EMERGING INFECTIOUS DISEASES

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

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Global Warming

Dengue in Florida

Morbillivirus

DEPARTMENT OF HEALTH AND HUMAN SERVICES

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

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Cover: Pieter Bruegel the Elder. Jäeger im Schnee (Hunters in the Snow) (1563). Reprinted with permission of the Kunsthistorisches Museum, Vienna, Austria.

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From Shakespeare to Defoe: Malaria in England in the Little Ice Age

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Present global temperatures are in a warming phase that began 200 to 300 years ago. Some climate models suggest that human activities may have exacerbated this phase by raising the atmospheric concentration of carbon dioxide and other greenhouse gases. Discussions of the potential effects of the weather include predictions that malaria will emerge from the tropics and become established in Europe and North America. The complex ecology and transmission dynamics of the disease, as well as accounts of its early history, refute such predictions. Until the second half of the 20th century, malaria was endemic and widespread in many temperate regions, with major epidemics as far north as the Arctic Circle. From 1564 to the 1730s—the coldest period of the Little Ice Age—malaria was an important cause of illness and death in several parts of England. Transmission began to decline only in the 19th century, when the present warming trend was well under way. The history of the disease in England underscores the role of factors other than temperature in malaria transmission.

The earth's climate has always been in a state of change. The past 250 to 300 years have seen a fairly steady warming trend. Average temperatures are now approaching those at the height of the Medieval Warm Period, near the end of the 12th century. The intervening centuries included a much colder period, the Little Ice Age, by far the most important climatic fluctuation in recent history (1). Such fluctuations, spanning several generations, are natural phenomena that have recurred several times in the past 10,000 years. They take place against a backdrop of episodes of longer duration and greater impact, such as the last Ice Age (1,600,000 to 10,000 years ago). In recent years, there has been growing concern that human activities may be modifying the natