986 from employees of Mama Yemo Hospital in Kinshasa, Zaire (currently the Democratic Republic of Congo) (11). WB bands were identified by using an HIV-1/2 WB assay (version 2.2, Genelabs Diagnostics, Singapore) and classified as indeterminate according to criteria for interpreting HIV-1 (http://www.cdc.gov/mmwr/preview/mmwrhtml/00001431.htm). Briefly, a positive WB result indicates reactivity to at least p24 and 1 of the 3 *env* (gp41, gp120, or gp160) proteins with banding intensity at least as strong as that seen in the weak-positive control; a negative WB result indicates no reactivity and an indeterminate WB result indicates reactivity to at least 1 protein.

Of the 70 WB-indeterminate serum specimens, 69 showed a broad range of HIV-1 WB-indeterminate band patterns, and 1 had an HIV-2 WB-indeterminate pattern with weak reactivity against gp36, p68, and gp80 proteins. Analysis of HIV-1 WB-indeterminate patterns provided several important observations. First, most of the specimens (41/69, 59%) showed reactivity to multiple viral proteins (Figure), whereas the remaining specimens (28/69, 41%) demonstrated reactivity against single HIV-1 proteins, including p24 (13/69, 18.8%), p17 (n = 8, 11.6%), gp160w (w = weak) (n = 4, 5.8%), and p51, p66, and gp41w (n = 1 each; 1.4% each). Second, in all but 3 specimens the WB-indeterminate patterns with reactivity to multiple viral proteins combined proteins of p24 or p17 gag or both with others, giving the following 21 profiles: p17/p24 (14/69, 20.3%); p24/p66 and p17/p24/p66 (n = 3 each, 4.3% each); p24/p51, p17/p24/p51, and p17/p24/

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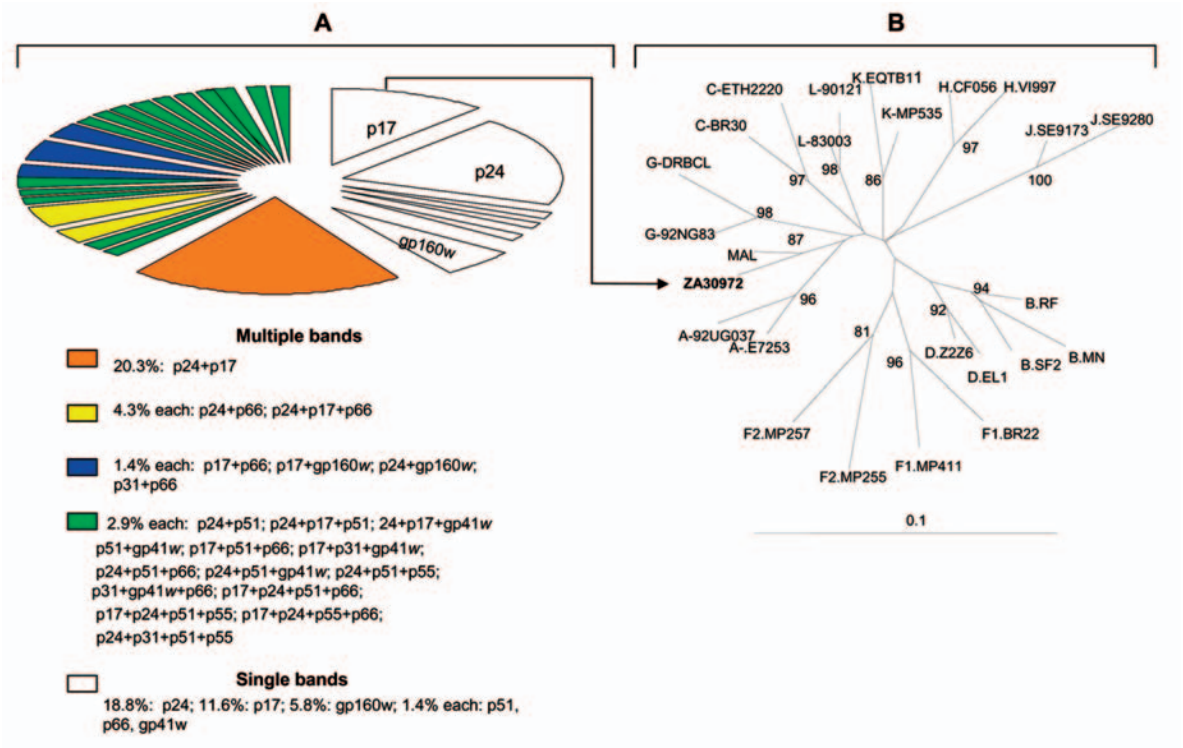

Figure. A) Distribution of HIV-1 Western blot–indeterminate patterns among 69 serum specimens from Kinshasa, Zaire, reactive by enzyme immunoassay. B) Phylogenetic classification of HIV-1 protease sequence ZA30972 (GenBank accession no. AY562558) isolated from the p17 *gag*-reactive serum. The phylogenetic tree was generated by the neighbor-joining method with the nucleotide distance calculated by Kimura 2-parameter method (12), included in the GeneStudio package (http://www.genestudio.com). Reference sequences were obtained from the Los Alamos database (http://hiv-web.lanl.gov/MAP/hivmap.html). The position of the outgroup (Simian immunodeficiency virus [SIV]cpz) is not shown. Values on branches represent the percentage of 100 bootstrap replicates. The scale bar indicates an evolutionary distance of 0.10 nucleotides per position in the sequence.

gp41w (n = 2 each, 2.9% each); p17/p66, p17/gp160, p24/gp160, p24/p51/gp41w, p17/p51/p66, p17/p31/gp41w, p24/p51/p55, p24/p51/p66, p17/p24/p51/p66, p17/p24/p51/p55, p17/p24/p55/p66, p24/p31/p51/ p55, p31/p66, p51/gp41w, and p31/p66/gp41w (n = 1 each, 1.4% each). Overall, this analysis indicated reactivity to 8 HIV-1 proteins that occurred as single bands or multiple combinations. Whereas reactivity to p24 might represent either early infection with HIV or reaction to nonspecific antigens (8), reactivity to other HIV-1 proteins (p17, p31, p51, p55, p66, gp41, or gp160) could reflect at least exposure to HIV-1, HIV-2, or SIV variants as well as cross-reactivity with yet unidentified HIV- or SIV-like strains (3,10).

To determine whether HIV- and SIV-like RNA could be detected in the 70 WB-indeterminate specimens, we attempted reverse transcription–polymerase chain reaction (RT-PCR) amplification of HIV-1, HIV-2, and SIV. However, because of a small volume (≤300 μL) of some serum specimens, a limited number of primers was used. We selected 4 sets of primers for 3 highly conserved gene regions, including *env*-gp41, *pol* protease, and *pol* integrase, which we previously developed and extensively tested for the efficient PCR amplification of field specimens. Briefly, gp41 primers allow the amplification of HIV-1 groups M (subtypes A–K and U [unclassifiable]) N, and O and SIVcpz with an efficiency of 95% (13); HIV-1 type specific protease primers efficiently (>95%) detect group M viruses (subtypes A–K and U [unclassifiable]), and allow successful amplification of some PCR–gp41 negative specimens. HIV-2 type-specific protease primers allow the amplification of HIV-2 at least subtypes A and B (13), and HIV-2/SIV integrase primers amplify HIV-2 and at least 5 major SIV lineages including SIVcpz, SIVsm, SIVagm (African green monkey), SIVmndBK12 (mandrill), and SIVsyk (Sykes monkey) (14). The integrase primers should also amplify other SIV lineages including SIVcol, SIVLhoest/SIVsun, SIVgsn, SIVmus, SIVmon, SIVtal, and SIVdeb (3). This prediction is based on the fact that genetic diversity of these sequences within the 3′-end of primer regions was the same as in strains previously used for testing (15), which was clearly visible through DNA alignment of the integrase primers with all SIV genomic integrase sequences deposited in GenBank. Positive controls for PCR amplification included HIV-1

Mn, HIV-2 ROD, and SIVsm. Using this approach, we amplified a 297-bp HIV-1 protease (PR) gene from only 1 (1.4%) of the 70 serum specimens. The remaining 69 specimens were PCR negative for all the primers tested. Phylogenetic analysis of the PR gene sequence showed a close phylogenetic relationship with the HIV-1 MAL strain (Figure), a recombinant virus that contains portions of subtypes A and D and an unclassifiable region that was identified in 1985 in Zaire (16).

Conclusions

The PR gene sequence was identified in the serum of the person with reactivity to only the p17 *gag* band by WB, which suggests that this person was recently infected and that antibodies to all the HIV-1 antigens had not yet developed. No information was available on demography, risk, or clinical status of this person. In the remaining 69 WB-indeterminate specimens, we could not rule out exposure to SIV from nonhuman primates, from handling infected animal meat before consuming it, or from keeping monkeys as pets. Although the limited molecular scope of this study and the quality of the old serum specimens may not be adequate for molecular confirmation of such viruses, our findings of complex HIV WB-indeterminate patterns with reactivity to multiple viral proteins in serum specimens from persons living in the Democratic Republic of Congo provide comprehensive insights into HIV WB-indeterminate sera in the mid-1980s.

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West Nile Virus Isolation in Human and Mosquitoes, Mexico

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West Nile virus has been isolated for the first time in Mexico, from a sick person and from mosquitoes (*Culex quinquefasciatus*). Partial sequencing and analysis of the 2 isolates indicate that they are genetically similar to other recent isolates from northern Mexico and the western United States.

Several recent reports (1–7) have documented the widespread geographic distribution of West Nile virus (WNV) in Mexico, but until now, no autochthonous human cases of illness due to this virus have been reported from the republic. Likewise, limited entomologic surveillance has been conducted in Mexico, and no information is available on the actual mosquito vectors of WNV in the republic. All Mexican WNV isolates studied to date have come from dead equines or birds (2,7,8). We report the first isolations of WNV from a sick person and from a pool of *Culex quinquefasciatus* mosquitoes and describe their phylogenetic relationship to other representative WNV strains from the United States and Mexico.

The Study

Mosquitoes were collected from June to September 2003 at the Ejido Francisco Villa, Municipality of Pesqueria, State of Nuevo Leon (25°47′N, 100°03′W), with CDC-type light traps baited with dry ice and mechanical aspiration from resting sites on vegetation and in houses. The area is located ≈40 km northeast of Monterrey and consists of mixed suburban housing and agriculture. Average annual rainfall in the region is 550 mm; the mean annual temperature is 28°C. After collection, the mosquitoes were placed on dry ice for transport back to the Medical Entomology Laboratory, Faculty of Biological Sciences, Autonomous University of Nuevo Leon, Monterrey, where they were separated into pools of ≈10 insects each, based on species, date, and method of collection (Table 1). The mosquitoes were stored in a mechanical freezer at –70°C and later transported on dry ice to the University of Texas Medical Branch (UTMB) to be processed for virus isolation. A total of 2,297 mosquitoes, representing 4 genera and 11 species, were tested in 238 pools (Table 1). Individual mosquito pools were titrated manually in sterile, Ten Broeck tissue grinders containing 1.0 mL of phosphate-buffered saline, pH 7.4, containing 30% fetal bovine serum and antimicrobial agents (penicillin, streptomycin, and amphotericin). The resultant suspension was centrifuged at 12,000 rpm for 5 min; then 200 µL of the supernatant was injected into a flask culture of Vero cells. After the solution was absorbed for 1 h at 37°C, maintenance medium (9) was added; cultures were maintained in an incubator at 37°C and examined daily for evidence of viral cytopathic effect (CPE) for 14 days.

A single pool of *Cx. quinquefasciatus* yielded a virus isolate, designated NL-54, which produced CPE on approximately day 7. The isolate was identified as WNV by immunofluorescence, hemagglutination-inhibition (HI) test, complement-fixation test, VecTest WNV/SLE antigen assay (Medical Analysis Systems, Camarillo, CA, USA), and reverse transcription–polymerase chain reaction (RT-PCR) (9,10).

The WNV human isolate was from a 62-year-old Mexican woman living in the municipality of Etchojoa (near Ciudad Obregon) in Sonora State. The patient had no history of travel during the preceding 2 months. She visited a local hospital in July 2004 with symptoms of fever, headache, vomiting, arthralgias, and myalgia. Her temperature was 38°C upon examination, and no neurologic symptoms were noted. An acute-phase blood sample was obtained, and a presumptive diagnosis of dengue fever was made. The patient was sent home and subsequently completely recovered. When RT-PCR using dengue primers was negative on the acute-phase serum, a culture was performed. WNV was isolated from the sample at the State Public Health Laboratory in Sonora and at UTMB, upon culture in Vero cells. HI tests conducted on the acute-phase serum at UTMB with West Nile, St. Louis encephalitis, yellow fever, dengue 1, and dengue 2 viral antigens were negative, which indicated that the patient had no preexisting flavivirus antibodies. An immunoglobulin (Ig) M

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Table 1.Summary of mosquitoes collected in Nuevo Leon, Mexico, during the summer of 2003 and tested for West Nile virus

Genus and species	No. pools	No. mosquitoes
Aedes aegypi	39	399
Ae. vexans	1	10
Ochlerotatus taeniorynchus	15	146
Anopheles pseudopunctipenis	1	8
An. quadrimaculatus	1	2
Culex coronator	13	118
Cx. quinquefasciatus	81	798
Psorophora ciliata	5	22
Ps. confinnis	2	8
Ps. cyanescens	9	89
Ps. ferox	70	697

enzyme-linked immunosorbent assay (11), performed on the acute-phase specimen and a 30-day convalescent-phase serum specimen in Sonora, demonstrated seroconversion and the presence of WNV-reactive IgM antibodies in the convalescent-phase serum sample.

Viral RNA was extracted from the 2 WNV strains after a single Vero cell passage directly from 140 μL of the infected cell culture supernatants, using the QIAamp viral RNA extraction kit (12). RT-PCR was performed by using 3 primer pairs to amplify the entire prM-E genes of each WNV isolate as previously described (12). PCR products were gel purified with the QIAquick kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol, and the resulting template was directly sequenced with the amplifying primers. Sequencing reactions were performed as described previously (8). Analysis and assembly of sequencing data were performed with the Vector NTI Suite software package (Informax, Frederick, MD, USA). Nucleotide and deduced amino acid sequences of the 2004-nucleotide region representing the prM-E genes from each isolate were aligned with the AlignX program in the Vector NTI Suite and compared to sequences of selected North American WNV isolates for which the prM-E genes were available in GenBank. Phylogenetic trees were constructed by Bayesian analysis with the program MRBAYES, version 2.0 (13), with the Metropolis-coupled, Markov chain, Monte Carlo algorithm run with 4 chains over 150,000 generations under a general time-reversible model with a burn-in time of 50,000 generations. Rate heterogeneity was estimated by using a γ distribution for the variable sites. The Bayesian consensus tree was compared to trees generated by neighbor-joining, maximum parsimony, and maximum likelihood analyses using PAUP, version 4.0b10 (14), and each method generated trees with the same overall topology. The consensus phylogram of the 40 WNV isolates generated by Bayesian analysis (13) is shown in the Figure, with confidence values at relevant nodes to demonstrate statistical support for each clade.

Conclusions

Comparison of the 2 Mexican isolates, NL-54 (GenBank accession no. AY963775) and human Sonora (GenBank accession no. AY963774), to the prototypical North American WNV isolate, WN-NY99 (GenBank accession no. AF196835), 2 previous Mexican isolates, MexNL-03 (GenBank accession no. AY426741) (7) and TM171-03 (GenBank accession no. AY371271) (2), and an isolate collected in Harris County, Texas, in 2002 (GenBank accession no. AY185906) (15) indicated nucleotide and deduced amino acid differences and similarities among each of the isolates. Table 2 shows the positions at which nucleotide and amino acid substitutions were found. Both the Mexican mosquito and human isolates reported herein shared a nucleotide mutation at position 660 (C to U) of the prM gene and 2 mutations at positions 1442 (U to C) and 2466 (C to U) of the E gene. Each of these 3 mutations was shared with a 2003 horse strain from Nuevo Leon (MexNL-03) (7) and a 2002 bird isolate from Harris County, Texas (TX-1) (15). The mutation at nucleotide 1442 also represented a deduced amino acid substitution in the envelope protein (V159A). The Mexican mosquito and human isolates reported herein shared a unique mutation at genomic position 1320 (A to G) in the E gene. The human isolate also had 3 additional

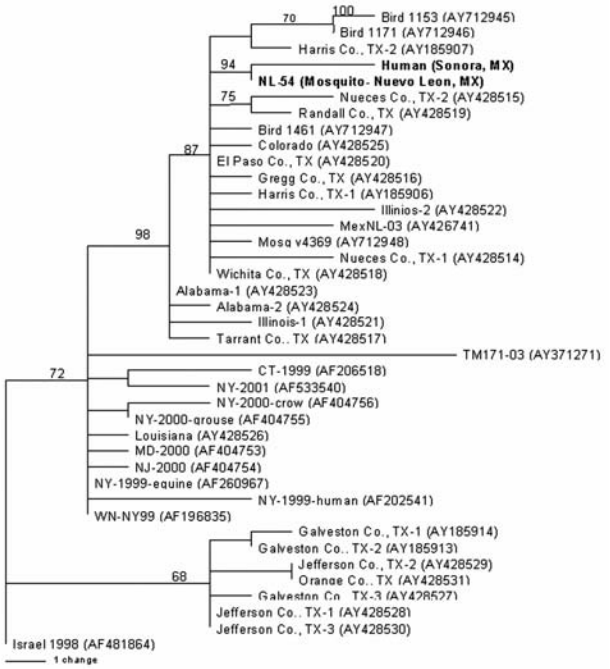

Figure. Phylogram of 2 West Nile viruses (WNV) isolated from a mosquito pool and human serum in Mexico (shown in **bold**). The phylogenetic tree was generated by Bayesian analysis of a 2004-nucleotide region of the prM and E genes of 40 WNV isolates rooted by the most closely related Old World strain, Israel 1998. Bayesian confidence values are shown to provide statistical support for each clade.

Table 2. Nucleotide and deduced amino acid differences in the prM-E genes of Mexican and Texas isolates compared to West Nile virus strain WN-NY99 (382-99)

Strain	Nucleotide (amino acid) substitutions in prM and E genes (nt 466–2469)*																		
	483	549	660	858	887	1074	1137	1179	1320	1356	1432	1442	1626	1656	1974	2328	2388	2392	2466
WN-NY99 (AF196835)†	C	U	C	C	U (Ile)	G	C	A	A	C	U (Ser)	U (Val)	C	U	C	C	C	G (Ala)	C
TM171-03 (AY371271)	U	‡		U	C (Thr)		U				C (Pro)		U			U	U		U
MexNL-03 (AY426741)		C	U					G		U		C (Ala)							U
NL-54 (Mosquito, MX)			U						G			C (Ala)							U
Human (Sonora, MX)			U			A			G			C (Ala)		C	U				U
Harris Co., TX-1 (AY185906)			U									C (Ala)						A (Thr)	U

*Nucleotide numbers correspond to WN-NY99; amino acid substitutions are in brackets.
†GenBank accession number.
‡Blank entries indicate no nucleotide or amino acid substitutions.

mutations in the E gene at positions 1074 (G to A), 1656 (U to C), and 1974 (C to U). Each of the additional nucleotide mutations was silent. The nucleotide mutations at nucleotide positions 660, 1442, and 2466 have also been described in most WNV isolates sequenced from Texas, Illinois, and Colorado in 2002 (12). This finding suggests that isolates obtained from northern states of Mexico (i.e., Nuevo Leon and Sonora) were derived from WNV strains circulating in the western United States. Only a single mutation at position nucleotide 2466 was shared by these 2 isolates and a 2003 bird isolate from Tabasco State (TM171-03). This finding supports results from earlier studies that suggest separate introductions of WNV into Mexico (2,7). Phylogenetic trees generated by a number of methods indicate that the recent Mexican mosquito and human isolates belong to the clade comprised of WNV isolates collected outside the northeastern United States after 2001, with the exception of isolates collected along the southeast coast of Texas. (Those isolates constitute a separate, sister clade relative to all other North American WNV isolates sequenced to date [Figure].). Because of a shared mutation between the recent Mexican mosquito and human isolates, these 2 virus strains constitute a distinct subclade within the larger US 2002 clade that is supported by strong Bayesian confidence values (94%). The accumulation of 3 additional nucleotide mutations in the 2004 Mexican human isolate is illustrated by longer branch lengths in comparison to the 2003 mosquito pool isolate NL-54, which suggests the continued microevolution of WNV in Mexico from year to year.

Our patient represents the first reported autochthonous human case of confirmed WNV infection in Mexico. The paucity of human cases reported to date from Mexico is curious for several reasons: 1) a large number of cases are reported from the United States, 2) available evidence indicates that WNV is now widely distributed in Mexico (1–7), 3) most of the WNV virus strains circulating in the republic are genetically similar to those in the United States (Figure). One explanation for this difference could be the failure of local health personnel to recognize the various clinical forms of WNV infection. As illustrated by our patient, West Nile fever can easily be mistaken for dengue fever. A second reason may be the difficulty of making a serologic diagnosis of WNV infection among persons living in geographic regions where several different flaviviruses circulate, and people have multiple flavivirus infections (11). A third and related possibility is that WNV infection may be less severe in persons with preexisting heterologous flavivirus antibodies (11).

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Cyclosporiasis Outbreak, Indonesia

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We describe an outbreak of *Cyclospora cayetanensis* infection among Dutch participants at a scientific meeting in September 2001 in Bogor, Indonesia. Fifty percent of the investigated participants were positive for *C. cayetanensis*. To our knowledge, this outbreak is the first caused by *C. cayetanensis* among susceptible persons in a disease-endemic area.

Cyclospora cayetanensis is a newly recognized protozoan parasite that causes gastrointestinal illness. *C. cayetanensis* infection is mostly characterized by a gradual onset of watery diarrhea, sometimes with explosive diarrhea, nausea, and abdominal cramping. Symptoms are often prolonged and can relapse after months (1). Oocysts of *C. cayetanensis* are, in comparison with those of other coccidian parasites, noninfectious in freshly excreted stool. Therefore, direct person-to-person transmission through fecal exposure is unlikely. Food and water contaminated with sporulated oocysts are the primary modes of transmission (1). Infections have been associated mainly with outbreaks from eating food such as raspberries, salads, and basil (1–3). Infections with *C. cayetanensis* are seasonal; in the tropics, the wet and cooler seasons provide conditions more favorable for sporulation than do the dry and warmer seasons (1,3).

A scientific meeting involving Dutch and Indonesian microbiologists was held September 2–6, 2001, in Indonesia. Immediately after the meeting, several participants reported mild-to-severe gastrointestinal symptoms, and *C. cayetanensis* infection was diagnosed in 4 participants. We investigated the extent of the outbreak among participants from our institute and identified *C. cayetanensis*–specific symptoms.

The Study

The meeting was held in a hotel near Bogor, Indonesia. All Dutch participants stayed in this hotel during the meeting and consumed the same meals (buffet). After the meeting, approximately half of the participants went home, while others took the opportunity to travel further.

Six weeks after the meeting, all members of our institute who visited the Bogor meeting were asked to participate in a cohort study. Participants were asked to complete a questionnaire and deliver 2 fecal samples. The questionnaire was set up to collect information about gastrointestinal symptoms, duration of stay, and results of earlier fecal diagnostic examination.

One of the 2 fecal samples was directly examined for *C. cayetanensis* after Ridley concentration by 2 microscopy methods. Presence of *C. cayetanensis* was demonstrated by nonrefractile spheres seen in a direct saline wet mount or by light pink–to–deep red, 8- to 10-µm long oocysts seen on modified acid-stained smears. Because the aim of the study was to determine the extent and duration of *C. cayetanensis* infection in our cohort, no special efforts were made to detect other possible pathogens. Another fecal sample was stored at –20°C until DNA was isolated for polymerase chain reaction (PCR). After DNA isolation, *C. cayetanensis*–specific real-time PCR was performed as described previously (4). The specific primers and probe were based on the known small ribosomal subunit RNA gene sequence for *C. cayetanensis*. This real-time PCR was specific when tested with a range of other intestinal parasites, and the DNA of ≥0.5 oocysts was estimated as the detection limit. Names and clinical conditions of the participants were blinded to laboratory technicians.

A case-control study was set up to investigate whether certain gastrointestinal symptoms were associated with *C. cayetanensis* infection. A case was defined as *C. cayetanensis*–positive microscopic result, positive PCR result, positive *C. cayetanensis* diagnosis 1–6 weeks before entering the study, or any combination. Only data from participants who submitted completed questionnaires were used for statistical analyses.

Analyses were performed by using the χ^2 test if variables were categorical. Continuous variables were not normally distributed; therefore, they were compared and tested with the Mann-Whitney *U* test. The ethics committee of the University Medical Center Utrecht approved the project; written informed consent was obtained from all participants before participation.

Thirty-two (94%) of the 34 attendees of our institute responded, and 29 completed the questionnaire; 3 participants delivered only fecal samples. Fourteen (48%) of the 29 attendees had cases that met the definition: 10 case-patients had a positive PCR result, 5 of which were also positive on microscopic analysis, and 4 case-patients had a positive diagnosis 1–6 weeks before entering the study. Fecal samples of these 4 case-patients were all negative on microscopic analysis; 2 were also negative on PCR

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analysis. Two samples were lost before PCR could be performed. Fecal samples from the 3 participants who did not return the questionnaire were all positive by PCR and negative by microscopic analysis. They were not included in the case-control study.

Symptoms of the 29 participants are listed in the Table. More women had cyclosporiasis than men (71% vs. 40%). Case-patients mentioned bowel disorders significantly more than noncase-patients: stomach cramps, nausea, and flatulence were common symptoms among the case-patients (71% vs. 40%, 93% vs. 27%, and 93% vs. 33%, respectively). Duration of symptoms was significantly longer for case-patients than for noncase-patients.

Conclusions

We showed that approximately half of the investigated meeting attendees were positive for *C. cayetanensis*. The number of proven infected persons would be higher if the investigations had started directly after the first symptoms appeared. The fact that 6 weeks after the probable exposure, *C. cayetanensis* DNA was still detectable in 13 persons corresponds to the known persistence of the parasite. Diagnosis in our study was primarily based on PCR-positive results. PCR is a much more reliable method to detect *C. cayetanensis* than diagnostic microscopy (4,5); however, ultraviolet fluorescence microscopy may be as sensitive as PCR (4).

An outbreak with a common source of infection has not been proven. We could not investigate potential food sources because we started surveillance 6 weeks after the assumed exposure in Indonesia, when participants had returned to the Netherlands. Only 2 of the participants could recall a meal that might have been a source of infection, and each suggested a different meal. Participants may have acquired *C. cayetanensis* infection on other occasions during their stay in Indonesia. Genotyping the different isolates to connect cases on a molecular basis was not possible because the number of oocysts detected in stools was small. However, assuming a joint exposure to *C. cayetanensis* is not unreasonable. First and most important, 3 of the participants in this study went to the conference and returned immediately to the Netherlands, and the conference was the only place where infection may have been acquired. Secondly, infections were acquired during the season with low incidence of transmission, so a common source is a more obvious route of infection than cases acquired at separate occasions. In our study, the main symptoms of cyclosporiasis were stomach cramps, nausea, and flatulence, without bloody diarrhea. The prolonged and relapsing character of the symptoms, especially diarrhea and abdominal cramps, was striking. This pattern of symptoms is mentioned in other outbreaks as well and seems to be characteristic for cyclosporiasis (1–3). Although nonindigenous persons are at risk for travelers' diarrhea in Indonesia, we believe that the gastrointestinal problems in the patients in our study were caused by cyclosporiasis. All 4 patients with an earlier diagnosis of *C. cayetanensis* infection were successfully treated with cotrimoxazole.

In conclusion, we report a possible outbreak of *C. cayetanensis* among Dutch microbiologists attending a meeting in Indonesia. At least 50% of participants were infected. To our knowledge, this outbreak is the first of *C. cayetanensis* among susceptible persons in a disease-endemic area.

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Table. Characteristics of meeting members exposed to *Cyclospora cayetanensis*, Bogor, Indonesia, 2001*

Variable	Cases (%), n = 14	Noncases (%), n = 15	p value†
Female sex	10 (71)	6 (40)	0.09
Symptoms			
Diarrhea‡	11 (79)	9 (60)	NS
Obstipation	3 (21)	0	NS
Stomach and abdominal cramps	10 (71)	6 (40)	0.09
Flatulence	13 (93)	5 (33)	0.001
Fever	2 (13)	1 (7)	NS
Nausea/appetite loss	13 (93)	4 (27)	<0.001
Median symptom duration (IQR), days§	41 (29–42)	1 (0–6)	<0.001
Median stay in Indonesia (IQR), days	14 (10.5–18.5)	16 (11–23)	NS

*NS, nonsignificant; IQR, interquartile range.
†Determined by χ^2 test for categorical variables and by Mann-Whitney *U* test for continuous variables.
‡Diarrhea was defined as ≥3 loose stools in 24 hours.
§If patients had symptoms when entering the study, the duration was fixed at 42 days.

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Plague from Eating Raw Camel Liver

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We investigated a cluster of 5 plague cases; the patients included 4 with severe pharyngitis and submandibular lymphadenitis. These 4 case-patients had eaten raw camel liver. *Yersinia pestis* was isolated from bone marrow of the camel and from jirds (*Meriones libycus*) and fleas (*Xenopsylla cheopis*) captured at the camel corral.

Human plague is acquired most often from the bites of infected fleas that leave their rodent hosts. Sporadic plague has also been attributed to domestic dogs and cats that may transport either *Yersinia pestis* in their mouths or infected fleas from rodent hosts to humans (1). Bubonic, pneumonic, or pharyngeal plague may develop in domestic cats and infect humans directly (2). However, humans rarely become infected when handling and preparing the carcasses of wild animals (3). Although domestic cats and other carnivores may be infected by eating infected animals, only 1 previous report raises the possibility of human plague infection from eating meat of an infected animal (4).

The Study

In February 1994, we investigated a cluster of 5 plague cases in Goriat, a town of 50,000 persons in a remote desert area in northwestern Saudi Arabia. On February 18, a 26 year-old-woman was admitted to the provincial hospital for severe pharyngitis and tonsillitis. Given the striking swelling of her neck, local clinicians suspected diphtheria. Since 2 of the patient's relatives had also been hospitalized in the previous 2 days with similar illnesses, the hospital called for assistance from the local preventive medicine specialist. He had seen similar cases in 1984 and suspected pharyngeal plague.

Through interviews with physicians and review of hospital admissions, we identified 5 patients, including the index case, who had been hospitalized with suspected plague or plague pharyngitis. The patients included a 9-year-old girl and 4 adults (2 men and 2 women, age range 18–35 years). Symptoms developed in 1 patient on February 15 and in 4 patients on February 16. All had fever (39°C–40°C), chills, malaise, myalgias, vomiting, headache, and delirium. Leukocyte counts ranged from 11,000 to 88,000/μL. Chest radiographs were normal in all 5 patients. Four had severe pharyngitis; 3 of them had dysphagia, tender submandibular lymphadenitis, and tonsillar enlargement. The fourth patient, the 9-year-old girl, had severe abdominal pain and tenderness on abdominal palpation, profound hypotension (blood pressure 60/30 mm Hg), and a generalized hemorrhagic rash. This patient and the 26-year-old index patient (blood pressure 90/60 mm Hg) died. These 4 patients with pharyngitis did not have buboes or lymphadenitis at any other site. The patient without pharyngitis had axillary lymphadenitis and cellulitis of his right arm; he had cut his arm while killing a sick camel on February 13. None of the patients had skin lesions that suggested recent flea bites.

Y. pestis was isolated from the blood of the patient with pharyngitis who died and from the spinal fluid of the patient with abdominal pain. Identification was confirmed by phage lysis and direct fluorescent antibody staining. Indirect hemagglutination for plague was positive in convalescent-phase sera from the 3 survivors from whom *Y. pestis* was not isolated.

The patients were from 4 related families, 2 from Goriat and 2 from a village 20 km from this town. The adult family members denied seeing rodents around their homes or being bitten by fleas or other biting insects. All families owned camels. The male head of each family traveled to the desert daily to allow his camels to graze. These men reported that several of their camels had recently died. We observed 3 camel carcasses in the desert near a corral where the camels were fed grain and hay to supplement their grazing.

The meat from the sick camel that had been butchered on February 13 was shared among 11 families (106 members). No other food was shared among these families. The 4 patients with pharyngeal plague were among 37 people who had eaten this camel meat; 1 patient with bubonic plague (the man who slaughtered the camel) was among the 69 people who had not eaten the meat (risk ratio [RR] 7.7, $p<0.05$, Fisher exact test). Moreover, pharyngeal plague developed in 4 of 6 patients who had eaten raw camel liver, but not in 31 persons who had eaten only cooked camel meat or liver (RR not defined, $p<0.01$, Fisher exact test).

We isolated *Y. pestis* from a sample of leftover camel meat containing bone and marrow. Jirds (*Meriones libycus*), jird carcasses, rodent burrows, and rodent excreta were found at the camel corral. *Y. pestis* was isolated from the blood and liver of live jirds collected from the camel corral and from fleas (*Xenopsylla cheopis*) combed from these jirds.

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Conclusions

This investigation confirms that human plague with pharyngeal and gastrointestinal symptoms can result from eating infected raw camel liver. Only 1 published report has proposed this method of infection. In 1976, in a small, remote Libyan village, 13 plague cases occurred after a sick camel was slaughtered and its meat eaten (4). However, as a source of infection eating camel meat could not be distinguished from droplet transmission, percutaneous exposure during camel killing or handling fresh meat, or flea bites. Moreover, plague infection was not found in the camel, and the human pharyngeal plague might have resulted from crushing fleas between the teeth while grooming (5).

The 4 patients with pharyngeal plague had symptoms similar to those of domestic cats with plague in New Mexico, where 46% of plague-infected cats had submandibular lymphadenitis (2). These feline cases were thought to result from eating infected prey. Similarly, since human plague patients had submandibular lymphadenitis, tonsillitis, pharyngitis and dysphagia, or severe gastrointestinal symptoms, this suggests ingestion as the route of exposure.

Christie et al. suggested that among domestic animals, camels may be an important plague host because their wide-ranging behavior increases the chance of coming into contact with natural plague foci (4). Our investigation indicates another scenario, with evidence of a plague epizootic at a fixed site where camels were corralled. Russian investigators have proposed several methods of natural infection of camels: bites from rodent fleas, mechanical transmission from ticks, eating feed contaminated with excreta of infected rodents, and eating dead rodents along with their feed (6). All of these possibilities existed in this outbreak.

This unusual profile of human plague was recognized because several related patients with life-threatening illnesses were seen at a hospital over a 2-day period and a medical officer recognized the illness. Sporadic cases of pharyngeal or gastrointestinal plague would be less likely to attract the attention of medical or public health workers. Clinicians and public health officers, particularly in the Middle East, North Africa, and Central Asia, should be alert for sporadic cases of pharyngeal or gastrointestinal plague so that curative and preventive measures can be promptly initiated.

Dr Bin Saeed is a graduate of the Saudi Arabian Field Epidemiology Training Program, where he performed this investigation. He has a doctorate in epidemiology from the University of Texas. His current research interest is the epidemiology of infectious diseases.

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Melioidosis, Northeastern Brazil

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Melioidosis was first recognized in northeastern Brazil in 2003. Confirmation of additional cases from the 2003 cluster in Ceará, more recent cases in other districts, environmental isolation of *Burkholderia pseudomallei*, molecular confirmation and typing results, and positive serosurveillance specimens indicate that melioidosis is more widespread in northeastern Brazil than previously thought.

Melioidosis is a fatal bacterial infection found in many parts of the tropical belt, particularly in Southeast Asia and northern Australia. Sporadic cases of the disease have been reported previously in Central and South America (1). In 2003 septicemic melioidosis was diagnosed for the first time in northeastern Brazil by culture of the causal agent, *Burkholderia pseudomallei* from a 10-year-old boy (2). That case is believed to be the first culture-confirmed case of melioidosis in Brazil and was part of a small cluster of cases (hereafter termed Brazil outbreak l). At first, evidence that >1 case of melioidosis had occurred was circumstantial. The diagnosis relied entirely on the phenotypic features of a blood culture isolate from the 10-year-old boy. A more detailed, multidisciplinary investigation obtained further evidence for the case cluster and clarified its likely relationship to infection in the surrounding population.

The Study

Outbreak l comprised 4 previously healthy children from the Municipality of Tejussuoca; the children were admitted to the hospital with clinical features of systemic infection over the course of 10 days (February 28–March 7, 2003) (Table 1; online Appendix Figure, available from http://www.cdc.gov/ncidod/EID/vol11no09/05-0493_app.htm). Three of the children died because of multiple organ systems failure. Patient l died shortly after admission to a local hospital, before any diagnostic microbiology tests could be arranged. Gram-negative bacilli were isolated by blood culture from 2 children, patient 2 and patient 3. For patient 2, the isolate did not survive preliminary laboratory analysis, but findings at autopsy were consistent with melioidosis (3). In patient 3, the isolate was presumptively identified as *B. pseudomallei*. Bacterial identification and susceptibility results came too late to guide the treatment of patient 3, who also died, but did lead to changes in antimicrobial drug therapy of patient 4, who was admitted to the hospital later than the other 3 patients, survived, and remains healthy. In her case, melioidosis was demonstrated by laboratory evidence of late seroconversion, detected by indirect hemagglutination assay. Preliminary epidemiologic investigations indicated that the children were probably infected when diving into an irrigation reservoir that filled shortly after the onset of the summer rains.

Environmental studies conducted shortly after the case cluster occurred (and then repeated with improved methods at a later date) did not isolate *B. pseudomallei* from this location or other nearby sites. A detailed review of surveillance methods was undertaken, and external advice was sought shortly before the ensuing rainy season. The presumptive *B. pseudomallei* isolate from patient 3 was sent to a reference laboratory for independent confirmation and molecular typing. A case definition was established for prospective epidemiologic surveillance, and seroepidemiologic studies began. External advice was sought for environmental isolation methods.

The first clinical isolate (outbreak 1, patient 3, the 10-year-old boy) was confirmed as *B. pseudomallei* by phenotypic and molecular methods, according to a validated discovery pathway (4). In brief, polymerase chain reaction (PCR)–based identification, gas-liquid chromatography of fatty acid methyl esters, and an agglutinating monoclonal antibody were used to confirm the isolate presumptively identified as *B. pseudomallei*.

Just over 1 year later, in 2004, several suspected cases of septicemic melioidosis occurred in another location in the State of Ceará (outbreak 2). The *B. pseudomallei* isolate from 1 such patient and 2 *B. pseudomallei* isolates from soil and water samples in the corresponding environmental study were sent for confirmation and molecular typing, as before. The patient was from the Municipality of Banabuiú, ≈400 km from the location of outbreak l. She used to wash clothing while sitting in a nearby river. She first complained of a perineal abscess, which persisted for 2 weeks before she was admitted to the hospital with septicemia. *B. pseudomallei* was isolated by blood culture after she died. The *B. pseudomallei* environmental isolates

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Table 1. Patients implicated in melioidosis case-cluster in northeastern Brazil, 2003 (outbreak 1)

Patient	Sex	Age, y	Outcome	Culture	Autopsy	Serology
1	M	15	Died	NA*	NA	NA
2	F	14	Died	Gram-negative bacteria	Melioidosis	Negative
3	M	10	Died	*Burkholderia pseudomallei*	Melioidosis	Negative
4	F	12	Survived	Negative	NA	Positive

*NA, not available.

were from river water taken near where she washed clothes and from soil from the compacted earth floor under the tub she bathed in at home.

*Eco*R1 ribotyping showed that the *B. pseudomallei* isolate from patient 3 in the 2003 outbreak 1 was similar to the Western Australian outbreak strain (Table 2, online Appendix Figure). *Eco*R1 ribotyping discriminated between 3 of the 4 Brazil *B. pseudomallei* isolates (Brazil outbreak 1, patient 3: ribotype 1; unrelated later case in second district: ribotype 6; and 2 environmental isolates from second district: ribotypes 1 and 4). However, ribotyping was not as discriminating as DNA macrorestriction typing (pulsed-field gel electrophoresis, PFGE), which showed that the Brazil and Western Australian outbreak isolates were distinct strains (Brazil outbreak, patient 3: PFGE type 2, Western Australian outbreak strain: PFGE type 1).

Autopsy results from patient 2 in the original case cluster were similar to those of the culture-positive third case-patient and were consistent with melioidosis (3). The 1 survivor of the case cluster (Brazil outbreak 1, patient 4) seroconverted (titer = 1:160) from an undetectable indirect hemagglutination assay (IHA) titer around the time of her infection. One parent was borderline positive by IHA (titer of 1:40), and one was negative by IHA (<10).

Of the 36 persons from both districts tested by IHA for serologic evidence of exposure to *B. pseudomallei*, 14 had titers ≥1:40; 7 had a titer of ≥1:160; and 2 had titers of 1:5,120. No significant associations occurred between seropositivity and district, or seropositivity and age. However, seropositivity and sex were significantly associated (Fisher exact test, p = 0.0159, relative risk = 0.320, 95% confidence interval [CI] = 0.12–0.83); 10 of 16 female patients had titers ≥40, the threshold titer, compared to 4 of 20 male patients. This apparent association between female sex and seropositivity is the reverse of the association expected from experience in Southeast Asia.

Conclusions

At the time of writing, sporadic human infection has been reported sporadically from other locations in northeastern Brazil, consistent with an emerging infectious disease. Prospective case surveillance, improved laboratory diagnosis, and targeted environmental bacteriologic testing will help clarify the epidemiology of melioidosis in this region. Why this disease has appeared in Brazil remains obscure, although our preliminary molecular typing results indicate a possible link with Australian and Southeast Asian infections through a putative common progenitor strain. Veterinary investigation may help identify a possible means of introduction of the disease, since the goats that feature in subsistence farming in Ceará may have been imported from parts of the Caribbean where caprine melioidosis is known to occur (5). An alternative hypothesis is that *B. pseudomallei* was introduced through rice cultivation. An environmental search for *B. pseudomallei* (then *Pseudomonas pseudomallei*) was conducted shortly after melioidosis was first reported in South and Central America (6): a large number of water samples from rice fields near São Paulo were cultured. No *P. pseudomallei* was found, which led to the conclusion that the temperature and moisture of the environment did not favor the microorganism. More recently, *B. pseudomallei* has been presumptively identified in the root soil of sugar cane in

Table 2. Molecular typing patterns for Brazil outbreak 1 strain (patient 3), subsequent northeastern Brazilian isolates, and unrelated strains*†

Connection	Location	Type	Source	Culture	Ribotype	PFGE type
Outbreak 1	Ceará, Brazil†	Clinical	Blood	*B. pseudomallei*	1	2
Later case, outbreak 2	Ceará, Brazil†	Clinical	Blood	*B. pseudomallei*	6	9
Later case, outbreak 2	Ceará, Brazil	Environ	Water	*B. pseudomallei*	4	6
Later case, outbreak 2	Ceará, Brazil	Environ	Soil	*B. pseudomallei*	1	3
Cluster	WA, Australia	Clinical	Blood	*B. pseudomallei*	1	1
Cluster	WA, Australia	Environ	Water	*B. pseudomallei*	1	1
Later case 1	WA, Australia	Clinical	Blood	*B. pseudomallei*	2	4
Later case 2	WA, Australia	Clinical	Blood	*B. pseudomallei*	3	5
Later case 3	WA, Australia	Clinical	Blood	*B. pseudomallei*	5	8
NCTC 10276	India	Clinical	Tissue	*B. pseudomallei*	5	7

*PFGE, pulsed-field gel electrophoresis; WA, Western Australia; Environ, environmental; *B. pseudomallei*, *Burkholderia pseudomallei*.
†Brazil outbreak 1 occurred at Tejusuoca; outbreak 2 occurred at Banabuiu, both in the state of Ceará

the São Paulo region (7). Rice is grown in parts of Ceará where melioidosis cases have been identified and is an important crop in other parts of the country. Further epidemiologic and environmental studies are needed to determine the extent of the environmental hazard and the risk it represents for the human population and their livestock in northern Brazil. Finally, the terrain in Ceará has many similarities to northern Australia, where the summer rains are known to coincide with most septicemic melioidosis cases (8). In some parts of the Caribbean, sporadic melioidosis cases appear to be a harbinger of more common disease when flooding or other climatic determinants prevail (9,10). The surveillance methods recently introduced in Ceará may therefore help predict future melioidosis events. Data on melioidosis serology results in an epidemic setting are limited. The seroepidemiology survey conducted after the Western Australian melioidosis outbreak relied on access to previous serum samples from the same community, fortuitously collected for other purposes before the outbreak (11). Results from a serologic survey based on single samples from each study participant, as in Ceará, will necessarily have much wider CI. The investigation into the Western Australian outbreak identified persons who seroconverted without clinical evidence of *B. pseudomallei* infection. Carefully planned prospective seroepidemiologic studies in northeastern Brazil will clarify the importance of these preliminary observations. Establishing the true prevalence of melioidosis in northeastern Brazil will help ascertain the threshold for serodiagnosis and the clinical relevance of borderline results.

In the absence of any obvious anthropogenic changes known to increase melioidosis risk, the unusual weather systems operating in early 2003 appear to be the most likely explanation for the apparent temporal and positional clustering of cases. The diversity of molecular types of *B. pseudomallei* and the seroprevalence of *B. pseudomallei* antibody–positive persons are more consistent with an endemic disease that has gone undetected for several years than a recent, point-source incursion. Further epidemiologic studies will need to address whether the apparent emergence of melioidosis in northeastern Brazil is due primarily to improved ascertainment, the regional impact of climate change, changes in land use, or a combination of these factors.

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Multidrug-resistant Tuberculosis Detection, Latvia

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To improve multidrug-resistant tuberculosis (MDR-TB) detection, we successfully introduced the *rpoB* gene mutation line probe assay into the national laboratory in Latvia, a country with epidemic MDR-TB. The assay detected rifampin resistance with 91% sensitivity and 96% specificity within 1 to 5 days (vs. 12–47 days for BACTEC).

Until recent years, global efforts to reduce the prevalence of multidrug-resistant tuberculosis (MDR-TB), defined as in vitro resistance to at least rifampin and isoniazid, have focused on preventing new cases of acquired MDR-TB. However, countries that already have a high incidence of MDR-TB must implement additional strategies, such as reducing transmission by detecting cases earlier and improving infection control in settings with shared air spaces. As yet undetermined are optimal methods to identify drug-resistant *Mycobacterium tuberculosis* in a timely and affordable way in resource-limited settings. Standard laboratory methods of detecting drug resistance, such as *M. tuberculosis* culture and drug susceptibility testing (DST) performed with Löwenstein- Jensen (LJ) medium, are inexpensive but slow; DST results are often not available for 3 to 4 months. Testing methods that use liquid media, such as BACTEC systems (Becton Dickinson, Sparks, MD, USA), can deliver DST results to clinicians within 3 to 4 weeks; however, this technology requires expensive equipment and media.

Several methods that work directly on respiratory specimens and that detect resistance to a limited number of drugs within 1 day to 3 weeks have been reported (1–5). One assay that is commercially available is a line probe assay, a reverse-hybridization assay that detects mutations in the *rpoB* gene (5–12). Among clinical *M. tuberculosis* isolates, those with mutations in the *rpoB* gene are associated with 80% to 90% rifampin resistance (5). Previously published studies using this assay have demonstrated 90%–100% concordance when results are compared to DST results among *M. tuberculosis* isolates from culture and 78%–98% sensitivity and 84%–100% specificity when applied to respiratory specimens that were positive for acid-fast bacilli (AFB) (5–12). However, these studies involved small numbers of respiratory specimens and were not performed in a national TB laboratory that supports diagnosis, treatment, and care for large numbers of MDR-TB patients.

Latvia is among those countries with the highest prevalence of MDR-TB in the world (13). Rifampin resistance in Latvia is closely associated with resistance to isoniazid; therefore, detecting rifampin resistance should also detect most MDR-TB cases (13). As part of a long-term project to integrate new assays into the Latvian national laboratory protocols to identify MDR-TB patients more quickly, we prospectively compared the results of the line probe assay for *rpoB* mutations to results with BACTEC DST technology.

The Study

We enrolled consecutive patients who were initially seen at or referred to the Latvian State Centre of Tuberculosis and Lung Diseases from January 2003 to March 2004 with AFB-positive respiratory specimens (sputum or bronchoalveolar lavage [BAL] specimens) and identified as being at high risk for MDR-TB. Patients at high risk were defined as those with a history of close contact to a known MDR-TB patient or with a history of previous TB treatment (14).

After sputum specimens were decontaminated (15), we tested for AFB (15) and set up 2 cultures for *M. tuberculosis*: 1 in LJ medium (15) and 1 in either the BACTEC Mycobacteria Growth Indicator Tube 960 or the BACTEC 460 system per manufacturer's instructions (Becton Dickinson). DNA was extracted from the remaining suspension with the QIAAMP DNA Mini kit (Qiagen, Valencia, CA, USA). Lysate was transferred to the line probe kit INNO-LiPA Rif.TB (Innogenetics, Ghent, Belgium) for amplification, including a second nested reaction with inner primers and the hybridization reaction (manufacturer's instructions). In general, the *rpoB* gene amplicons were incubated with immobilized, membrane-bound *rpoB* gene probes, including overlapping wildtype sequences (S1–S5) and 4 of the most frequent mutations (R2:Asp516Val, R4a:His526Tyr, R4b:His526Asp, and R5:Ser531Leu). The kit also includes a probe for *M. tuberculosis* complex.

DST was performed with the BACTEC 460 system (manufacturer's protocols). We then compared line probe results to *M. tuberculosis* culture and BACTEC DST results for each patient. We also set up DST on LJ media by using the proportion method (15). All laboratory testing was performed at the Latvian State Centre of Tuberculosis

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and Lung Diseases, Riga, Latvia. Line probe results were not provided to physicians. Patient identifiers were removed before analysis. The protocol underwent institutional ethical review by the Latvian State Centre of Tuberculosis and Lung Diseases, was determined not to be human subjects research, and was approved as programmatic evaluation by the Centers for Disease Control and Prevention.

In total, 89 (37%) of 243 patients who met the case definition for being at high risk for MDR-TB had AFB-positive respiratory specimens; 77 (87%) were sputum specimens, and 12 (13%) were BAL specimens. *M. tuberculosis* isolates grew in BACTEC cultures from 86 (97%) of the AFB-positive specimens. Mycobacteria other than *M. tuberculosis* were identified in 3 of the remaining BACTEC cultures. No dual infections were found. *M. tuberculosis* complex was also detected by line probe assay in 86 specimens, although for 2 patients *M. tuberculosis* grew in respiratory specimens in BACTEC cultures but was not detected by the line probe assay, and 2 specimens that were positive for *M. tuberculosis* complex by line probe assay did not grow in BACTEC but did grow on LJ media. These isolates were injected into the BACTEC 460 system for DST. The line probe assay correctly separated *M. tuberculosis* complex and nontuberculosis mycobacteria.

The line probe assay had good sensitivity, high specificity, and positive predictive value and negative predictive value for rifampin resistance compared to BACTEC (Table 1). Among the isolates resistant to rifampin by BACTEC DST, the *rpoB* mutations detected by the line probe included 20 (61%) R5 (Ser531Leu), 8 (24%) R2 (Asp516Val), 1 (3%) R4b (His526Asp), and 1 (3%) ΔS5 (absence of hybridization to 1 wildtype sequence). One rifampin BACTEC DST-susceptible isolate had a line probe result read as ΔS1, ΔS2 (absence of hybridization to 2 wildtype sequences).

Most patients considered high risk for MDR-TB had resistance to at least 1 drug (Table 2). Rifampin resistance was highly correlated with classification as MDR-TB; 32 (97%) of 33 patients with rifampin resistance had MDR-TB. The predictive value of the line probe *rpoB* mutation result for MDR-TB was 91% (95% confidence interval 92–100).

The line probe assay performed directly on DNA extracted from respiratory specimens gave quicker results for rifampin resistance (median = 4 days, range 1–5) than other methods (BACTEC 460 median = 28 days, range 12–47; LJ median = 58 days, range 47–65). While DST results from the BACTEC liquid culture system were available considerably faster than were results from LJ media, *rpoB* gene mutation results were available in <1 week.

Table 1. Comparison of results from line probe assay for *rpoB* gene mutations to rifampin susceptibility results on acid-fast bacilli–positive respiratory specimens*†

	BACTEC 460 System	
Line probe *rpoB* gene mutation results	Rifampin-resistant	Rifampin-susceptible
Resistant	31	1
Susceptible	2	52
No amplification	1	1

*N = 88; Includes 86 *Mycobacterium tuberculosis* isolates from BACTEC plus 2 isolates that grew on Löwenstein-Jensen media.
†Compared to results from BACTEC drug susceptibility testing, the line probe assay had a sensitivity of 91% (95% confidence interval [CI] 83–99) and a specificity of 96% (95% CI 92–100). The positive predictive value of the line probe *rpoB* mutation result for rifampin resistance was 94% (95% CI 88–100), and the negative predictive value was 96% (95% CI 92–100).

Conclusions

In Latvia, where nearly 40% of patients had a history of TB treatment and 10% of all new patients without a history of treatment have MDR-TB (13,14), integrating a line probe assay for *rpoB* gene mutations into regular laboratory services could enhance MDR-TB control efforts. Results from this study demonstrated that in persons considered at high risk for MDR-TB, the line probe assay detected rifampin resistance with 91% sensitivity and 97% specificity on respiratory specimens within 1 to 5 days of specimen collection in a busy clinical laboratory. Additionally, 91% of patients at high risk for MDR-TB, with line probe assay results consistent with *rpoB* mutations, were ultimately confirmed as having MDR-TB.

In addition, we compared the timeliness of acquiring rifampin DST results between a liquid and solid media system and a line probe assay. Although liquid media were considerably faster than solid media, the line probe assay for *rpoB* mutations performed directly on respiratory specimens gave results consistent with MDR in <1 week.

Therefore, integrating the use of the line probe assay on AFB-positive respiratory specimens into the Latvian national laboratory could permit much earlier segregation and isolation of infectious patients who have a high

Table 2. Drug-susceptibility profiles for patients at high risk for MDR-TB with acid-fast bacilli–positive respiratory specimens*†

Drug resistance	No. (%)
None	35 (40.0)
Any resistance	52 (60.0)
Rifampin (total)	34 (38.6)
Mono-rifampin	1 (1.1)
MDR (total)	33 (37.5)
R, H	2 (2.3)
R, H, S	17 (19.3)
R, H, S, E	14 (15.9)
Isoniazid (total)	52 (59)
Mono-isoniazid	5 (5.7)
H, S	14 (15.9)

*N = 88; includes 86 *Mycobacterium tuberculosis* isolates from BACTEC plus 2 isolates that grew on Löwenstein-Jensen media.
†R, rifampin; H, isoniazid; S, streptomycin; E, ethambutol; MDR-TB, multidrug-resistant tuberculosis.

likelihood of MDR-TB (thereby reducing MDR-TB transmission) and could facilitate more focused DST practices for first- and second-line TB drugs and more efficient use of resources. The high specificity is reassuring; the use of line probe assay results to inform drug treatment selections would rarely result in missed opportunities to treat with rifampin. Conversely, only 9% of patients infected with a rifampin-resistant isolate would not benefit from early detection of resistance and would, in turn, receive care similar to the current standard.

Several other assays that detect drug resistance within 1 to 3 weeks have been described (1–5). Some of these may perform as well as the line probe assay and be less expensive. We chose the line probe assay for our project because it was commercially available and had been evaluated by several investigators (5–12). Also, the equipment and skills could be applied toward other molecular epidemiologic studies to better understand ongoing transmission of MDR-TB in Latvia. We will evaluate the cost-effectiveness of integrating this assay into the Latvian State Centre of Tuberculosis and Lung Diseases and may also model the cost of new assays as they become available. This study, part of a larger project to reduce the prevalence of MDR-TB in Latvia, is a first step in identifying optimal methods to identify drug-resistant *M. tuberculosis* in a timely and affordable way in resource-limited settings with high MDR-TB prevalence.

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β-Lactam Resistance and *Enterobacteriaceae*, United States

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Extended-spectrum cephalosporins (ESC) are an important drug class for treating severe *Salmonella* infections. We screened the human collection from the National Antimicrobial Resistance Monitoring System 2000 for ESC resistance mechanisms. Of non-Typhi *Salmonella* tested, 3.2% (44/1,378) contained bla_{CMY} genes. Novel findings included bla_{CMY}-positive *Escherichia coli* O157:H7 and a bla_{SHV}-positive *Salmonella* isolate. CMY-positive isolates showed a ceftriaxone MIC ≥2 μg/mL.

Extended-spectrum cephalosporins (ESC) are important for treating persons with severe *Salmonella* infections (1). This drug class is particularly important for pediatric therapy because fluoroquinolones are not approved for use in children. In 2000, 25% (8,153/32,022) of laboratory-confirmed *Salmonella* cases reported to the Centers for Disease Control and Prevention (CDC) occurred in children <5 years of age (2).

The National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria began monitoring for resistance to cephalosporins and other drugs among human-derived *Salmonella* and *Escherichia coli* O157 isolates in 1996. *Shigella* was added to the surveillance in 1999. From 1996 to 1998, 15 (0.4%) of 4,093 non-typhi *Salmonella* isolates tested by NARMS were resistant to ESC, and none of the 675 *E. coli* O157 isolates tested were ESC resistant (3). Thirteen (87%) of 15 ESC-resistant *Salmonella* isolates exhibited a bla_{CMY-2}-mediated mechanism of resistance (3,4), including 11 serotype Typhimurium, 1 Thompson, and 1 Newport. One *S.* Cubana isolate exhibited a bla_{KPC-2} carbapenemase (5), and the remaining *S.* Typhimurium isolate exhibited a yet-uncharacterized extended-spectrum β-lactamase (3). To determine the dynamics and mechanisms of cephalosporin resistance among species and serotypes, we examined the 2000 NARMS collection and determined mechanisms of ESC resistance in isolates exhibiting elevated cephalosporin MICs.

The Study

As NARMS participants, 17 state and local public health laboratories representing 40% of the US population submitted every tenth non-Typhi *Salmonella* isolate, every tenth *Shigella* isolate, and every fifth *E. coli* O157 isolate received in 2000 to CDC for antimicrobial susceptibility testing. Identification and serotyping were conducted at submitting laboratories. The MIC was determined for 17 antimicrobial agents at CDC by using partial range broth microdilution (Sensititre, Westlake, OH, USA). Isolates were chosen for further study based on the following MIC criteria: cefoxitin (≥16 μg/mL), ceftiofur (≥4 μg/mL), or ceftriaxone (≥16 μg/mL).

Isoelectric Focusing (IEF) for β-Lactamases

β-Lactamase content was determined for all isolates that met the MIC criteria for further study. The IEF methods of Rasheed et al. (6) were used with modification. Crude cellular protein extracts were prepared by pelleting 3-hour trypticase soy broth cultures (grown at 37°C with shaking at 300 rpm), resuspending in 0.2% sodium acetate at 5% of original culture volume, and freeze-thawing 4 times in a dry ice/ethanol bath and a 37°C water bath. Preparations were then diluted twofold with distilled water and placed on ice for 30 min with occasional swirling. The supernatant was collected after centrifuging for 30 min at maximum relative centrifugal force (14,000 rpm) in a Beckman 5417R microcentrifuge (Palo Alto, CA, USA). Three- to 5-μL aliquots of each preparation were resolved by focusing for 1.5 h on an Ampholine PAGplate polyacrylamide gel, pH range 3.5–9.5 (APBiotech**,** Piscataway, NJ, USA), according to manufacturer's instructions. Gels were stained by overlaying with a 500 μg/mL solution of nitrocefin (Becton Dickinson, Franklin Lakes, NJ, USA). Isoelectric points were estimated by comparison with the following standard β-lactamases: TEM-12 (pI 5.25), SHV-3 (pI 7.0) and MIR-1 (pI 8.4).

Polymerase Chain Reaction (PCR) for β-Lactamase Genes

For isolates that were IEF-positive for a β-lactamase with a pI ≥8.4, amplification of bla_{CMY} genes was attempted. Internal primers were used to amplify a 369-bp portion of bla_{CMY} genes from crude colony lysates. The forward primer anneals to nucleotide (nt) 271–289 of the 1,146-nt bla_{CMY-2} sequence from *Klebsiella pneumoniae* (NCBI accession no. X91840) and has a sequence of 5′-GGCGTGTTGGGCGGCGATG-3′. The reverse primer anneals to nt 621-639 of bla_{CMY-2} and has a sequence of 5′-CAGCGGAACCGTAATCCAG-3′. APBiotech Ready-to-Go

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Beads (Piscataway, NJ, USA) were used to formulate 25-μL reactions, which were run in an MJResearch thermocycler (Waltham, MA, USA) under the following conditions: 1 cycle of 94°C for 5 min followed by 25 cycles of: 94°C for 30 s, 59°C for 1 min, 72°C for 1 min. Amplicons were resolved by electrophoresis in 1% agarose gels. For isolates exhibiting a β-lactamase with a pI = 8.0, bla_{SHV} genes were amplified using primers 4 and 5 from Rasheed et al. (7) with Perkin-Elmer Amplitaq Gold 2X Master Mix (Boston, MA, USA).

Conclusions

In 2000, 2,152 non-Typhi *Salmonella, Shigella,* and *E. coli* O157 isolates were received and tested. Of these, 57 (2.6%) met the MIC criteria for additional testing to determine mechanisms of extended-spectrum cephalosporin resistance: 46 non-Typhi *Salmonella* isolates, 7 *Shigella* isolates (all *S. sonnei*), and 4 *E. coli* O157:H7 (Table). bla_{CMY} genes were identified in 44 (96%) of the 46 *Salmonella* isolates. One *S.* Nienstedten isolate produced a bla_{SHV} enzyme with a pI of 8.0. One *S.* Muenchen isolate with a cefoxitin MIC = 16 μg/mL yielded no β-lactamases by IEF. This isolate exhibited very low MIC of ceftriaxone and ceftiofur (≤0.25 and ≤0.5 μg/mL, respectively).

The 7 *S. sonnei* isolates included in the study met only the cefoxitin MIC criterion (≥16 μg/mL). All 7 showed a ceftiofur MIC 1.0 μg/mL or less, and a ceftriaxone MIC ≤0.25 μg/mL. Six of these were also resistant to ampicillin, amoxicillin-clavulanate, and cephalothin. Each isolate was IEF-positive for a β-lactamase enzyme with a pI≥8.4, but was polymerase chain reaction–negative for a bla_{CMY} gene. We suspect the resistance is related to overproduction of chromosomal *amp*C genes known to be present in *Shigella* species (8); however, porin deficiency (9) and penicillin-binding protein changes (10) are worth exploration as well. Efflux mechanisms (11,12) are possible, but multidrug-resistance pumps might be less likely since none of the 7 isolates were resistant to chloramphenicol, nalidixic acid, or ciprofloxacin, and only 4 were resistant to tetracycline.

Eight isolates (5 *Salmonella* and 3 *S. sonnei*) produced putative TEM enzymes in addition to an enzyme with a pI≥8.4. The pI in each case was 5.3 or 5.4. Plasmidborne bla_{TEM-1} enzymes have been identified in several *Salmonella* serotypes (13).

Twenty-seven (61%) of 44 bla_{CMY}-containing *Salmonella* in 2000 were serotype Newport. This finding coincides with emergence of a multidrug-resistant strain of *S.* Newport called MDRAmpC (14). MDRAmpC increased from 1% (1/77) of *S.* Newport isolates tested by NARMS in 1998 to 22% (27/124) in 2000 (15). In addition, bla_{CMY} genes were found in 5 other *Salmonella* serotypes (Typhimurium, Heidelberg, Agona, Infantis, and Reading) in 2000. This contrasts with 1996–1998, when bla_{CMY} was found in 3 serotypes (Newport, Typhimurium, and Thompson), which indicated that these genes or the mobile elements that house them have been disseminated. Furthermore, bla_{CMY} genes were identified in each of the 4 *E. coli* O157:H7 isolates that met the MIC criteria in 2000. To our knowledge, this is the first report of bla_{CMY} in this *E. coli* serotype.

All 48 bla_{CMY}-positive isolates (44 *Salmonella* and 4 *E. coli* O157:H7) exhibited a ceftiofur MIC ≥8 μg/mL; however, their ceftriaxone MIC ranged from 2 to 32 μg/mL. Since the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) breakpoint for ceftriaxone resistance is 64 μg/mL, none of these isolates were interpreted as ceftriaxone-resistant, and only 48% (23/48) were intermediate (16 or 32 μg/mL). In contrast, all 48 CMY-producing isolates showed a cefoxitin MIC ≥16 μg/mL (intermediate or resistant according to CLSI guidelines).

NARMS sampling in 2000 showed that bla_{CMY} genes continue to be the major mechanism of extended-spectrum cephalosporin resistance among non-Typhi *Salmonella*; other mechanisms of ESC are rare. The increasing diversity of bla_{CMY}-positive *Salmonella* serotypes and the discovery of bla_{CMY} genes in *E. coli* O157:H7 highlight the mobility of these mechanisms. This finding is not unexpected since these genes have been shown to be present on

Table. Characterization of isolates exhibiting increased MIC to extended-spectrum β-lactams among 2000 NARMS*

Isolate/serotype	Total no. met MIC criteria/ total no. tested (%)	Total PCR-positive for bla_{CMY} (%)	Total IEF-positive for pI ≥ 8.4 (%)	No. that produce other β-lactamases
Salmonella				
Newport	27/124 (22)	27 (100)	27 (100)	1
Typhimurium	11/303 (3.6)	11 (100)	11 (100)	2
Heidelberg	3/79 (3.8)	3 (100)	3 (100)	1
Other nontyphoidal *Salmonella* (Agona, Infantis, Muenchen, Nienstedten, Reading)	5/872 (0.57)	3 (60)	3 (60)	1
Shigella sonnei	7/367 (1.9)	0	7 (100)	3
Escherichia coli O157:H7	4/407 (0.98)	4 (100)	4 (100)	0
Total	57/2,152 (2.6)	48 (84)	55 (96)	8

*NARMS, National Antimicrobial Resistance Monitoring System; PCR, polymerase chain reaction; IEF, isoelectric focusing.

large plasmids, some of which are transferable by conjugation (4). Since *S.* Newport MDRAmpC and *E. coli* O157:H7 have been associated with bovine reservoirs, we hypothesize that bla_{CMY} genes may be circulating among cattle. This remains to be proven and warrants more intensive study of bla_{CMY} prevalence and movement in bovine production settings. Further research is also necessary to determine factors that contribute to dissemination of the mobile elements carrying these genes and selection of bla_{CMY}-positive strains such as *S.* Newport MDRAmpC. Notably, isolates exhibiting this extended-spectrum cephalosporinase may show a ceftriaxone MIC as low as 2 μg/mL, but MIC to ceftiofur and cefoxitin fall more reliably in the intermediate or resistant range. For this reason, we currently performed subsequent β-lactamase analysis on any isolate exhibiting a ceftriaxone or ceftiofur MIC ≥2 μg/mL.

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Dr Whichard is a molecular biologist with the NARMS/FoodNet laboratory at CDC. Her research interests include β-lactamases, multidrug-resistant *Salmonella* isolates, bacteriophages, and other mobile genetic elements.

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Perinatal Group B Streptococcal Disease Prevention, Minnesota

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In 2002, revised guidelines for preventing perinatal group B streptococcal disease were published. In 2002, all Minnesota providers surveyed reported using a prevention policy. Most screen vaginal and rectal specimens at 34–37 weeks of gestation. The use of screening-based methods has increased dramatically since 1998.

Group B streptococci (GBS) emerged as the leading cause of invasive bacterial infections in newborns in the United States in the 1970s. Although the incidence of GBS disease has declined substantially, it remains the leading cause of serious infection in newborns (1). Perinatal GBS transmission can be reduced dramatically by diagnosing maternal GBS colonization and administering intrapartum antimicrobial prophylaxis (IAP) during labor and delivery (2).

In 1996, the Centers for Disease Control and Prevention (CDC) published consensus guidelines recommending 2 methods of perinatal GBS disease prevention. The screening-based approach recommends obtaining vaginal and rectal cultures at 35–37 weeks of gestation. Women with GBS-positive cultures are offered IAP during labor. The risk-based approach recommends administering IAP to women with GBS risk factors when they go into labor (3). These guidelines are believed to have increased use of GBS disease prevention approaches by prenatal care providers, which has led to a decrease in the incidence of GBS disease (1,4). A 2002 study further indicated that routine screening for GBS would prevent ≈50% more newborn GBS infections than would a risk-based approach (5). This study, along with other data, led CDC to publish revised guidelines in August 2002 recommending universal prenatal screening (6).

As part of the Minnesota Department of Health Emerging Infections Program, prenatal care providers in Minnesota were surveyed in April 1998 to determine strategies to prevent perinatal GBS disease (7). In November 2002, a similar survey was undertaken to determine the extent to which Minnesota providers have adopted the revised 2002 CDC guidelines.

The Study

In 2002, all licensed obstetricians and certified nurse midwives in Minnesota were surveyed. All family practitioners who listed obstetrics as a secondary specialty and a 20% random sample of the remaining licensed family practitioners were surveyed. In 1998, surveys were mailed to a random sample of 50% of obstetricians and 25% of family practitioners who indicated on their licensure application that they provided prenatal care. All midwives were surveyed. Statistical analysis was performed with EpiInfo software (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Three mailings were sent during each study period. A total of 463 surveys (60% of those mailed) were completed in 2002, and 515 surveys (80% of those mailed) were completed in 1998. Providers who did not provide prenatal care were excluded from further analysis. The final sample included 97 midwives, 189 obstetricians, and 64 family practitioners in 2002 and 102 midwives, 128 obstetricians, and 201 family practitioners in 1998. No significant differences were found in provider characteristics (location, practice type, and number of deliveries performed) from 1998 to 2002.

In 2002, all providers surveyed indicated they had a policy to prevent perinatal GBS disease. Of these, 318 (91% [96% of obstetricians, 92% of midwives, and 73% of family practitioners]) indicated their policy was based upon at least 1 previously published guideline. Family practitioners ($p<0.05$) and midwives ($p<0.05$) were significantly more likely to follow published guidelines during 2002 than during 1998.

In 1998, the risk-based approach was the most common method of preventing GBS disease (Table 1). In 2002, the screening-based approach was the most common method. In 2002, providers were significantly more likely to have adopted a screening-based approach to prevention than they were in 1998 ($p<0.001$). In 2002, when risk-based providers were questioned, 14 (52%) of 27 midwives, 5 (50%) of 10 family practitioners, and 6 (32%) of 19 obstetricians indicated they planned to implement the new guidelines.

In 2002, among those who reported a screening-based approach, 262 (89%) of 293 providers routinely collected specimens from both vaginal and rectal sites. Midwives (97%) were more likely than obstetricians (90%) and family practitioners (77%) to collect specimens from both sites. In 2002, midwives ($p<0.001$) were significantly more likely to use both vaginal and rectal sites to screen

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Table 1. Change in policy types to prevent perinatal group B streptococci infection, Minnesota, 1998 and 2002

	Obstetricians		Midwives		Family practitioners	
Policy	1998, n (%) (N = 127)	2002, n (%) (N = 189)	1998, n (%) (N = 104)	2002, n (%) (N = 97)	1998, n (%) (N = 200)	2002, n (%) (N = 64)
Screening-based*	46 (36)	170 (90)	13 (13)	70 (72)	84 (42)	53 (83)
Risk-based*	74 (58)	12 (6)	75 (72)	13 (13)	87 (43)	5 (8)
Risk-based, planning to implement screening-based	–	5 (3)	–	14 (15)	–	5 (8)
Other/unknown†	7 (6)	2 (1)	16 (15)	0	29 (15)	1 (1)

*p<0.001, change from 1998 to 2002 among all prenatal care provider groups.
†p<0.005, change from 1998 to 2002 among all prenatal care provider groups.

for GBS than in 1998. No significant increase was seen in the proportion of obstetricians or family practitioners who screened vaginal and rectal specimens from 1998 to 2002 (Table 2).

Among providers who used a screening-based approach to prevent perinatal GBS infection in 2002, most (88%) obtained cultures at 35–37 weeks of gestation. No change was seen in the proportion of providers who screened at 35–37 weeks of gestation when responses from the 1998 and 2002 surveys were compared (Table 2).

In 2002, when providers were asked if their laboratories used a selective broth to isolate GBS, 171 (58%) of 293 indicated that they did. Obstetricians were significantly more likely than midwives and family practitioners to report selective broth use in their laboratories. Obstetricians ($p<0.001$) were significantly more likely to report that their laboratory used selective broth in 2002 than in 1998 (Table 2). Little change was seen among midwives and family practitioners regarding their knowledge of selective broth use from 1998 to 2002.

In 2002, a total of 225 (77%) of 292 providers reported using penicillin most often for IAP. Midwives and obstetricians were more likely than family practitioners to report using penicillin. Midwives ($p<0.01$) were significantly more likely to use penicillin in 2002 than they were in 1998. Little change was seen in the proportion of family practitioners and obstetricians who used penicillin in 1998 versus 2002 (Table 2).

Conclusions

The results of this survey suggest that all Minnesota providers have adopted a policy on preventing perinatal GBS disease, and most follow established, published guidelines. In 1998, a risk-based approach to GBS disease prevention was the most common strategy identified by providers. In 2002, screening all pregnant women for GBS was the predominant strategy.

The effectiveness of the screening-based approach depends partly on the sensitivity and specificity of the specimens collected. A previous study by Philipson et al. indicated that swabbing both vaginal and rectal sites significantly increased the sensitivity of isolating GBS compared with swabbing the vagina only (8). In our study, 89% of Minnesota providers indicated they routinely collected specimens for GBS screening from both vaginal and rectal sites. Because vaginal and rectal swabs are likely to yield diverse bacteria, selective broth is recommended to limit growth of other organisms, thus increasing the chance of isolating GBS (9). In a study by Silver and Struminski, ≈32% of women had false-negative culture results when direct agar plating was used instead of selective broth to isolate GBS (10). In our study, most obstetricians (72%) indicated that their laboratories used selective broth; however, less than half of midwives and family practitioners reported using selective broth. Many providers (41%) did not know whether their laboratories used selective broth. A recent survey found that 89% of laboratories that process GBS specimens use selective enrichment broth media for GBS isolation (11). Preliminary data from a 2004 survey

Table 2. Change in group B streptococci (GBS) screening characteristics among prenatal care providers reporting a screening-based approach, Minnesota, 1998 and 2002

	Obstetricians		Midwives		Family practitioners	
Characteristic	1998, n (%) (N = 45)	2002, n (%) (N = 170)	1998, n (%) (N = 13)	2002, n (%) (N = 70)	1998, n (%) (N = 84)	2002, n (%) (N = 53)
Vaginal/rectal screening	41 (91)	153 (90)	6 (46)	68 (97)*	61 (73)	41 (77)
Screening at 35–37 weeks of gestation	42 (93)	152 (89)	10 (77)	62 (89)	77 (92)	44 (83)
Use selective broth†	12 (27)	122 (72)‡	2 (15)	28 (40)	34 (40)	21 (40)
Penicillin first IAP choice§	35 (78)	138 (81)	7 (54)	60 (86)¶	32 (38)	27 (51)

*p<0.001, use of vaginal/rectal screening from 1998 to 2002.
†Prenatal care providers were asked if their laboratory used selective broth to isolate GBS.
‡p<0.001, use of selective broth from 1998 to 2002.
§IAP, intrapartum antimicrobial prophylaxis.
¶p<0.01, penicillin as first choice for IAP from 1998 to 2002.

of laboratories in Minnesota indicated that 92% of laboratories use a selective enrichment broth media to isolate GBS (Minnesota Department of Health, unpub. data).

Collecting cultures late in the gestational period is more likely to detect women who are colonized when they deliver, compared to screening at an earlier stage of a woman's pregnancy. In 2002, most (88%) providers who reported a screening-based approach to perinatal GBS disease prevention obtained cultures at 35–37 weeks of gestation.

Research in the 1980s showed that administering antimicrobial prophylaxis to women who are colonized with GBS was effective in preventing disease in newborns. Because of its narrow spectrum, penicillin remains the preferred drug of choice. Ampicillin, a broader-spectrum agent, is considered an acceptable alternative. In our study, >80% of obstetricians and midwives reported using penicillin as their first choice for IAP. Although family practitioners were significantly more likely to use penicillin in 2002 than in 1998, only 51% of family practitioners listed penicillin as their first choice.

Several factors should be considered when interpreting the results of this study. First, the survey was conducted only among Minnesota providers, so the results may not be generalized to other states. Second, the overall response rate was 80% in 1998 and 60% in 2002. This decrease is most likely explained by a sampling change in which a greater proportion of family practitioners with a history of providing prenatal care were sampled in 1998 than in 2002. We suspect that most family practitioners who failed to complete the survey in 2002 did so because they did not provide prenatal care. When characteristics of responders in 1998 and 2002 were compared, no significant differences were noted regarding location of practice, practice type, size of practice, and median number of deliveries performed. Finally, surveys are measures of reported practices and may not reflect actual services provided.

Prenatal care providers, especially family practitioners, should continue to discuss and establish policies regarding perinatal GBS disease prevention. Providers should be educated about optimal specimen sites and timing of screening. Education on using selective broth medium to isolate GBS should be provided to clinicians and laboratories. In addition, clinicians should be familiar with the appropriate antimicrobial agents used for IAP and ensure rapid drug administration when it is indicated.

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Characterizing Vancomycin-resistant Enterococci in Neonatal Intensive Care

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Repetitive sequence–based polymerase chain reaction fingerprinting was used to characterize 23 vancomycin-nonsusceptible enterococcal isolates from 2003 to 2004. Five genetically related clusters spanned geographically distinct referring centers. DNA fingerprinting showed infant-to-infant transmission from referring institutions. Thus, community healthcare facilities are a source of vancomycin-nonsusceptible enterococci and should be targeted for increased infection control efforts.

Vancomycin-resistant enterococci (VRE) are a cause of nosocomial infections in US hospitals. The National Nosocomial Infections Surveillance system of the Centers for Disease Control and Prevention reported vancomycin resistance in 28.5% of nosocomial enterococcal intensive care unit infections in 2003 (1). In a recent study, 14% of VRE-colonized patients progressed to infection within 15 days of a positive surveillance culture (2). Moreover, VRE can transfer the *vanA* gene for vancomycin resistance to more virulent pathogens such as *Staphylococcus aureus* both in vitro and in vivo (3).

Risk factors for VRE colonization in children include young age, use of invasive devices, antimicrobial drug administration, immunosuppression, low birth weight, and underlying malignancy (4). Interfacility transfer of patients colonized with VRE is common, and previous hospitalization is a risk factor for harboring VRE at the time of hospital admission (5,6). Active surveillance culture (ASC) programs for VRE and aggressive implementation of infection control measures reduce VRE transmission among adult and pediatric patients (7,8).

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We identified our first VRE-infected patient (bacteremia and urinary tract infection) in our neonatal intensive care unit (NICU) in 2000. This occasion prompted the initiation of our own ASC program. During the next 3 years (2000–2002), 65 patients with VRE colonization or infection were identified among the 1,820 patients admitted to our NICU. Of the VRE-colonized or -infected patients, some experienced serious infections, such as meningitis and bacteremia, while others were completely asymptomatic (9). By 2002, a multifaceted intervention greatly reduced the intrahospital spread of VRE. We continued our ASC program in the NICU during 2003–2004. An additional 25 patients were found to be colonized with vancomycin-nonsusceptible enterococci (VNSE); this group includes VRE and enterococci with intermediate susceptibility to vancomycin. All infants colonized with VRE had been admitted directly from regional hospitals in the Washington, DC, metropolitan area. This is the first study in which an ASC program and repetitive sequence–based polymerase chain reaction (Rep-PCR) fingerprinting were used to characterize the genetic relatedness and document intrahospital spread of VNSE.

The Study

The NICU at Children's National Medical Center (CNMC) is a level III/IV 40-bed unit that provides care for critically ill patients in the first 6 months of life. CNMC has no obstetrics service; therefore, all NICU admissions are referrals from other hospitals. Our ASC program for VNSE included rectal swab cultures performed upon admission to the NICU. Repeat cultures were collected weekly from patients with negative admission cultures unless they became colonized or were discharged. Dacron-tipped swabs were moistened with sterile trypticase soy broth before rectal sampling. All neonates with VRE were placed on contact isolation during their NICU hospitalization.

Rectal swab specimens were added to *Campylobacter* blood agar plates containing 10 μg/mL vancomycin (Becton Dickinson Diagnostic Systems, Sparks, MD, USA). VRE were identified by using standard laboratory procedures. Species identification and vancomycin susceptibility were determined by using MicroScan Gram-Positive Breakpoint Combo Panels (Dade Behring, Deerfield, IL, USA) with a 24-hour incubation. Vancomycin susceptibility results were categorized according to the standards published by the Clinical and Laboratory Standards Institute (10). Susceptible isolates had vancomycin MICs $\leq$4 μg/mL, intermediate isolates had MICs 8–16 μg/mL, and resistant isolates had MICs $\geq$ 32 μg/mL. *Enterococcus faecium* and *E. gallinarum* were differentiated by using standard laboratory tests for motility and detection of acid production from xylose (11).

The genetic relatedness of 23 VNSE was determined with Rep-PCR DNA fingerprinting by procedures recently described (12). Briefly, DNA was extracted from 2 μL of an overnight VNSE culture by using the Ultraclean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). The extracted DNA was amplified by Rep-PCR by using the DiversiLab Enterococcus Kit (Bacterial Barcodes, Spectral Genomics, Houston, TX, USA).

The Rep-PCR products were analyzed by using the DiversiLab System and Software (Bacterial Barcodes, Spectral Genomics). The resulting DNA fingerprint patterns were viewed as virtual electropherograms. Analysis was performed with DiversiLab software version 2.1.6.6 by using Pearson correlation coefficients to determine genetic similarities and the unweighted pair group method with arithmetic mean to create dendrograms. Samples were classified into 3 groups: indistinguishable (similarity >97%), similar (similarity 95%–97% with fingerprint patterns displaying 1–2 band differences), and different (similarity <95% with >2 band differences) (12).

Of 1,333 NICU patients admitted during 2003–2004, a total of 25 were colonized with VNSE, yielding a colonization rate of 2%. The median age of these patients was 45 days (range 14–200 days). Twenty-three isolates were available for DNA analysis. Fifteen (65%) of the 23 isolates were *E. gallinarum*, and all had intermediate susceptibility to vancomycin. The remaining 8 isolates (35%) were *E. faecium* and all were vancomycin resistant. Rep-PCR analysis identified 5 distinct fingerprinting patterns with >95% similarities (Figure). The genetically related clusters are grouped as C1 (isolates 1–5, vancomycin-resistant *E. faecium*), C2 (isolates 9–12, vancomycin-intermediate *E. gallinarum*), C3 (isolates 13–14, vancomycin-intermediate *E. gallinarum*), C4 (isolates 15–20, vancomycin-intermediate *E. gallinarum*), and C5 (isolates 22–23, vancomycin-intermediate *E. gallinarum*). *Enterococcus* isolates 6, 7, and 8 (vancomycin-resistant *E. faecium*) and 21 (vancomycin-intermediate *E. gallinarum*) were genetically unique and unrelated to all other isolates tested. The similarity coefficients among members of each of the dominant clusters were >95% and the band differences were minor (i.e., only 1 or 2 band differences were found when gel images were compared with those of other cluster members). In cluster C1, 2 of the 4 isolates of *E. faecium* (isolates 1 and 2) had similarity coefficients >99% and were recovered from infants transferred from the same medical center. Both infants were admitted to our NICU within 2 weeks of each other. In cluster C4, 2 of 6 isolates of *E. gallinarum* (isolates 16 and 18) had similarity coefficients >98% and were recovered from patients transferred from the same medical center.

Conclusions

Because VRE can colonize the gastrointestinal tract for a prolonged period without progressing to clinically apparent disease, early recognition of colonization is essential for preventing patient-to-patient transmission. Of the 25 infants colonized with VNSE identified by our ASC program in 2003–2004, 23 isolates were available for DNA fingerprinting and further characterization. Thirty-five percent of the patients harbored vancomycin-resistant *E. faecium*, and 65% had vancomycin-intermediate *E. gallinarum*. Although *E. gallinarum* has low-level intrinsic vancomycin resistance and, thus, provokes fewer infection control concerns than high-level vancomycin-resistant *E. faecium*, invasive infections with this pathogen have been documented (13). The initiation of our ASC program, coupled with accurate and timely identification of VNSE, was associated with a sharp decrease in transmission of these bacteria to other NICU patients.

Molecular typing of VRE isolates with PCR and contour-clamped homogeneous electric field electrophoresis to conduct restriction fragment length polymorphism

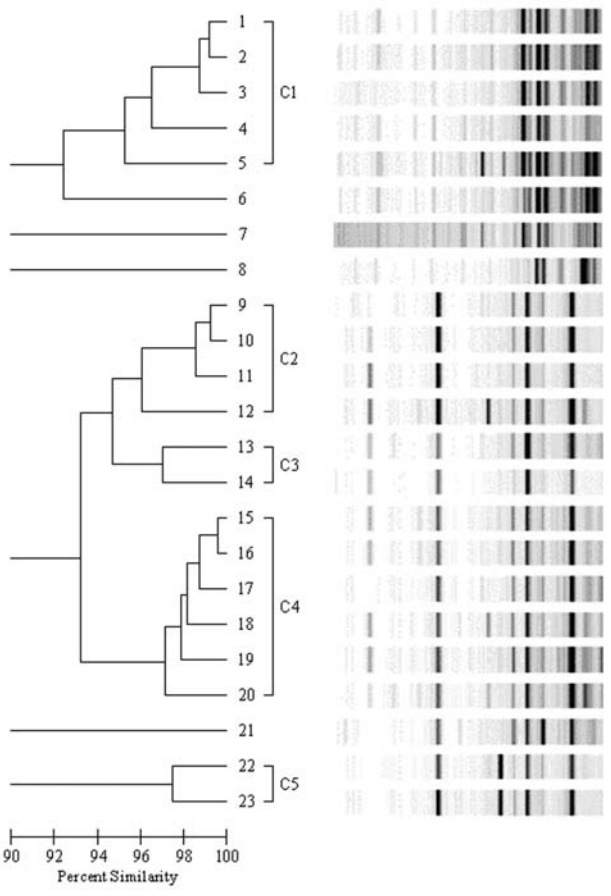

Figure. Dendrogram of 23 nonsusceptible enterococci isolates. Genetically related clusters are labeled C1–C5. Isolates represented by lanes 6, 7, 8, and 21 are genetically distinct.

analysis of specific enterococcal genes has been described (14). These techniques enable targeted analysis of specific portions of the VRE genome and strain characterization. However, the overall genetic similarity of strains is unknown because only a small portion of the genome is assessed by these methods. Rep-PCR DNA fingerprinting is faster and easier to use than the other methods and results in a high level of genetic discrimination, making it a useful molecular epidemiologic tool.

Rep-PCR DNA fingerprinting identified 5 dominant clusters of VNSE in our NICU study patient population. Two instances of strong genetic relatedness were observed in isolates from neonates who were transferred from the same referral center within a limited period of time. The close relatedness of other VNSE was independent of the patient's hospital of origin. Infants from the same referral center usually did not have strains that were genetically related. One study described 7 VRE strains from 3 different locations within the same institution (14). Another study characterized VRE isolates from 6 different hospitals and found 23 isolates of 3 related types at 1 institution, while all isolates from another hospital were genetically distinct (15).

Referring centers that had transferred patients with VNSE to our NICU were informed of our results. As a result of our investigation, which showed no patient-to-patient transmission in our NICU during the study period, we established the following procedures: 1) we obtained ASC for VRE only from infants >14 days of age on admission to the NICU and placed them on contact isolation pending results, and 2) we no longer perform weekly surveillance cultures on previously culture-negative patients. This approach is cost-effective and sustainable.

Our ASC program identified a significant number of neonates admitted to our NICU who had been previously unrecognized as colonized with VNSE. Molecular fingerprinting of their isolates identified the existence of 5 clusters and several unique strains of VNSE circulating among newborns born in Washington, DC, metropolitan area hospitals. Our data also suggested that referring centers had experienced infant-to-infant spread based on similar Rep-PCR DNA fingerprint patterns. Our ASC program in tandem with the implementation of appropriate infection control measures led to a decrease in transmission of VRE to other NICU patients.

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Human Infection with *Rickettsia honei*, Thailand

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Human spotted fever rickettsiosis was detected molecularly by 2 real-time polymerase chain reaction (PCR) assays performed on DNA extracted from a Thai patient's serum sample. Sequences of PCR amplicons from 5 rickettsial genes used for multilocus sequence typing were 100% identical with those deposited with GenBank for *Rickettsia honei* TT-118.

The original Thai tick typhus isolate, TT-118, was obtained from a mixed pool of *Ixodes* sp. and *Rhipicephalus* sp. larval ticks from *Rattus rattus* trapped in Chiangmai Province, Thailand, in 1962 (1) and has recently been determined to be a strain of *Rickettsia honei*, the etiologic agent of Flinders Island spotted fever (2). No isolate has been associated with Thai tick typhus in humans, and TT-118 was found only to be moderately pathogenic for guinea pigs and gerbils (1). However, evidence of spotted fever rickettsiosis has been seen in Thailand; this evidence comes from 2 reports of a total of 11 cases, 3 cases from Chiangmai and 8 cases from the Thailand-Burma border. All 11 patients had signs and symptoms characteristic of spotted fever rickettsiosis, and their sera were reactive to spotted fever group (SFG) rickettsial antigens, including those derived from TT-118 (3,4). Additional proof of the presence of spotted fever rickettsiae in Thailand derives from rodent (5) and human (6,7) serosurveys. In addition, spotted fever agents have been demonstrated in Thai ticks by using molecular biology techniques to detect rickettsiae (8–10). Collectively, these reports indicate that SFG rickettsiae and rickettsioses exist within Thailand. However, at the time of this writing, detection of an SFG rickettsia from a human source had not been reported in Thailand.

The Study

We describe the first detection of *R. honei* TT-118 or a very similar strain from a 36-year-old male freelance photographer living in Bangkok, Thailand, who complained of a febrile illness and was admitted to a nearby hospital on December 21, 2002 (V. Sangkasuwan et al., unpub. data). Blood was collected and submitted to the Armed Forces Research Institute of Medical Sciences, Thailand, where the serum was determined to be positive for antibodies to spotted fever rickettsiae. A portion of this serum sample, without identifiers, was subsequently sent to the Naval Medical Research Center for confirmatory serologic and molecular diagnosis. To confirm the original serologic report, the patient's serum was tested for spotted fever, typhus, and scrub typhus group-specific immunoglobulin (Ig) G by enzyme-linked immunosorbent assay using *R. rickettsii* R (ATCC VR891), *R. typhi* Wilmington whole cell antigen, and KpKtGm r56 recombinant antigen, as previously described (11,12). The patient's serum was confirmed to have IgG to SFG rickettsiae (titer >1:6,400) but not to have antibodies to *Orientia tsutsugamushi* or *R. typhi*.

To ascertain whether the patient's serum contained molecular evidence of SFG rickettsia, DNA was extracted from 150 μL of the patient's serum (DNeasy Tissue Kit, Qiagen, Valencia, CA, USA). Three micrograms Poly(dA) (Sigma Chemical Co., St. Louis, MO, USA) was added as a DNA carrier. Two real-time polymerase chain reaction (PCR) assays were performed to determine if rickettsial nucleic acid was detectable in the patient's serum sample: 1) the *Rickettsia* genus–specific real-time PCR assay amplified and detected a 115-bp segment of the 17-kDa antigen gene and 2) the rickettsial SFG-specific real-time PCR amplified and detected a 128-bp segment of the *ompB* with a SmartCycler (Cepheid, Sunnyvale, CA, USA), as previously described (13). Both assays demonstrated the presence of the target sequences in the serum sample with Ct values of 36.22 and 36.87, respectively.

To identify which SFG rickettsia was responsible for the patient's febrile illness, segments of 5 rickettsial genes were amplified by PCR, and the amplicons produced were sequenced and compared to reported sequences of other SFG rickettsiae. New oligonucleotide primers were selected from the conserved regions of *ompB*, *ompA*, and *sca4* after alignment of at least 19 rickettsial sequences: RompB11F (ACCATAGTAGCMAGTTTTGCAG), Rak1452R (SGTTAACTTKACCGYTTATAACTGT), RhoA1F (GAATAACATTACAAGCTGGAGGAA), RR657R (TATTTGCATCAATCSYATAAGWA), RhoA4336F (AGTTCAGG AACGACCGTA), RrD749F (TGGTAGCATTAAAAGCTGATGG), RrD2685R (TTCAGTAGAAGATTTAGTACCAAAT), RrD1826R (TCTAAATKCTGCTGMATCAAT), and RrD1275R (TGTTAAACCTGYATTTACATAAT). All other primers used have been previously described (2,14,15).

To produce the amplicons used for sequencing, 2 μL or 4 μL of the sample DNA preparation was added to either a

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25- or 50-μL reaction mixture, respectively, containing 0.5 μmol/L (for *ompA* and *ompB*), or 0.3 μmol/L (for 17-kDa gene, *gltA* and *sca4*) of forward and reverse primers, and PCR SuperMix High Fidelity (Invitrogen, Carlsbad, CA, USA). Two microliters of PCR products was used as template in the nested PCRs. Each PCR was performed on a TGradient Thermocycler (Whatman Biometra, Göttingen, Germany) and incubated at 94°C for 1 min followed by 40 cycles of denaturation at 94°C for 30 s; annealing at 48°C (*gltA*), 50°C (*ompA* and *ompB*), 52°C (sca4), or 58°C (17-kDa gene) for 1 min; and elongation at 70°C for 1–2 min. After the amplification steps were completed, the reaction mixtures were exposed to a final elongation step at 72°C for 7 min, and PCR products were visualized with ethidium bromide (GIBCO BRL Life Technologies, Inc., Gaithersburg, MD, USA) on 1.5% agarose gels after electrophoresis. Each mastermix for PCR was prepared in a clean room separated from where the DNA templates were added; no research with *R. honei* TT-118 had been conducted in our laboratory. A positive control DNA (*R. parkeri* genomic DNA) and a negative control (molecular biology grade water, GIBCO) were run at the same time under the same condition as the sample. The negative control consistently produced no detectable product. A 1,328-bp PCR product of *ompB* from the positive control DNA was sequenced and showed 100% identity with the published *R. parkeri ompB* sequence. The sample PCR products from the 17-kDa gene and the nested PCR products of *gltA*, *ompA*, *ompB*, and *sca4* were purified by using a QIAquick PCR purification kit (Qiagen). The BigDye Terminator v 3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) was used in subsequent sequencing reactions, according to manufacturer's instructions. Sequencing products were purified by using Performa Gel Filtration Cartridges (EdgeBioSystems, Gaithersburg, MD, USA), and sequencing was performed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The primers used for PCR amplification were the same as those used for the sequencing reactions. At least 2 sequencing reactions were performed for each strand of DNA. Sequences were assembled with Sequencher 4.0 (Gene Codes Corporation Inc., Ann Arbor MI, USA), and basic local alignment search tool (BLAST) searches were managed on the NCBI Web site (http://www.ncbi.nlm.nih.gov/blast/).

A 434-bp fragment from the 17-kD antigen gene was amplified by standard PCR, and the sequence between bases 67 and 458 of the open reading frame (ORF) was determined to be 100% identical with the published sequences of *R. honei* strain TT-118 and *R. honei* strain RB, and 99.7% identical with *R. honei* strain "south Texas *A.* [*Amblyomma*] *cajennense* SFG rickettsia." Two PCR fragments were produced for *gltA* by nested PCR that included bp 82 to 1178 of the ORF. A 1,069-bp sequence was obtained after assembling the sequences of the 2 fragments. BLAST search showed this sequence to be 100% identical with that of *R. honei* TT-118 and 99.9% with *R. honei* strain RB. A 741-bp *ompB* fragment was amplified by nested PCR. Sequence of this fragment was found to have 100% identity with *R. honei* TT-118 and 99.8% with *R. honei* RB. The 1,403-bp *ompA* amplicon sequence had 100% identity with *R. honei* TT-118 and *R. honei* RB *ompA* published sequences. The 1,090-bp sequence determined for *sca4* was 100% identical with that published for *R. honei* TT-118.

Conclusions

Molecular detection of *R. honei* TT-118 in a clinical specimen from a patient suspected of having spotted fever rickettsiosis was achieved after clinical diagnosis and serologic analysis. To determine the identity of SFG agent, standard PCR and nested PCR procedures were conducted to produce amplicons from 5 rickettsial genes for multilocus sequence typing (MLST) (10,15). The 17-kDa antigen gene, a highly conserved gene among members of the genus *Rickettsia*, was used to confirm the relationship of the unknown agent to known rickettsiae. Similarly, DNA encoding *gltA* was used to ascertain the relationship of the unknown agent to other agents within the genus *Rickettsia*. The sequences of the other 3 gene segments (*ompB*, *ompA*, and *sca4*) used in the MLST scheme are much more variable among the rickettsiae and, therefore, they provided more information regarding the identity of the unknown agent.

BLAST searches against the determined sequences of segments amplified from the 17-kDa antigen, citrate synthase, OmpB, OmpA, and Sca4 genes of the unknown agent showed 100% identity with those sequences deposited within GenBank for *R. honei* TT-118. The closest other neighbors to the unknown agent included *R. honei* RB (the type strain of *R. honei*) and *R. honei* "south Texas *A. cajennense* SFG rickettsia." Thus, the patient at the time of his illness was infected with *R. honei* TT-118 or a very similar strain of SFG rickettsiae.

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Rickettsia

[rĭ-ketʹse-ə]

Genus of gram-negative, rod-shaped or coccoid bacteria that are transmitted by lice, fleas, ticks and mites. Named after American pathologist Howard Taylor Ricketts; despite the similar name, *Rickettsia* spp. do not cause rickets (from the Greek *rhakhis*, "spine"), a disorder of bone development caused by vitamin deficiency.

Sources: Dorland's illustrated medical dictionary. 30th ed. Philadelphia: Saunders; 2003. and Merriam-Webster's collegiate dictionary. 11th ed. Springfield (MA): Merriam-Webster's, Inc; 2003.

Streptococcus pneumoniae and *Haemophilus influenzae* type b Carriage, Central Asia

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A study of children was conducted in 3 Central Asian Republics. Approximately half of the *Streptococcus pneumoniae* isolates were serotypes included in available vaccine formulations. Approximately 6% of children carried *Haemophilus influenzae* type b (Hib). Using pneumococcal and Hib conjugate vaccines may decrease illness in the Central Asian Republics.

Streptococcus pneumoniae and *Haemophilus influenzae* cause a large percentage of acute respiratory and invasive bacterial infections throughout the world (1). Acute respiratory infection is the leading cause of childhood death in the Central Asian Republics of the former Soviet Union (2,3), a region that includes Kazakhstan, Uzbekistan, Turkmenistan, Tajikistan, and the Kyrgyz Republic. These deaths occur despite the availability and use of antimicrobial drugs throughout the former Soviet Union (4,5).

To prevent illness from *S. pneumoniae* in the United States, the 7-valent pneumococcal conjugate vaccine (Prevnar, Wyeth Pharmaceuticals, Philadelphia, PA, USA) was added to the routine infant immunization schedule in 2000. Prevnar contains *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. Higher valency formulations (9-, 11-, and 13-valent) are under evaluation. The 9-valent formulation (including types 1 and 5) was successful in South Africa (6) and The Gambia (7), and an 11-valent formulation (including types 1, 3, 5, and 7F) is being studied in the Philippines. An accelerated development and introduction plan for pneumococcal conjugate vaccines for use in developing countries is supported by the Global Alliance for Vaccines and Immunization (www.preventpneumonia.com).

H. influenzae type b (Hib) conjugate vaccines have been recommended for infants in the United States since 1990. Widespread use of these vaccines has dramatically reduced Hib invasive disease in both industrialized and developing countries (8,9). The World Health Organization (WHO) has recommended use of the Hib conjugate vaccine in regions of the world where the extent of Hib disease has been established. Prevalence of Hib invasive disease must be assessed in countries in the Central Asian Republics before introducing the Hib conjugate vaccine.

Laboratory data to determine prevalence of *S. pneumoniae* and Hib are not collected in the Central Asian Republics. To determine the benefits of using the pneumococcal and Hib conjugate vaccines in these countries, we conducted a nasopharyngeal swab survey of pediatric patients to identify the most prevalent serotypes and penicillin-resistance patterns of *S. pneumoniae* and to assess the presence of Hib.

The Study

In January 1997, we obtained nasopharyngeal swabs from a convenience sample of both ill and well children, ages 2–59 months, who were visiting outpatient clinics in Taraz City (formerly Djambul), Kazakhstan; Fergana, Uzbekistan; and Osh, Kyrgyz Republic. Before swabs were obtained, written parental consent was obtained in Russian, Kazak, Kyrgyz, or Uzbek under a protocol approved by a local institutional review board and the Centers for Disease Control and Prevention (CDC).

Nasopharyngeal swab collection and pathogen isolation have been described previously (1). Briefly, a flexible calcium alginate swab was inserted through the nares to the nasopharynx, rotated ≈180°, and withdrawn. While in the field, the swabs were first streaked on chocolate agar (CA) plates containing bacitracin to isolate *H. influenzae*, and then onto Trypticase soy 5% sheep blood agar plates containing gentamicin to isolate *S. pneumoniae*. All plates were brought back to the laboratory and incubated appropriately. Pure *H. influenzae* cultures were isolated and spread onto quad plates. Those colonies that grew on only the XV and blood quadrants were considered to be *H. influenzae* and were saved on CA slants. Suspected *S. pneumoniae* colonies were streaked onto conventional 5% sheep blood agar plates with an optochin disk added. After appropriate incubation, α-hemolytic isolates with an optochin inhibition zone >14 mm were considered to be *S. pneumoniae* and saved

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on CA slants. CA slants of both *H. influenzae* and *S. pneumoniae* were transported to CDC in Atlanta. Isolates of *H. influenzae* were serotyped with Difco *H. influenzae* serotype-specific rabbit antisera (BD, Sparks, MD, USA), and *S. pneumoniae* isolates were serotyped with CDC-prepared antiserum. *S. pneumoniae* cultures were tested for antimicrobial susceptibility to penicillin with broth dilution MIC testing by using the guidelines of the Clinical and Laboratory Standards Institute (formerly NCCLS) and customized MIC panels.

Results were similar in all 3 sites, so data were combined. The method of isolate storage and transport resulted in different survival rates among isolates (Tables 1 and 2). Low rates of *S. pneumoniae* isolates among children receiving antimicrobial drugs prevent any conclusions about that group. Among *S. pneumoniae* and *H. influenzae* isolates, survival was negatively associated with duration of storage. Among *S. pneumoniae* isolates, survival was positively associated with increasing age. However, the lack of any trends in Hib colonization and *S. pneumoniae* nonsusceptibility by age and duration of storage suggests that differential survival did not produce bias.

Of 630 children swabbed, 375 (59%) were colonized with *S. pneumoniae*. Of the 375 isolates, 224 *S. pneumoniae* isolates were available for susceptibility testing and serotyping. Of the 224 isolates, 54 (24%) were nonsusceptible to penicillin. The 9 most common serotypes in decreasing order were 19F (17% of isolates), 6B (15%), 6A (9%), 14 (6%), 23B (4%), 19A (3%), 23F (3%), 18C (2%), and 4 (2%). These accounted for 61% of all isolates.

In our sample, the 7-valent pneumococcal conjugate vaccine would cover 47% of pneumococcal isolates, the 9-valent would cover 48%, and the 11-valent would cover 51%. Of all the serotypes covered in these vaccines, serotypes 6B, 14, 19F, and 23F account for all nonsusceptible strains. Because all 3 vaccines contain these 4 serotypes, each vaccine would cover 33 (61%) of 54 nonsusceptible isolates of *S. pneumoniae*. An additional 13% of nonsusceptible strains are vaccine-related (strains 6A [4 of 54, 7%] and 23B [3 of 54, 6%]).

Of the 630 children from whom nasopharyngeal swabs were obtained, 357 (57%) were carrying *H. influenzae*. Of the 300 isolates available for serotyping, 34 (11%) were Hib. When Hib carriage is determined by multiplying the percentage of children colonized with *H. influenzae* times the percentage of Hib among all *H. influenzae* isolates tested, the carriage rate is 6% (Table 2).

Conclusions

Our survey showed that most children in these Central Asian Republics were colonized with at least 1 potential respiratory pathogen. Approximately half of the *S. pneumoniae* isolates and more than half of the penicillin-nonsusceptible *S. pneumoniae* isolates are included in the available pneumococcal conjugate vaccine formulations. Approximately 6% of the children in this convenience sample were carrying Hib.

The colonization rate of Hib found in our study is similar to rates observed in industrialized populations before Hib conjugate vaccines were widely used. Carriage rates for Hib before widespread vaccination in Finland, the

Table 1. *Streptococcus pneumoniae* in convenience sample, Central Asian Republics, January 1997*

Variable	% SP colonization (n/N)	% SP isolate survival (n/N)	% SP PCN nonsusceptible isolates (n/N)	Calculated % colonization with PCN-nonsusceptible SP†
Age (mo)				
2–5	49 (47/95)	49 (23/47)	17 (4/23)	8
6–11	64 (74/115)	54 (40/74)	38 (15/40)	24
12–23	66 (94/142)	60 (56/94)	27 (15/56)	18
24–35	62 (65/105)	62 (40/65)	23 (9/40)	14
36–47	58 (61/106)	64 (39/61)	18 (7/39)	10
48–59	51 (34/67)	79 (27/34)	19 (5/27)	10
Sex				
Male	60 (197/331)	60 (119/197)	27 (32/119)	16
Female	60 (178/299)	60 (106/178)	22 (22/106)	13
Reported use of antimicrobial drugs in past 7 days				
Yes	49 (34/70)	29 (10/34)	10 (1/10)	5
No	61 (335/552)	64 (213/335)	24 (52/213)	15
Weeks storage before transport				
3	65 (72/110)	25 (18/72)	11 (2/18)	7
2	56 (175/315)	62 (108/175)	24 (27/108)	13
1	62 (128/205)	77 (99/128)	26 (26/99)	16
Total	59 (375/630)	60 (225/375)	24 (55/225)	14

*SP, *S. pneumoniae*; PCN, penicillin.
†Result obtained by multiplying the percentage of children colonized with SP times the percentage of SP isolates that are nonsusceptible to penicillin (percentages in column 1 multiplied by the percentages in column 3).

Table 2. *Haemophilus influenzae* in convenience sample, Central Asian Republics, January 1997*

Variable	% HI colonization (n/N)	% HI isolate survival (n/N)	% Hib among all HI isolates (n/N)	Calculated % colonization with Hib†
Age (mo)				
2–5	45 (43/95)	77 (33/43)	6 (2/33)	3
6–11	59 (68/115)	76 (52/68)	17 (9/52)	10
12–23	60 (85/142)	89 (76/85)	8 (6/76)	5
24–35	60 (63/105)	87 (55/63)	13 (7/55)	8
36–47	58 (62/106)	85 (53/62)	13 (7/53)	4
48–59	54 (36/67)	86 (31/36)	10 (3/31)	5
Sex				
Male	57 (187/331)	82 (154/187)	13 (20/154)	7
Female	57 (170/299)	86 (146/170)	10 (14/146)	8
Reported use of antimicrobial drugs in past 7 days				
Yes	57 (40/70)	82 (33/40)	9 (3/33)	5
No	56 (310/552)	84 (260/310)	12 (31/260)	7
Weeks storage before transport				
3	59 (65/110)	71 (46/65)	20 (9/46)	12
2	57 (178/315)	83 (147/178)	10 (14/147)	6
1	56 (114/205)	94 (107/114)	10 (11/107)	6
Total	57 (357/630)	84 (300/357)	11 (34/300)	6

*HI, *Haemophilus influenzae*; Hib, *H. influenzae* type b.
†Result obtained by multiplying percentage of children colonized with HI times the proportion of Hib isolates (percentages in column 1 multiplied by percentages in column 3).

United Kingdom, and the United States were 2%–6% (10–13). In these countries, introduction of the Hib vaccine virtually eliminated Hib invasive disease (13).

Assessing the prevalence of disease due to specific respiratory pathogens is difficult; blood cultures are insensitive, and other diagnostic tests are not specific. Nasopharyngeal colonization surveys of groups of children identify the predominant organisms circulating in the community and the presence or absence of antimicrobial-drug resistance. The presence of *S. pneumoniae* serotypes found in the pneumococcal conjugate vaccine suggests this vaccine may decrease some illness from acute respiratory infection. The experience in other countries with similar prevaccination Hib nasopharyngeal carriage rates suggests that the Hib conjugate vaccine may also decrease illness. These findings may be helpful in the decision-making process regarding the value of introducing conjugate vaccines for Hib and pneumococcal disease prevention.

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Human Herpesvirus 8 and Pulmonary Hypertension

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Human herpesvirus 8 (HHV-8) antibodies were detected in 1 of 33 patients with pulmonary hypertension (including in 1 of 16 with idiopathic pulmonary arterial hypertension), 5 of 29 with cystic fibrosis, and 3 of 13 with interstitial lung disease. No relationship between HHV-8 infection and pulmonary hypertension was found.

Human herpesvirus 8 (HHV-8) has been detected in patients with Kaposi sarcoma, primary-effusion B-cell lymphomas, and Castleman disease (1). Recently, 2 articles from 1 group suggested that HHV-8 has a role in the pathogenesis of idiopathic pulmonary arterial hypertension (IPAH) (2,3). IPAH has been reported in 2 patients with HHV-8–associated Castleman disease; lung tissue from 1 of these patients was positive for latency-associated nuclear antigen-1 (3). HHV-8 latency-associated nuclear antigen-1 and HHV-8 viral cyclin gene were identified in the lung tissue of 10 (62.5%) of 16 patients with IPAH, whereas only 1 (7.1%) of 14 patients with associated pulmonary hypertension (PH) had HHV-8 gene sequences in lung tissue (2). Conversely, Japanese researchers failed to confirm the detection of HHV-8 latency-associated nuclear antigen-1 in 10 IPAH patients (4).

Identifying HHV-8 as a cofactor in IPAH pathogenesis could raise relevant therapeutic and preventive issues. We conducted a seroprevalence study aimed at detecting antibodies to HHV-8 among lung transplantation candidates; we assessed the HHV-8 seroprevalence among PH patients with and without IPAH, and we compared results with those of non-PH patients.

The Study

We retrospectively analyzed data from 75 patients referred to the Department of Cardiovascular and Respiratory Sciences of the University of Rome La Sapienza from January 2001 to February 2004 for clinical and serologic (hepatitis C virus, hepatitis B virus, HHV-8, and cytomegalovirus) evaluation for lung transplantation. The diagnosis of PH was based on international criteria (5,6). Echocardiographic data, including the right ventricular end-diastolic diameter (RVEDD), were available for all patients. Informed consent for medical and surgical procedures was obtained for all enrolled patients.

Thirty-three of 75 patients had significant PH (mean pulmonary arterial pressure [PAP] >25 mm Hg assessed by right heart catheterization). Sixteen of them had IPAH (PAP 53.4 ± 17.1 mm Hg), whereas among the 17 patients with secondary PH, 7 patients had chronic thromboembolic PH (PAP 40.3 ± 7.8), and the remaining 10 patients (PAP 37.1 ± 12.2 mm Hg) had PH associated with connective tissue disease (4 patients), HIV infection (3 patients), and lung disease (3 patients). The 42 patients without PH included 29 patients with cystic fibrosis (PAP 21.1 ± 3.3 mm Hg) and 13 patients with interstitial lung disease (PAP 18 ± 4.6 mm Hg) (8 patients with idiopathic pulmonary fibrosis, 2 with sarcoidosis, 3 with pulmonary fibrosis secondary to bleomycin treatment).

We performed assays for antibodies directed to lytic antigens of HHV-8 in plasma samples, according to a previously well-described method (7). Briefly, we used an in-house indirect immune fluorescent assay based on BCBL-1 cell line. Samples reactive at 1:40 dilution in the antilytic test were considered positive. As assessed in a large-scale multicenter study that employed a consensus-based method for defining the "true" status of specimens, this assay had the highest sensitivity of the assays evaluated (97.1%) and a specificity of 83.2% (8).

Conclusions

The 75 patients (38 male), who were candidates for lung transplantation, were all born and living in Italy. Their mean age was 40.5 years (range 14–74). Antibodies against lytic antigens of HHV-8 were detected in 9 (12.0%) patients (median HHV-8 antibody titer 1:160, range 1:80–1:320).

No significant differences in age, sex, current residency, and cardiopulmonary symptoms (e.g., dyspnea, orthopnea, peripheral edema) were found between patients with or without HHV-8 antibodies. Nevertheless, patients with HHV-8 antibodies were generally younger (36.1 years ± 13.0 vs. 41.0 ± 14.3 years, $p = 0.3$) and more likely to be male (6 [66.7%] of 9 vs. 35 [50.7%] of 69, $p = 0.4$) than patients with no HHV-8 infection. All 3 patients with HIV infection were HHV-8 negative.

A higher heart rate and lower RVEDD, evaluated by echocardiography, were found in HHV-8–seropositive patients compared to HIV–seronegative patients (112 ± 20 vs. 89 ± 15 beats/min, $p<0.001$ and 22.8 ± 4.5 mm vs. 30.9 ± 6.9 mm, $p = 0.02$, by analysis of variance). No further

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difference in echocardiographic parameters was reported between patients with or without HHV-8 antibodies.

A difference in the HHV-8 seroprevalence was found between the PH patients (3.0%) and the patients without PH (19.0%). Patients with PH were older (47.3 years ± 12.3 vs. 33.9 ± 12.7 years, $p<0.001$) and more like to be male (9 [27.3%] of 33 and 30 [71.4%] of 42, $p<0.001$). Among the 33 patients with PH, 1 (6.3%) of 16 with IPAH had serologic HHV-8 antibodies, whereas no patient with secondary PH had HHV-8 antibodies (Table).

Among the 42 patients with no clinical or diagnostic evidence of PH, 5 (17.2%) of the 29 with cystic fibrosis and 3 (23.1%) of the 13 with interstitial lung disease had HHV-8 antibodies, all of them affected by idiopathic pulmonary fibrosis. No difference in HHV-8 seroprevalence rate was found in patients with cystic fibrosis and in patients with interstitial lung disease (Table).

We found an 11.5% prevalence of HHV-8 antibodies and a 1:160 median HHV-8 antibody titer among a population of Italian patients who were candidates for lung transplantation. The seroprevalence and the range of the median end point dilution are similar to those found in the Italian general population of blood donors, <1:40–1:160. (8,9).

Our findings are different from those found by 1 group of researchers (2,3) among IPAH patients and consistent with other results (4,10). HHV-8 antibodies were detected more frequently among young boys and patients without PH. IPAH patients had a low HHV-8 seroprevalence rate (6.3%) with a female prevalence (87.5%). The HHV-8 prevalence rate in Europe and the United States is higher in men who have sex with men than in the general population (11), whereas consistent with our results, the specific literature on PH shows a female predominance in IPAH patients (1.7 female/male ratio) (6).

The higher heart rate observed in patients with HHV-8 antibodies is likely due to the higher prevalence of patients with cystic fibrosis in this subgroup (69%). Conversely, the higher RVEDD observed in patients without HHV-8 antibodies is likely related to the higher prevalence of diseases, such as IPAH and chronic thromboembolic PH, that cause severe right ventricular dysfunction in these patients. The limited sample size of the population does not allow an appropriate relevant analysis, but similar findings were found in a previous study (12) and could be related to an hyperdynamic circulation due to chronic infection.

Several limitations of our study should be mentioned. First, the high prevalence of HHV-8 antibodies among non-PH lung transplantation candidates could be the result of these patients' pulmonary disease and of their previous exposures to medical and surgical procedures not investigated in this study. Indeed, as previously reported (13), HHV-8 DNA was detected with significantly higher frequency in lung tissue samples of patients affected by idiopathic pulmonary fibrosis. Second, we separated IPAH from PH in the setting of autoimmune disease. We recognize that distinguishing IPAH from secondary PH is not always possible because underlying autoimmune disease can go undiagnosed. Additionally, the pulmonary and systemic pathologic features of the lung diseases being compared differ, even if the result is IPAH. This observation may affect some of the clinical physiologic parameters reported. In conclusion, demographic and virologic issues did not provide evidence of a direct relationship between HHV-8 infection and PH, either idiopathic or secondary.

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Table. Human herpesvirus 8 (HHV-8) seroprevalence among candidate patients for lung transplantation

Diagnosis	Patients	Female sex (%)	Median age (range)	HHV-8 seroprevalence (%)
Patients with pulmonary hypertension				
Idiopathic pulmonary arterial hypertension	16	14 (87.5)	46 (28–74)	1 (6.3)
Secondary pulmonary hypertension	17	11 (64.7)	44 (22–65)	0 (0)
Patients without pulmonary hypertension				
Cystic fibrosis	29	9 (31.0)	23 (14–28)	5 (17.2)
Interstitial lung disease	13	3 (23.1)	47 (43–74)	3 (23.1)

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Gram-positive Rod Surveillance for Early Anthrax Detection

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Connecticut established telephone-based gram-positive rod (GPR) reporting primarily to detect inhalational anthrax cases more quickly. From March to December 2003, annualized incidence of blood isolates was 21.3/100,000 persons; reports included 293 *Corynebacterium* spp., 193 *Bacillus* spp., 73 *Clostridium* spp., 26 *Lactobacillus* spp., and 49 other genera. Aound-the-clock GPR reporting has described GPR epidemiology and enhanced rapid communication with clinical laboratories.

Identifying intentional *Bacillus anthracis* exposures quickly is essential for limiting human illness and death (1). During the 2001 anthrax attack, inhalational anthrax developed in 11 persons, and 5 died (2). Initial laboratory evidence of anthrax infection came from routine diagnostic blood cultures, which yielded *B. anthracis* in all 8 patients, who had not received antimicrobial drug therapy before blood cultures were obtained (3,4). Less than 24 hours elapsed from the time each patient's blood was drawn and the culture inoculated, until their culture was initially noted to have bacterial growth and preliminarily identified as gram-positive rods by immediate microscopic examination of a Gram stain. However, species-specific identification generally took several more days since additional laboratory testing of the bacterial isolate was required.

The Connecticut inhalational anthrax patient was intubated for mechanical ventilation during the 2-day delay between preliminary identification of gram-positive rods in blood culture and laboratory results specifically suggesting *B. anthracis*. According to then-existing requirements, the Connecticut Department of Public Health (CDPH) was not notified until *B. anthracis* was suspected. Public health officials were unable to interview the patient, who never recovered.

Since January 1, 2003, Connecticut laboratories and physicians have been required to report any gram-positive rod (GPR) identified from blood or cerebrospinal fluid (CSF) to CDPH. CDPH requested that laboratories call immediately if the isolate was identified within 32 hours of inoculation. This was the first time CDPH required laboratories to report a finding immediately by telephone. Surveillance objectives were to detect anthrax septicemia or meningitis more quickly, ensure around-the-clock laboratory reporting of potential bioterrorism events, and describe the epidemiology of GPR septicemia and meningitis in the absence of an intentional *B. anthracis* release.

Across the nation, local, state, and federal agencies have been pilot testing a variety of surveillance approaches to detect intentional disease outbreaks more quickly (5–10). Approaches have included syndromic surveillance (6–8) and environmental air monitoring for potential bioterrorism agents (9,10). We describe results from the inaugural year of CDPH's unique laboratory-based surveillance system.

The Study

At the end of January 2003, Connecticut clinical laboratories were notified by mail that GPR isolates identified from CSF or blood within 72 hours of culture inoculation must be reported to CDPH Epidemiology Program. CDPH asked laboratories to call the department immediately if the isolate was identified within 32 hours of inoculation and collected either from an outpatient or an inpatient within 3 days of admission. Other GPR reports were to be mailed to CDPH. Although CDPH was most interested in timely telephone reporting of isolates identified within 24 hours of inoculation, we chose 32 hours to identify isolates missed in laboratories lacking sufficient staff to continuously examine blood cultures during night shifts (generally 8-hour periods). Blood cultures were processed according to each clinical laboratory's usual culture practices since reported culture isolates were obtained from routine diagnostic testing. In clinical settings, blood cultures are generally performed by filling commercially manufactured bottles, primed to promote either anaerobic or aerobic bacterial growth, with the patient's blood at the time of phlebotomy. Culture bottles are then brought to the clinical laboratory for incubation.

Immediate clinical follow-up was conducted whenever >1 of the patient's blood culture bottles yielded the isolate within 32 hours of inoculation and for all CSF isolates. This follow-up involved clinically characterizing the patient's illness through telephone discussion with the patient's physician or inpatient nurse to determine whether the illness was suspicious for anthrax (e.g., respiratory

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symptoms or widened mediastinum seen on chest radiograph). Laboratory follow-up was conducted for all isolates, with daily laboratory contact until genus identification. For *Bacillus* spp., laboratories were asked to report isolates' hemolysis and motility characteristics, and, if necessary, isolates were forwarded to Connecticut's state laboratory to rule out *B. anthracis* by γ-phage lysis.

Laboratory audits were conducted to ensure complete reporting of qualifying isolates; 33 of Connecticut's 34 clinical laboratories participated. We provided laboratories a list of GPR genera, and they provided a list of blood and CSF cultures that had yielded these genera within 72 hours of inoculation during 2003. We compared patient names, culture dates, and results with the 2003 GPR reports to identify unreported isolates.

Chart reviews were performed for *Clostridium* isolates to obtain etiology and underlying medical conditions. Health department labor resources were estimated by staff questionnaire administered October 2003. Because laboratories required several weeks to implement the reporting requirement after notification, the analysis period was limited to March–December 2003. In addition, only the first isolate from a given patient's illness was counted in this analysis.

From March to December 2003, a total of 623 GPR isolates were identified. CSF isolates were few (5 total: 2 *Listeria* spp., 2 *Bacillus* spp., and 1 *Corynebacterium* sp.). By genus, blood isolates included 293 *Corynebacterium* spp., 193 *Bacillus* spp. (none *B. anthracis*), 73 *Clostridium* spp., 26 *Lactobacillus* spp., 14 *Listeria* spp., 10 *Propionibacterium* spp., and 9 other genera (Table 1). Annualized incidence of GPR blood isolates was 21.3/100,000 persons. Twenty-three of the 195 *Bacillus* isolates were forwarded to Connecticut's state laboratory to rule out *B. anthracis* by γ-phage lysis (all were negative).

Among the 498 blood isolates with available incubation period, 171 (34%) isolates grew in ≤24 hours. Of these, 131 (76%) were reported to CDPH: 97 by telephone (61% reported on date detected and 42% reported outside office hours), 31 by mail, and 2 by unknown reporting method. Overall, 82% of these rapid-growing isolates were either *Bacillus* (52%) or *Clostridium* spp. (30%).

Unreported isolates (n = 304) identified by laboratory audit only grew more slowly (80% incubation period >24 hours versus 54% of reported isolates, p<0.001) and/or presumed contaminants (65% *Corynebacterium* spp.). Nearly all (98%) unreported isolates were from clinical laboratories that had reported other isolates but failed to report all isolates. *Corynebacterium* isolates (all nondiphtheria species, i.e., "diphtheroids") were less likely to be reported than other genera (30% vs. 70%; p<0.001).

Clostridium isolates grew significantly more quickly in blood culture than other genera (median incubation 15.3 hours; Table 2) and more frequently in inoculated anaerobic culture bottles (68%) than in aerobic culture bottles (13%). Annualized incidence of clostridial bacteremia was 2.3/100,000 persons, excluding 6 postmortem cultures likely due to agonal bacteremia. The 67 patients were elderly (median age 76 years) and frequently critically ill (22 deaths). Many (56%) had an intraabdominal source identified. Underlying immune-compromise (49%) and malignancy (60%) were common; 24% had neither condition.

From March to September 2003, an average of 56 staff hours was required per month to receive, respond to, and process reports. For September 2003 specifically, the most recent month assessed, aggregate personnel time was 45 hours (20% outside office hours).

Table 1. Characteristics of gram-positive rod bacterial isolates from blood culture, Connecticut, March–December 2003

			Time from inoculation to growth				No. inoculated bottles with growth by bottle type		
Genus	Total	Reported n* (%)	No. isolates†	Median (h)	Range (h)	% positive ≤24 h	No. isolates†	No. aerobic inoculated (% aerobic positive)	No. anaerobic inoculated (% anaerobic positive)
Bacillus‡	193	134 (69)	161	23.5	2.7–70.3	56	134	242 (43)	218 (21)
Clostridium	73	47 (64)	69	15.3	1.4–71.9	75	70	134 (13)	134 (68)
Corynebacterium‡	293	94 (32)	220	42.9	2.8–71.9	8	94	178 (49)	174 (15)
Lactobacillus	26	14 (54)	20	31.7	9.0–70.0	35	14	25 (52)	25 (56)
Listeria	14	14 (100)	13	26.1	9.3–65.0	38	13	26 (58)	24 (71)
Propionibacterium	10	7 (70)	7	49.2	18.0–68.1	13	4	9 (100)	9 (33)
Other§	9	4 (44)	8	41.3	14.8–70.3	14	7	13 (85)	11 (45)
All	618	314 (51)	498	33.6	1.4–71.9	34	336	627 (41)	595 (34)

*n = number identified by mandated reporting. The remainder of isolates were identified by laboratory audit.
†No. of isolates for which information on time from inoculation to growth and number of bottles to which samples had been added and number of bottles yielding isolate were available, respectively. Not all laboratories were able to retrieve these data retrospectively for laboratory audits.
‡No *Corynebacterium diphtheriae* or *Bacillus anthracis* organisms were reported.
§Other category includes *Bifidobacterium* (2), *Brevibacterium* (2), *Actinomyces* (1), *Aureobacterium* (1), *Erysipelothrix* (1), *Eubacterium* (1), and *Oerskovia* spp. (1).

Table 2. Genus as predictor of incubation time, Connecticut gram-positive rod surveillance, March–December 2003

Genus	No. isolates	Mean incubation (h)	Mean incubation difference* (h)	Standard error	p value*
Clostridium	69	21.1	Ref	Ref	Ref
Bacillus	161	28.1	6.99	2.20	0.002
Listeria	13	30.7	9.60	4.63	0.038
Lactobacillus	20	33.3	12.19	3.89	0.002
Corynebacterium	220	43.8	22.62	2.11	<0.001
Other†	15	43.1	21.94	4.36	<0.001

*Mean difference is β-coefficient of univariate linear regression comparing each genus to *Clostridium* spp., the reference group; p value is the p value associated with that β-coefficient. Ref, reference.
†Other category includes *Propionibacterium* (7), *Bifidobacterium* (2), *Brevibacterium* (2), *Actinomyces* (1), *Aureobacterium* (1), *Erysipelothrix* (1), and *Oerskovia* spp. (1).

Conclusions

A major public health preparedness challenge is increasing the sensitivity and timeliness of recognition of individual, potentially sentinel cases of category A bioterrorism agent disease. Each category A agent has unique clinical and diagnostic features: no one system can meet the challenge for all agents. For anthrax, we attempted to shorten the time from occurrence of the earliest specific diagnostic finding, GPR identified by Gram stain of blood or CSF culture, to notification of the public health system. In doing so, we established an around-the-clock GPR laboratory reporting system with <1 full-time staff position. The system has enhanced rapid communication between CDPH and laboratories and provided baseline information on GPR sepsis epidemiology.

The first system objective was earlier detection of anthrax septicemia and meningitis. Additional anthrax cases have not occurred to test this system, and most *Bacillus* isolates are attributable to culture contamination. However, through auditing, we determined that 62% of *Bacillus* isolates identified within 24 hours of inoculation were reported by telephone. Improvement is needed, but, through auditing, the system tracks the timeliness and completeness of reporting and speciation of all *Bacillus* organisms, including, potentially, the next *B. anthracis* isolate.

Overcoming laboratory personnel's reticence to report results that are likely spurious culture contaminants has been a challenge of implementing the system. This reticence is reflected by the low reporting rate for *Corynebacterium* spp. (i.e., "diphtheroids") with their unique Gram stain appearance and rare association with pathology. Despite this, our analysis indicates that the system has met its second objective of ensuring around-the-clock laboratory reporting of potential bioterrorism events, given that many GPR reports were made by telephone outside office hours.

The third system objective was to describe baseline GPR septicemia and meningitis epidemiology. Most clinically important isolates were *Clostridium* spp. Like *B. anthracis*, *Clostridium* spp. grow rapidly in blood culture and can produce a life-threatening sepsis syndrome. However, during a repeat anthrax attack, the distinct epidemiology of clostridial sepsis could help differentiate clostridial sepsis from inhalational anthrax among persons who are critically ill with a GPR sepsis. *Clostridium* spp. predominately grow in anaerobic culture bottles, and clostridial sepsis usually affects elderly persons with abdominal conditions, malignancy, or immune suppression (11,12). Notably, recent clostridial sepsis outbreaks involving contaminated tissue transplants and illicit drugs have an epidemiology different from this baseline, in which illness predominately affects persons <50 years of age (13–15).

An ongoing challenge to this surveillance approach is that no precise clinical algorithm exists for how to readily identify whether a bacterium isolated from blood culture is from culture contamination. This uncertainty complicates the triage of isolates' clinical importance even with physician consultation.

The GPR surveillance system continues with modification. Beginning January 2004, Connecticut laboratories are now required to report by telephone any blood or CSF specimen with growth of GPRs within 32 hours of inoculation. Growth after 32 hours is no longer reportable, to reduce reporting of culture contaminants without significantly sacrificing sensitivity to detect anthrax or clostridial infections. Immediate clinical follow-up is conducted on isolates most likely to be sentinel events: aerobic bottle isolates (possible anthrax event) and anaerobic isolates in patients < 50 years of age (unusual *Clostridium* event).

The earliest possible knowledge of an anthrax attack could minimize illness and death by allowing more lead time for intervention. Connecticut has successfully implemented a laboratory-based system that allows for early detection of even a single case of inhalational anthrax.

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Dr Begier was an Epidemic Intelligence Service Officer assigned to the Connecticut Department of Public Health when this work was conducted. Her research interests include vaccine-preventable diseases and traditional and alternative approaches to disease surveillance.

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Syndromic Surveillance in Bioterrorist Attacks

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The article by Nordin et al. (1) in this issue of Emerging Infectious Diseases describes the use of syndromic surveillance to detect inhalational anthrax resulting from a hypothetical covert release of *Bacillus anthracis* spores at a major shopping mall. This study is an important evaluation of syndromic surveillance's utility in detecting an inhalational anthrax epidemic against a background of real patient presentations. Based on historical clinical data from a large health maintenance organization (HMO), the authors evaluated the sensitivity of a syndromic surveillance system to detect an incident by season of the year, day of the week when the release occurred, and attack rate in mall patrons.

Although numbers of persons exposed and becoming ill, as modeled in the study, are not specified, the effect can be inferred from the specified methods. On the basis of information from the mall's Web site (2) and the methods stated in the article, the number of cases associated with a 15% attack rate in mall visitors (115,000 daily average) and workers (12,000) would be ≈19,000 (if no additional exposures occurred after day of release). Of these patients, 59% would be from the metropolitan area in which the mall was located, an additional 6% would reside within a 150-mile radius of the metropolitan area, and the remainder would be from more distant points, including international visitors. Syndromic surveillance, with the HMO patient database, would detect 50% of such incidents by day 5, with only 20% detected by day 4. Lesser attack rates would notably lower the probability of detection. Even more problematic, the syndromic surveillance systems, as modeled, would fail to detect the outbreak in 13% of releases in summer and 47% of releases in winter. Performance would improve markedly with higher attack rates. After detection of an aberrant signal, the occurrence of a syndrome must be investigated to determine the cause, and exposure history of patients must be determined to discover the source. These investigations could result in additional delay before a targeted response could be mounted to prevent more illnesses. Such delays are problematic because the effectiveness of postexposure prophylaxis for inhalational anthrax is related to speed of implementation (3).

The authors point out that an astute clinician might diagnose inhalational anthrax in a patient before syndromic surveillance detected that an outbreak of some type was occurring. If, as the 15% attack rate scenario suggests, ≥100 patients had onset of illness on day 2 after exposure, a correct diagnosis could be established for at least 1 patient by day 4. By this time, hundreds of inhalational anthrax patients would be seen at hospitals, at least 1 day before the syndromic surveillance system, as modeled, would have a 50% probability of signaling the outbreak.

The issue now becomes whether or not syndromic surveillance can augment the public health response to an outbreak. For example, if a syndromic surveillance system allowed follow-up of individual cases, it might accelerate case finding and investigation into the source of infection. This potential role of syndromic surveillance was not included in the modeled scenario.

Syndromic surveillance systems, of the type modeled by Nordin et al., may be too slow to allow public health officials and policy makers to mount a sufficiently rapid postexposure prophylaxis campaign. Therefore, the ability of many current syndromic surveillance systems to rapidly detect bioterrorist attacks needs to be improved. Another reason to improve syndromic surveillance systems is that the systems may have public health value other than detecting bioterrorist attacks, such as tracking the course of seasonal diseases. We should not forget, however, that clinical care providers will continue to have a critical role in detecting bioterrorist attacks, and communications must be maintained with these first-line sentinels.

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Telithromycin-resistant *Streptococcus pneumoniae*

To the Editor: In recent years, antimicrobial drug resistance in *Streptococcus pneumoniae* has increased worldwide and is a major health concern**.** Resistance to β-lactams and macrolides, considered to be first-line therapeutic agents, is particularly high in France and many Asian countries (1–3). Resistance to new fluoroquinolones is reported with increasing frequency, which emphasizes the need for new effective drugs.

Telithromycin, the first member of a new macrolide family, the ketolides, has been developed to overcome macrolide resistance. In vitro data have shown that telithromycin remains active against 98% to 100% of erythromycin-resistant strains (2,3). However, resistant mutants have been isolated in vitro, and a few poorly documented clinical failures have been reported in the treatment of pneumococcal infections. We report the first isolation of telithromycin-resistant *S. pneumoniae* from a blood culture after therapy.

An 87-year-old woman was admitted on March 28, 2004, to St Joseph Hospital in Paris with typical upper left lobar pneumonia, as inferred from auscultatory results, radiologic findings, and laboratory data: leukocytes 37,300 cells/μL, C-reactive protein 455 mg/L, and positive urinary pneumococcal antigen (BinaxNOW, Binax, Inc., Portland, ME, USA). She was not febrile. She had been followed for many years for chronic obstructive pulmonary disease (COPD), with acute exacerbation only in 2001. At that time, she was treated with the macrolide roxithromycin, without bacteriologic documentation, in addition to acetylcysteine (3 × 200 mg/d) and aerosolized terbutaline. On March 13, her COPD was exacerbated. On March 20, she visited her general practitioner and received 800 mg/day telithromycin for 5 days without improvement. Because of a cutaneous rash attributed to telithromycin, she received 20 mg prednisolone. After 48 hours, she was admitted to St Joseph Hospital because her respiratory syndrome was aggravated. A blood culture drawn on admission yielded a *S. pneumoniae* serotype 14 with decreased β-lactam susceptibility (MICs: penicillin G: 1 μg/mL; amoxicillin: 0.75 μg/mL; cefotaxime: 0.5 μg/mL, as determined by Etest). The strain was resistant to tetracyclines, cotrimoxazole, macrolides, and lincosamides (erythromycin and clindamycin MIC >32 μg/mL). The MIC of telithromycin, performed on Mueller-Hinton agar + 5% horse blood by serial 2-fold dilution, was equal to 2 μg/mL in air and 8 μg/mL under CO^2 (0.01–0.03 μg/mL for control strains ATCC 49619 and 10 clinical isolates, including 5 that were MLSB [macrolide-lincosamide-streptogramin B]–resistant). The patient was treated with 100 mg/kg/day intravenous amoxicillin and improved within 48 hours. She was discharged from the hospital 1 week later in good condition but remained a healthy carrier of resistant *S. pneumoniae*.

Resistance to macrolides has been documented in France since our first report in 1978 (4). In the last 10 years, resistance has increased to ≈50% of the strains in adults and ≥70% in children, the highest in the Western world. More than 98% of the strains are of the MLSB phenotype, conferring high-level resistance to macrolides, lincosamides, and streptogramin B, in contrast to the situation in the United States, where most strains are of the *mefE* type (efflux), which confers low-level resistance to 14- and 15-membered macrolides only**.** However, <2% of the macrolide-resistant strains have a decreased susceptibility to telithromycin (2,3). Resistance to β-lactams is also very frequent (≈50%), particularly in erythromycin-resistant strains (<90%); these figures explain why macrolides may more likely select a penicillin-resistant strain than most β-lactams (5).

Since resistance to telithromycin was documented before ketolides were introduced in clinical practice, we cannot exclude the possibility that the telithromycin- resistant strain was selected in 2001, while our patient was treated with roxithromycin.

The clinical impact of macrolide resistance has been occasionally questioned since these antimicrobial agents achieve high tissue and intracellular levels. However, *S. pneumoniae* is an extracellular bacterial pathogen; well-designed clinical studies have documented the failure of macrolides in treating high-level resistant strains with an MLSB phenotype (6). After an 800-mg oral dose, telithromycin achieves serum and epithelial lining fluid concentrations of 2.2 and 15 μg/mL, respectively, yielding a free drug concentration of 0.7 μg/mL in the serum and 15 μg/mL in the epithelial lining fluid. In an excellent in vitro model, telithromycin eradicated *S. pneumoniae* of the *mefE* phenotype with MICs ≥0.25 and 1 μg/mL (7). The drug was not effective against strains with MICs 2–8 μg/mL, as was seen in our patient. When incubated under CO^2, MICs of macrolides increase by 1 dilution compared to the MIC in air, against both susceptible and resistant strains. With telithromycin, the MIC increase is 2–6 dilutions but only for macrolide-resistant strains (8). The clinical impact of this finding is still to be determined. This report emphasizes the need for routine testing of *S. pneumoniae* isolates for resistance to telithromycin.

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Integrated Human-Animal Disease Surveillance

To the Editor: Early identification of zoonotic disease occurrence through simultaneous monitoring of human and animal disease surveillance systems is critical to protect health in both populations. We assessed the surveillance and reporting needs of a small but diverse group of Michigan veterinarians by examining their perspective of the current animal disease reporting system, the system enhancements desired, and their computer and Internet accessibility.

Developing systems that link human and animal disease reporting systems can help identify and facilitate a response to known and emerging zoonotic diseases. A system that allows simultaneous electronic capture and assessment of human and animal disease reports is being implemented in Michigan. The system will be based on the Michigan Disease Surveillance System platform, a Web-based human disease reporting system implemented by the Michigan Department of Community Health (1,2).

To ensure an integrated system that meets both human and veterinary public health needs, we developed a questionnaire for veterinarians to collect information on the current animal disease reporting system, system enhancements that are desired, and computer and Internet access capabilities (University Human Research Committee approved). In July 2003, a total of 112 questionnaires was sent to a convenience sample of Michigan veterinarians who represent a variety of practice types and sizes, participate in organized veterinary medicine and academia, and would be motivated to participate in system development. Of the 112 questionnaires, 84 (75%) were completed. Of 79 practices represented, 19 (24%) treated companion animals, 15 (19%) treated equids, 4 (5%) treated food animals (dairy, beef, small ruminant, poultry, or swine), 32 (41%) treated a variety of animals (no patient type >75%), 4 (5%) treated zoo animals, and 5 (6%) veterinarians were not in clinical practice.

Of 81 respondents, 75 (93%) indicated that they were aware of the Michigan reportable animal disease list. Yet, 37 (47%) of 79 respondents have reported no cases and 32 (41%) of 79 have reported 1 to 5 cases annually. Furthermore, only 19 (25%) of 76 respondents reported that their clients might have confidentiality issues that could impede disease reporting. Therefore, it will be important to continue educating veterinarians to ensure that suspected disease cases are reported as well as confirmed cases, which may have been reported by the laboratory also.

Positive feedback was received regarding the current animal disease reporting system. Respondents evaluated the availability of a published list of reportable diseases (68 [89%] of 76) and the availability and quality of laboratory testing and disease confirmation (57 [75%] of 76) as Strongly Like/Like (Scale = Strongly Dislike, Dislike, No Opinion, Like, Strongly Like). However, case submission feedback may need to be assessed as only 34 (45%) of 76 respondents evaluated this service as Strongly Like/Like and 13 (17%) of 76 evaluated it as Strongly Dislike/Dislike.

Of 73 respondents, 68 (93%) reported that routinely published animal disease surveillance data would be beneficial for themselves or their clients. Of 55 respondents, 24 (44%) indicated that these data would be used to increase disease awareness and 23 (42%) indicated the data would be used for risk assessment and prevention planning. Outbreak alert information, easy access to regulatory requirements, and animal disease information were also desired.

Virtually all practices had a computer (79 [99%] of 80), Internet access (70 [88%] of 80), and email (53 [75%] of 71). Most (68 [91%] of 75) were comfortable using a Web-based application to submit case reports. However, because Internet access and email may not be universal, multiple modes of communication must be utilized. Furthermore, of 81 respondents, 29 (36%) indicated lack of time, and 24 (30%) indicated lack of staff as a barrier to online reporting. Therefore, the reporting system should be efficient.

Rabies, bovine tuberculosis, and West Nile virus, all zoonotic diseases, were listed by >50% of the 79 respondents as 1 of the 6 diseases they felt were most important in Michigan. Simultaneous tracking of human and animal diseases was considered useful by 32 (84%) of 38 respondents because animals are sentinels of human (zoonotic) disease and by 7 (18%) respondents because of the threat of agroterrorism. In general, respondents are aware of the importance of animal disease reporting to public health.

In Michigan, the human and animal disease reporting systems are similarly structured, although there is no local level animal health agency (Figure). These similarities can provide the basis for a system that is functionally appropriate to track diseases in humans and multiple animal species and meet multiple agency surveillance objectives.

Overall, this group of Michigan veterinarians considers developing a Web-based disease reporting system as useful as long as the following issues are addressed: 1) quality case report feedback; 2) access to correct and coordinated human and animal disease information; and 3) computer system reliability and efficiency. Based on the results of this study, the second phase of this project, construction of the animal disease surveillance portion of Michigan's reporting system, will be implemented with continued input from local, state, and federal stakeholders.

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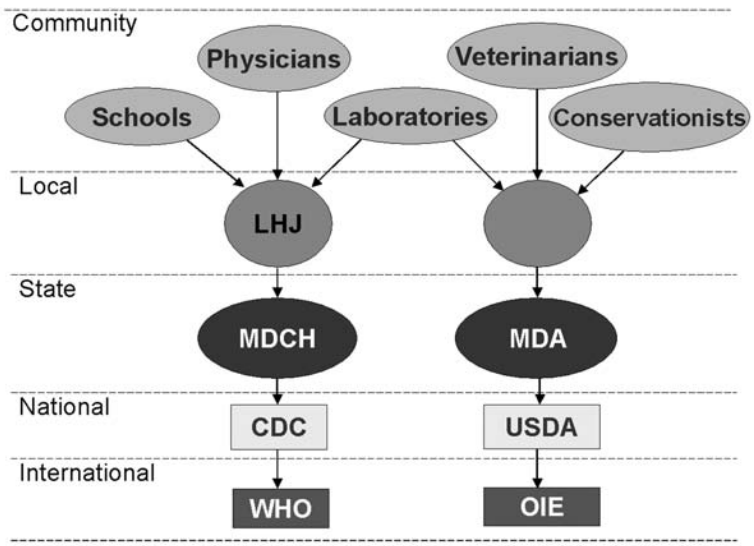

Figure. Comparison of Michigan human and animal disease reporting system structures. LHJ, Local Health Jurisdiction; MDCH, Michigan Department of Community Health; CDC, Centers for Disease Control and Prevention; WHO, World Health Organization; MDA, Michigan Department of Agriculture; USDA, US Department of Agriculture; Animal Plant Health Inspection Service, Veterinary Services; OIE, Office International Epizooties.

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*Van*B-*Van*C1 *Enterococcus gallinarum*, Italy

To the Editor: We report detecting a *van*B determinant in *Enterococcus gallinarum* in poultry in Italy. High-level v*an*A-mediated glycopeptide resistance has been described for *E. gallinarum* and *E. casseliflavus* (1–4), and v*an*B-mediated vancomycin resistance has been frequently described for *E. faecalis* and *E. faecium*. However, v*an*B-mediated resistance in isolates of *E. gallinarum* has been described only in sporadic nosocomial cases of infection or colonization (5,6).

In January 2005, a study of contamination by foodborne organisms in

slaughtered broiler carcasses was conducted in Sicily. To detect glycopeptide-resistant enterococci (GRE), each carcass was placed in a bag with 100 mL sterile buffered peptone water and shaken vigorously for 60 sec. After overnight incubation at 37°C, 0.5 mL rinsate was added in duplicate to 5 mL ethyl violet azide broth (Oxoid, Basingstoke, United Kingdom) with 4 mg/L vancomycin. Broth cultures were further incubated at 37°C for 48 h, and 0.1 mL aliquots were spread onto duplicate plates of VRE (commercial denomination product, Oxoid) agar.

A vancomycin-resistant isolate of *E. gallinarum* was identified in a carcass from a broiler farm in eastern Sicily. The biochemical tests of API 20 Strep (bioMérieux, Marcy l'Etoile, France) and motility test at 30°C were used to characterize the isolate at the species level. The MICs of vancomycin and teicoplanin were 64 μg/mL and 1 μg/mL, respectively. The isolate was subjected to a multiplex polymerase chain reaction followed by an endonuclease cleavage of amplicons by *Msp*I (Invitrogen, Carlsbad, CA, USA) as previously described (7) to detect *van* gene determinants; this process demonstrated a simultaneous presence of *van*C1 and *van*B determinants.

E. gallinarum and the other motile enterococci are thought to infrequently cause infection. However, the recent involvement of *van*C1-*van*A *E. gallinarum* in person-to-person spread in a long-term-care facility (8) and in an intensive care unit (2), along with identification of *van*C1-*van*B isolates in some patients treated with prolonged courses of glycopeptides (5,6), suggests reassessment of their possible pathogenic role.

For the first time, 1 isolate of *E. gallinarum* has been found harboring the *van*B gene in poultry. Our findings confirm that *E. gallinarum* can capture the genetic determinants of high-level glycopeptide resistance, probably under selective pressure conditions that do not permit survival of a host organism with constitutive low-level resistance (3). Previous studies have demonstrated that *E. gallinarum* can transfer these determinants to *E. faecium* by conjugation (2).

The role of food animals as reservoirs of GRE and the causes of their persistently high prevalence in poultry carcasses in some European countries are being investigated (9). Moreover, the public health risk associated with consumer exposure to GRE when handling raw animal foods is poorly understood. In Europe, the food chain is thought to be the major source of GRE since avoparcin was used as a food additive for animals until the European Union ban in 1997. Previous studies in Italy showed that avoparcin withdrawal successfully reduced GRE contamination of poultry meat products (10). However, our finding, 7 years after the European Union ban, highlights that resistance genotypes in motile enterococci should be closely monitored (11).

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Empyema Thoracis from *Salmonella* Choleraesuis

To the Editor: The clinical presentations of nontyphoidal *Salmonella* infection are protean, including gastroenteritis (most common), bacteremia, septic arthritis, osteomyelitis, and endovascular infection. (1). Despite the growing number of patients with invasive infection due to nontyphoid *Salmonella*, reports of thoracic empyema due to these organisms remain rare (2–6).

We searched the computer database of our microbiology laboratory for patients with positive pleural effusion culture from June 1997 to February 2004. Patients were included if they met the following criteria: 1) thoracentesis recovered purulent pleural fluid; 2) microorganisms identified by microscopic examination; and 3) a *Salmonella* species isolated from a pleural effusion specimen.

Isolates of *Salmonella* were identified to the serotype level, according to the Kauffman and White scheme, using somatic and flagellar antigens (Denka Seiken Co., Ltd., Tokyo, Japan) and also by conventional methods and the Phoenix System (panel type, NMIC/ID4) (Becton Dickson, Sparks, MD, USA) (7). Susceptibilities of *Salmonella* isolates to ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole were determined by the disk diffusion method. Organisms were categorized as susceptible or resistant to the antimicrobial agents tested on the basis of National Committee for Clinical Laboratory Standards (NCCLS) guidelines (8). Antimicrobial therapy was considered to be appropriate when the antimicrobial agent was active in vitro by the disk diffusion susceptibility method against a *Salmonella* isolate.

During the study, 973 patients with a diagnosis of empyema thoracis were identified; 12 (1.23%) of these patients, including 9 men and 3 women, were infected with *Salmonella* species. The clinical characteristics of the 12 patients are summarized in the online Appendix Table (available from http://www.cdc.gov/ncidod/eid/vol11no09/05-0030_app.htm). The median age was 49 years; 1 patient was >65 years of age. Underlying diseases were present in all patients, including 7 with malignancy, 5 with gallstones, and 3 each with diabetes mellitus and chronic renal failure. Five patients had used antacids and 3 patients had received chemotherapy or steroids. Ten patients (83.3%) were immunocompromised and had a variety of illnesses, including malignancy, liver cirrhosis, and diabetes mellitus. Common symptoms were dyspnea (83.3%), fever (75%), and cough (50%). Analysis of pleural effusion showed a median leukocyte count of 25,600/μL, a lactate dehydrogenase level of 513 U/L, and a glucose level of 88 mg/dL. Gram staining was conducted on 3 patients' pleural effusion but none of them showed positive results.

Twenty-three *Salmonella* isolates were recovered as the sole pathogen from various clinical specimens, including pleural effusion (15 isolates), blood (6 isolates), ascites (1 isolate), and aortic wall (1 isolate). Among the 12 patients with empyema thoracis, 4 had *Salmonella enterica* serotype Typhimurium (*S.* Typhimurium) and 1 had group C2 *Salmonella* during 1997–1999; 7 patients had *Salmonella enterica* serotype Choleraesuis (*S.* Choleraesuis) after 1998. All *S.* Typhimurium and group C2 *Salmonella* were isolated from pleural effusion specimens, but *S.* Choleraesuis was isolated from multiple extrapulmonary sites including blood, ascites, and aortic wall (Online Table). Although the number of study cases is limited, it may suggest that *S.* Choleraesuis is more invasive than 2 other *Salmonella* species.

Among the *S.* Choleraesuis isolates recovered from 7 patients, 2 were resistant to ampicillin and sulfamethoxazole-trimethoprim, 3 were resistant to chloramphenicol, 1 was resistant to ciprofloxacin, and all were susceptible to cefotaxime. All *S.* Typhimurium isolates were susceptible to sulfamethoxazole-trimethoprim, ciprofloxacin, and cefotaxime. Two of the 4 patients had isolates that were resistant to chloramphenicol, and 2 other patients had isolates that were resistant to ampicillin. The group C2 *salmonella* isolate was resistant to chloramphenicol only.

Among the 12 *Salmonella* isolates from patients with empyema thoracis, 9 were resistant to >1 commonly used antimicrobial. Treatment and outcome information was available for 11 of the 12 patients. All 11 patients received antimicrobials drugs (median duration 35 days); this therapy was appropriate in 9 of 11 patients. Six patients had thoracentesis, 2 had close tube thoracostomy, and 1 had open drainage. One of the 4 patients with *S.* Typhimurium empyema who did not receive appropriate antimicrobial drugs died. In contrast, 4 (57%) of the 7 patients with *S.* Choleraesuis infection, including 1 who did not receive appropriate antimicrobial therapy, died. Another factor related to outcome was drainage. One (20%) of the 5 patients who underwent tube thoracostomy or thoracoscopy died, while 3 (50%) of the 6 patients who underwent thoracentesis died. All 3 of these patients had *S.* Choleraesuis.

Most (92%) of our patients were <65 years of age. These data indicate that *Salmonella* should be considered as a potential cause of thoracic empyema, even in younger patients, especially in the presence of malignancy or hepatobiliary disease. More than half of our patients had used antacids or had suffered from gallstones. This finding suggests that susceptibility to

Salmonella infection may be increased by alterations in the gastrointestinal tract, including decreased gastric acidity and chronic gastrointestinal disease. Leukocytosis was noted in 25% of patients. In fact, two thirds of the patients had a normal leukocyte count with immature leukocytes, which may be attributable to their relatively impaired cell-mediated immunity.

The predominant organism in this series was *S.* Choleraesuis, followed by *S.* Typhimurium. In Taiwan, the rate of resistance of *S.* Choleraesuis to ampicillin, chloramphenicol, or sulfamethoxazole-trimethoprim increased to approximately 90% for all 3 drugs and the rate of resistance to ciprofloxacin was from 7.7% to 59% (5–7). The resistance rate of *S.* Choleraesuis to ciprofloxacin in this study was similar to our previous report (7).

Nine of the 11 patients who completed follow-up information received appropriate antimicrobial drugs with drainage; however, 4 died. These 4 deaths (57%) were due to *S.* Choleraesuis-related empyema; 3 patients had underlying malignancy. Although appropriate antimicrobial drugs were used, our data suggest that more aggressive treatment with open drainage of the pleural effusion might have contributed to a better outcome than closed tube thoracostomy or simple thoracocentesis alone. In contrast to *S.* Choleraesuis-related infection, all 4 patients with non–*S.* Choleraesuis-related thoracic empyema survived. One of these patients did not receive appropriate antimicrobial drug treatment, but did have adequate drainage with simple thoracocentesis. This suggests adequate and aggressive drainage of pleural effusion may be as important as appropriate antimicrobial drugs. However, the overall death rate (36%) in this study was still higher than that of other reports (9). This might have been due to the high number of immunocompromised patients in this study.

In conclusion, thoracic empyema is a rare complication of nontyphoid *Salmonella* infection and is closely associated with an immunocompromised condition, even in patients <65 years of age. Higher rates of resistance and death were noted in patients with empyema thoracic caused by *S.* Choleraesuis. Early diagnosis, appropriate antimicrobial drug therapy, and aggressive drainage are necessary to improve the outcome of patients with thoracic empyema due to *S.* Choleraesuis.

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Asymptomatic *Yersinia pestis* Infection, China

To the Editor: Plague is one of the oldest identifiable diseases. Modern public health measures and effective antimicrobial treatments have led to a decrease in plague cases worldwide. However, plague remains endemic in many natural foci. Since the early 1990s, the World Health Organization (WHO) has reported a steadily increasing trend in human plague cases, which has resulted in the recognition of plague as a reemerging disease (1). The emergence of antimicrobial drug–resistant strains of *Yersinia pestis*, along with an increasing number of plague cases, remind us that plague still poses a serious public health threat (2,3). In China, human cases of plague have been reported to WHO nearly every year from 1989 to 2003; these account for 9.5% of cases and 15.5% of deaths from this disease in Asia (1). Human cases of plague in China are usually caused by contact with plague-infected rodents. Here, we report the results of a serologic survey by using 3 methods (passive hemagglutination assay, Western blot, and protein microarray analysis) in marmot hunters in Qinghai Province, China.

One hundred twenty serum samples were collected in 2 villages in

Huangyuan County, Qinghai Province, from marmot hunters (63 samples) and their family members (57 samples); none had a history of fever in the past 2 years. One hundred nineteen serum samples were collected from persons with no history of marmot hunting in 2 nearby counties in Qinghai Province in which plague was not endemic. Thirty serum samples were collected from persons in Beijing and used as negative controls.

All serum samples were initially screened with a passive hemagglutination assay to detect immunoglobulin (Ig) G antibody against F1 antigen of *Y. pestis*, by using a standard protocol (4). We then used an F1 antigen–based Western blot to analyze all serum samples. The protein microarray analysis was performed with 149 purified recombinant proteins of *Y. pestis* (5).

The results of the serologic survey are summarized in the Table. The passive hemagglutination assay showed 17 positive samples in the marmot hunter population. None of the control serum samples were positive for F1 antigen in this assay. Western blot identified 9 additional positive samples in the marmot hunter population, resulting in a seropositivity rate of 21.7% (26/120). We also found positive samples in 4 (3.4%) of 119 serum samples by using Western blot in persons from areas in which plague was not endemic. Identical results were also obtained by using protein microarray analysis, which validated the results of Western blot.

Previous studies have shown that plague antibodies were more prevalent in males in the exposed population, and differences in the age, sex, or ethnic group of plague patients are the result of variations in exposure to the pathogen, not intrinsic factors (6,7). Our study showed that in the marmot hunter population, the plague seropositivity rate was significantly higher in males (36.8%, 25/68) than in females (2.0%, 1/52, $p<0.01$). Among the marmot hunter population, 63 (92.6%) of 68 males were hunters. Plague antibodies were also more prevalent in marmot hunters (39.7%, 25/63) than in their family members (1.8%, 1/57, $p<0.01$).

This is the first serologic survey of plague in the marmot hunter population. The plague seropositivity rate of 21.7% (26/120) in hunters and their families is much higher than the 3.4% (4/119) in the population from regions in which plague was not endemic ($p<0.01$). Seroprevalence in marmot hunters was even higher (39.7%), which suggests that marmot hunting is a risk factor for plague infection.

The marmot (*Marmota himalayana*) is the main host of *Y. pestis* in Qinghai Province. Plague-infected marmots are more easily captured by hunters. When persons hunt and butcher marmots without any effective protection, *Y. pestis* can be transmitted through tiny wounds in the skin, by bites of infected fleas, or by the respiratory route. Asymptomatic plague infection in marmot hunters might be explained by prophylactic use of antimicrobial drugs. Most hunters usually take sulfamethoxazole or tetracycline as a prophylactic measure. Even if the hunters were infected with *Y. pestis*, they would likely not develop symptomatic plague. However, if the antimicrobial drugs are not effective or hunters do not use prophylaxis, symptomatic infections will occur. Most reported human cases of plague in Qinghai Province were caused by hunting or butchering marmots, as shown by a recent outbreak of plague in October 2004 in Qinghai, in which 19 cases were reported and 8 persons died (M. Li et al., unpub. data).

Table. Analysis of sera for plague antibody by 3 methods

	Marmot hunter population, no. positive/no. tested (%)			Population from nonendemic areas, no. positive/no. tested (%)		
Method*	Male	Female	Total	Male	Female	Total
PHA	16/68 (23.5)	1/52 (1.9)	17/120 (14.2)	0/60 (0)	0/59 (0)	0/119 (0)
WB	25/68 (36.8)	1/52 (1.9)	26/120 (21.7)	3/60 (5.0)	1/59 (1.7)	4/119 (3.4)
PMA	25/68 (36.8)	1/52 (1.9)	26/120 (21.7)	3/60 (5.0)	1/59 (1.7)	4/119 (3.4)

*PHA, passive hemagglutination assay; WB, Western blot; PMA, protein microarray analysis.

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Sporotrichosis, Plain of Jars, Lao People's Democratic Republic

To the Editor: In May 2003, a previously healthy, 42-year-old rice farmer and miller, living on the Plain of Jars (Xieng Khuang Province) in northeast Lao People's Democratic Republic (PDR) (Laos), dehusked and polished glutinous rice in her hand-operated rice mill. While milling, her hand slipped, removing the skin covering the interpharyngeal joint of her right index finger, on a dusty, wooden part of the machine. She did not recall the implantation of a wood splinter. During the following 4 weeks, multiple firm, erythematous lesions developed, which were not tender, fluctuant, or itchy, at the site of the injury and on the medial and anterior aspects of the lower and upper arm (Figure). The lesions spread proximally from the site of injury, but they remained confined to her right arm. She had no fever, and no lymphadenopathy developed. Her household had no domestic animals, including cats. No systemic disease developed, and she showed no evidence of immunosuppression, diabetes, or alcoholism. While waiting for a diagnosis, she persuaded a surgeon to excise all the lesions, but they soon recurred. She believed that the only solution would be to have her arm amputated. Initial biopsy specimens demonstrated no organisms and showed no growth on Sabouraud dextrose agar. Without facilities for further fungal diagnostic work in Lao PDR, but with a probable clinical diagnosis of sporotrichosis, we sent one of the excised lesions to Taiwan for molecular analysis by previously described methods (1,2). Polymerase chain reaction (PCR) was negative for mycobacteria but positive for *Sporothrix schenckii*, the cause of sporotrichosis, and the diagnosis was confirmed by sequencing the 18S rRNA gene, which showed 100% identity to that of *S. schenckii* (1,2). The lesions resolved with 6 months of oral itraconazole therapy (100 mg every 12 h).

S. schenckii is a dimorphic fungus found in soil, hay, decaying vegetation, and moss. Persons exposed to these environmental foci, such as farmers and gardeners, are especially at risk. Percutaneous inoculation is presumably the main method of infection, although inhalation and insect and mammal bites and scratches, especially from armadillos and cats, have been implicated (3,4). Our patient presumably contracted the fungus from the wood frame of the milling machine. In the 1940s, contamination from untreated wood was responsible for an epidemic that affected ≈3,000 gold miners in South Africa (from timbers in the mine). Lymphocutaneous sporotrichosis is the most frequent presentation, and the traditional treatments are oral saturated potassium iodide solution and local hyperthermia, but oral itraconazole for 3 to 6 months is now recommended (3,4).

Sporotrichosis has been described from North and South America, Europe, and Japan. In Asia and Australasia, it has been described from India (5), Taiwan (1), Australia (6), and Thailand (7), but apparently not from Laos, Cambodia, and Burma (Myanmar). Serologic evidence for human sporotrichosis infection is found in highland areas of southwest Vietnam (8). At least in part, the relative paucity of reports probably reflects the lack of sophisticated fungal diagnostic techniques in much of Southeast Asia. Some evidence

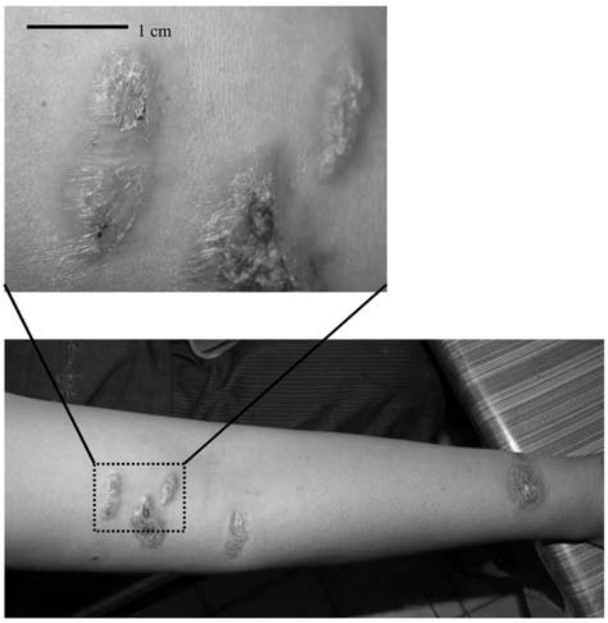

Figure. Lesions on the right arm of the patient.

suggests that sporotrichosis is more prevalent in tropical environments with relatively cool temperatures and high humidity such the Peruvian Andes (9), northwest India (5), southwestern Vietnam (8), and in Laos in the Plain of Jars. If this environmental association is correct, sporotrichosis may occur more extensively in the cooler humid areas of Asia, such as the highlands of China, Laos, Vietnam, and Burma. Sporotrichosis can disseminate in HIV-infected patients, and this syndrome may increase as the prevalence of HIV infection rises in these areas.

With 73% of the Lao population living on <US$2/day (10) and one accessible microbiologic culture laboratory in Laos, PCR is not an available local routine diagnostic technique. We were fortunate to have access to an overseas diagnostic facility, which allowed confirmation of the clinical diagnosis before the patient received a prolonged course of a drug with adverse effects and drug interactions.

Diagnosis by histopathologic examination and culture may be difficult, and identifying laboratories in different regions of the subtropics and tropics with an interest in diagnosis of sporotrichoid lesions and the capability to perform culture and PCR would facilitate the diagnosis and awareness of this disease. Itraconazole, which has become the drug of choice for lymphocutaneous sporotrichosis, is expensive. Saturated solution of potassium iodide is an inexpensive alternative and appears to be effective, although adverse effects occur frequently (3,4).

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West Nile Virus Antibodies in Colombian Horses

To the Editor: West Nile Virus (WNV) is rapidly spreading in the Western Hemisphere (1). We report the first evidence for WNV transmission in South America.

WNV is serologically related to the Japanese encephalitis complex of flaviviruses (Flaviviridae), which includes Saint Louis encephalitis virus (SLEV) (in North and South America), Japanese encephalitis virus (Asia), and Murray Valley encephalitis virus (Australia) (2). Because of antigenic cross-reactivity within this complex, WNV serologic diagnosis requires highly specific assays, such as the plaque-reduction neutralization test (PRNT) (3). We used PRNT to evaluate serum collected from 130 healthy equines (horses and donkeys) in Colombia, where WNV had not been previously reported. These equines were sampled between September 15 and October 29, 2004, in the northern departments of Córdoba and Sucre in the Caribbean region of Colombia. Samples were heat-inactivated and titrated by PRNT for antibodies to WNV, SLEV, and 3 other South American flaviviruses: Rocio, Ilhéus, and Bussuquara. Twelve specimens (9%) from 10 different premises tested positive for WNV (Table). None of these animals had been vaccinated against WNV or had traveled outside of the region. An equine immunoglobulin (Ig) M-capture enzyme-linked immunosorbent assay (ELISA) that used WNV antigen detected anti-WNV IgM in 2 of the 12 specimens, which indicated that some of these infections were relatively recent (probably within 3 months of sampling). The positive findings in both Córdoba and Sucre were corroborated by a WNV-specific blocking ELISA (4). Numerous other samples exhibited flavivirus reactivity

Table. $PRNT_{90}$ antibody titers to WNV and other South American flaviviruses for Colombian equine sera*†

Equine ID‡	Department	Age (y)	WNV	SLEV	ILHV	ROCV	BSQV
3	Córdoba	4	1:40	<1:10	<1:10	<1:10	<1:10
35	Córdoba	5	1:160	<1:10	<1:10	<1:10	<1:10
39§	Córdoba	4	1:40	<1:10	<1:10	<1:10	<1:10
41	Córdoba	6	1:40	<1:10	<1:10	<1:10	<1:10
48	Córdoba	4	1:640	<1:10	<1:10	<1:10	<1:10
76	Sucre	5	1:80	<1:10	<1:10	<1:10	<1:10
85	Sucre	9	1:80	<1:10	<1:10	<1:10	<1:10
94	Sucre	3	1:40	<1:10	<1:10	<1:10	<1:10
101	Sucre	4	1:40	<1:10	<1:10	<1:10	<1:10
109§	Sucre	7	1:160	1:40	1:40	1:10	<1:10
123	Córdoba	6	1:40	<1:10	<1:10	<1:10	<1:10
125	Córdoba	4	1:160	<1:10	<1:10	<1:10	<1:10

*These 12 specimens were considered positive for WNV infection; a 4-fold WNV $PRNT_{90}$ titer compared to that of other flaviviruses was required for a positive determination of previous WNV infection.
†$PRNT_{90}$, 90% plaque reduction neutralization test; WNV, West Nile virus; SLEV, Saint Louis encephalitis virus (South American strain); ILHV, Ilhéus virus; ROCV, Rocio virus; BSQV, Bussuquara virus.
‡All equines were horses except for 76 and 85, which were donkeys.
§Also positive for anti-WNV immunoglobulin M by antibody-capture enzyme-linked immunosorbent assay.

in the neutralization and blocking ELISA assays, mostly because of SLEV. Complete test results from these horses, as well as from Colombian cattle and chickens, will be presented elsewhere.

These serologic data should be considered indirect evidence of WNV activity in Colombia. We encourage Colombian human and animal health authorities to enhance surveillance for human, equine, and avian disease attributable to WNV. Efforts are needed to isolate the virus or detect specific viral RNA to confirm this finding and to identify vectors and vertebrate hosts involved in WNV transmission in Colombia.

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Wild Poliovirus Type 1, Central African Republic

To the Editor: In this article we summarize the investigation and response to the reemergence of wild poliovirus (WPV) type 1 in the Central African Republic (CAR) in 2003. Since 2000, reported annual routine vaccination coverage with >3 doses of oral polio vaccine (OPV) has been very low in CAR (<50%); National Immunization Days have been conducted every year since 1996, except in 2002 (1).

From December 2003 to November 2004, the active acute flaccid paralysis surveillance system reported 112 cases of acute flaccid paralysis suspected to be polio-myelitis and 4 deaths (case-fatality ratio 4%). Fecal samples were collected and sent to the Institut Pasteur de Bangui. WPV type 1 (WPV1) was isolated in 30 cases (27%), vaccine polioviruses in 15 cases (5 type 1, 5 type 2, and 6 type 3) (13%), and nonpolio enteroviruses in 18 cases (16%). Epidemiologic investigations showed that 97% of patients with poliomyelitis received <3 doses of OPV and 93% of patients were <5 years of age. Isolates were sent to the National Institute for Virology in Johannesburg, South Africa, for sequencing. All viruses were type 1 and could be traced to common ancestral strains that circulate in disease-endemic reservoirs shared by northern Nigeria and southern Niger (WEAF-B genotype). The first importation occurred in Chad in August 2003 from northeastern Nigeria, and the outbreak spread to the adjacent countries of Cameroon in October 2003 and the CAR in December 2003.

In CAR, the first case occurred in a 19-month-old child living in Ndjoh village north of Bossembélé in Ombela M'Poko. A special mission by the World Health Organization/ CAR officer determined that the child had not

received OPV and had traveled 200 km into a northern region a few weeks before the onset date of December 16, 2003. The last case of acute flaccid paralysis in this region was noted on November 23, 1999. Supplemental immunization activities were conducted from March to April 2004. However, collected funds were not enough to cover the entire country, and only sanitary regions 1, 3, 4, 5, and 7 were included. The OPV coverage rate was estimated at 104% for the first round and 141% for the second round (CAR Ministry of Health, unpub. data). The second case occurred in April 2004 in Gadzi in sanitary region 2 in a 6-year-old nomad child who had not received OPV. The third case occurred in May 2004, in a village near where the second case was diagnosed, in a 23-month-old child who had not received OPV. Twenty-five other cases occurred between July and November in sanitary region 2 (Figure).

This outbreak is the largest epidemic of WPV1 in CAR since July 2000, when the last case of WPV1 was isolated (2). Probable reestablishment of endemic poliovirus and possible diffusion of WPV1 to countries further south, such as the Democratic Republic of Congo, is a concern. Four main reasons may explain this outbreak: 1) the close links with countries, such as Chad and Cameroon, where WPV1 recently reemerged; 2) declining rates of routine vaccination and low population immunity after disruption of health service infrastructures and road networks; 3) displaced persons' living in crowded areas with little sanitation and poor water supply; and 4) lack of response preparedness to WPV importation. Epidemiologic investigation of the first case was not conducted until >1 month after onset and implementation of the polio immunization initiative in a limited area.

In May 2004, a decision was made by African Union health ministers to conduct a series of synchronized poliovirus campaigns across the African continent. Four rounds of National Immunization Days were conducted in CAR from August to December 2004. The OPV coverage rate in 600,000 children <5 years of age was estimated to be 89% in August, 98% in September, 102% in November, and 100% in December. Since November 2004, only 1 WPV1 case has been virologically confirmed in sanitary region 2. WPV1 has not been isolated in 2005.

To restore the gains made in polio eradication in Central Africa, WPV transmission must be interrupted in Nigeria and Niger (3). Until then, neighboring countries must implement high routine vaccination coverage and high-quality, supplemental immunization activities. In 2002, these steps successfully prevented importation of WPV into Bangladesh and Nepal during a resurgence of polio in India. Surveillance standards must also be maintained to ensure the rapid detection of any WPV importation, thus allowing timely response and containment.

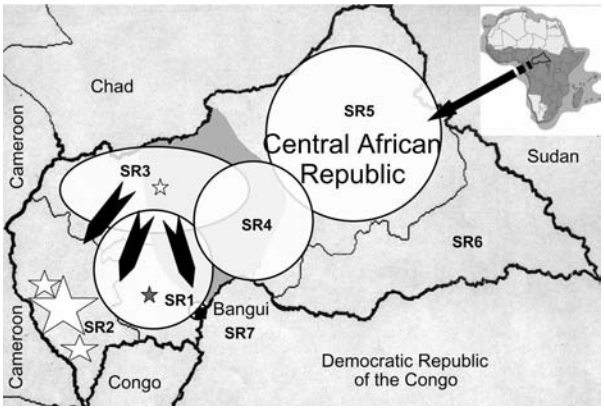

Figure. Supplementary immunization activities (SIA) areas, military conflicts, and migration movements, Central African Republic, 2001–2004. Gray star indicates first case of wild poliovirus type 1, Ombella-M'Poko (SR1), 2003; white stars indicate 2004 cases (1 in Ouham [SR3], 3 in Nana-Mambere [SR2], 3 in Sangha-Mbaere [SR2], and 23 in Mambere-Kadei [SR2]); circles indicate SIA areas, March–April 2004; arrows indicate 2001–2003 migration; dark gray shading indicates military conflict areas, 2001–2003.

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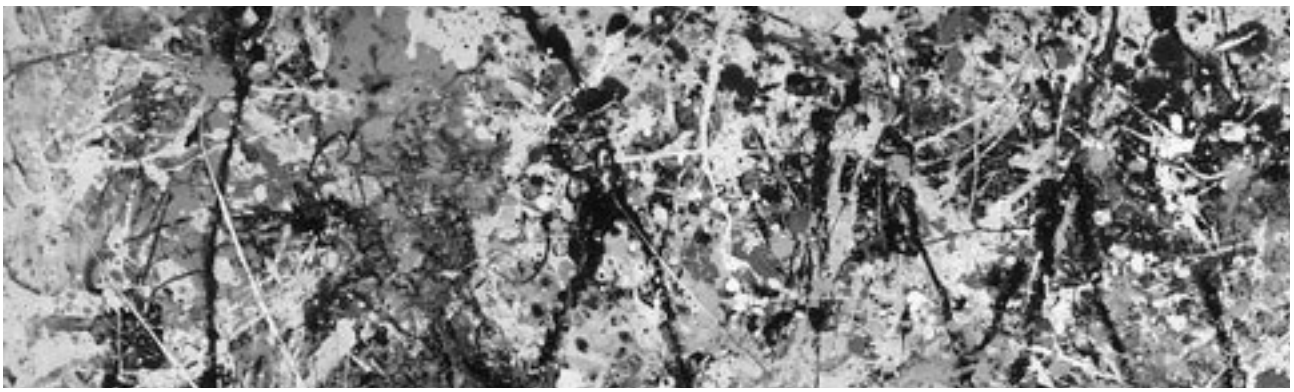

Jackson Pollock (1912–1956). Autumn Rhythm (Number 30) (detail) (1950)
Enamel on canvas (266.7 cm × 525.8 cm). The Metropolitan Museum of Art, George A. Hearn Fund, 1957 (57.92)

Oneness, Complexity, and the Distribution of Disease

Polyxeni Potter*

"On the floor," said Jackson Pollock, "I feel more at ease. I feel nearer, more a part of the painting… I can walk around it, work from the four sides and literally be in the painting…. akin to the… Indian sand painters of the West" (1). Immediacy to the art work is hallmark of abstract expressionism or the New York School, a revolutionary art movement that shifted the center of artistic avant-garde from Europe to the United States. Born in the aftermath of World War II, this movement valued the inner world over external objects and articulated an emotional landscape fraught with uncertainty and despair.

"Modern art to me is nothing more than the expression of contemporary aims of the age that we're living in," Pollock wrote (1). The age was the postwar period, filled with anxiety, shocked by the atom bomb, changed by machines, immersed in Freudian theory and psychoanalysis, swinging with improvisational jazz. To this age, Pollock brought abstraction suited to his radical prototype, volatile personality, roughness, and impatience. Along with Willem De Kooning, Barnett Newman, Mark Rothko, and others, he elevated the act of painting, advocating that it should be as direct and fundamental as what it was trying to express and that it could, itself, promote emotional expression.

"I continue to get further away from the usual painter's tools such as easel, palette, brushes….I prefer sticks, trowels, knives and dripping fluid paint or a heavy impasto with sand, broken glass…" (1), said Pollock about his expressive technique, which came to be known as action painting. The need to make an original statement, always at the heart of artistic and other human endeavor, permeated not only the subject matter of abstract expressionism but also its technical execution. Well-versed in the language of art, he knew and admired the work of Picasso, the surrealists, and Mexican muralists and experimented with several styles, seeking a new idiom.

"I shall be an Artist of some kind. If nothing else I shall always study the Arts…," wrote Pollock as a young man (2). He was born in Cody, Wyoming, the youngest of 5 children and migrated with his family to Arizona and California, where he studied art at Manual Arts High School in Los Angeles. At 18, he moved to New York City, settled in Greenwich Village, and enrolled in the Art Students League, where he studied with American Regionalist painter Thomas Hart Benton, later his mentor and friend.

"Thank you, but not so hard, not so hard," he admonished a colleague who hit him in frustration (2). Pollock was known to use his artist's hands in anger. He was excitable, contentious, and mistrustful of authority. He got in fights and more than once slugged his instructors in school. Those who knew him in his early years thought he could not draw. As an adult, he struggled with creative blocks, depression, and alcoholism and was torn with self-doubt. Two of his brothers, who lived with him in Manhattan, encouraged him to seek psychoanalysis, thinking that if he could "hold himself together," his work

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would become "of real significance," because his painting was "abstract, intense, evocative…" (3). Jungian psychoanalysis, particularly the conflict between reason and the unconscious, made Pollock aware of how central his emotions had become in his life and work (4).

In 1943, Pollock's work attracted the attention of Peggy Guggenheim, influential art dealer and patron, who commissioned a large mural. He tore down the walls of his Greenwich apartment to accommodate the huge canvas and completed the painting in a marathon 15 hours. In 1945, he left the city for East Hampton, Long Island. There in the countryside and away from distractions, he did his most innovative work. He died at age 44 of injuries in a car wreck, not knowing what he had accomplished or could have accomplished in a full life span.

"The modern artist is working with space and time and expressing his feelings rather than illustrating," Pollock believed (1). For hours he sat on the back porch of his farm house, taking in the natural environment, absorbing its shapes and complexity, which would later find their way into his thick interwoven designs. His studio, a converted barn, allowed space for large yachting canvases he bought at a nearby hardware store (5). He invented a new way to apply paint, one that combined maximum spontaneity with rigid control and produced images unprecedented in the history of art. "Dancing" on the spread canvas, Pollock created on its surface spontaneous images shaped by the trajectory of his motions. He controlled, adjusted, and modified color and shapes in paintings that were continuous, complex, and provocative and contained none of the traditional elements of composition—perspective, balance, borders, beginning, end.

"Is this a painting? Is this a painting?" he agonized (6). Not alone in his bewilderment, the artist was torn between marveling at and doubting his creation. "Jack the dripper," he was dubbed by the critics, who knew not what to make of these awesome tangles of paint. Was he making a profound statement or "flinging paint in the public's face?" (7).

Autumn Rhythm, on this month's cover, is characteristic of Pollock's most mature work. The painting contains no illusion of physical space or shapes, only textured color and seemingly unrelated lines. Reaction can only come from what the viewer feels or sees in the painting, from the striations of color and texture, which come to life as the eye moves from web cluster to color mass, discovering depth in flat surfaces, relationships in unconnected lines, muted softness, deep darkness, a delicate weave of lines, dots, swirls, and curves, overlapping into a labyrinth of infinite possibilities.

"I am nature," Pollock asserted (6). Through his creative "dance" he saw himself as one, not only with the painting but with its subject as well. By pouring a constant stream of paint directly out of a can, he produced a continuous trajectory, a web of crisscrossing trajectories, filled with energy and motion and reminiscent of organic shapes, trees, woods, designs of increasing complexity—nature's fingerprint as seen from his back porch in East Hampton.

Viewing Autumn Rhythm is a personal experience, one shared with Pollock in his moment of inspiration. Caught in the colorful web, we follow the infinite enamel skeins until the complex interconnections levitate off the canvas and we become part of the web.

The oneness and complexity of Pollock's paintings, his spontaneous dance to the rhythm of nature and the riddles of the inner world, speak to the biomedical scientist in a direct and fundamental way the artist would have applauded. For, in science as in art, entangled trails lead to unanticipated discoveries.

Disease distribution follows the complex, repetitive, and cumulative patterns of nature. Like Pollock's creations, it traverses spontaneous routes and arrives at unpredictable destinations. In some infectious diseases, malaria for one, incidence and spread have been defined by environmental factors: rainfall, temperature, elevation, and distribution of vector mosquitoes. Emergence of HIV unveiled additional interconnections as it inflated immunocompromised populations. No longer defined by vector distribution alone but now linked to distribution of HIV, malaria cases and deaths have increased (8), turning an old scourge and a new one into unlikely partners.

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EMERGING INFECTIOUS DISEASES

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The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles see below and visit http://www.cdc.gov/eid/ncidod/ EID/instruct.htm.

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Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. Begin each of the following sections on a new page and in this order: title page, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

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). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

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Types of Articles

Perspectives. Articles should be under 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), a one-sentence summary of the conclusions, and a brief biographical sketch of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and 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. Articles should be under 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), a one-sentence summary of the conclusions, and a brief biographical sketch of first author—both authors if only 2. 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 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), a one-sentence summary, and a brief biographical sketch of first author—both authors if only 2. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 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), a one-sentence summary of the conclusions, and brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., “The Study” and “Conclusions.” Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and a brief biographical sketch of first author—both authors if only 2. Dispatches 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.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. All letters should contain material not previously published and include a word count.

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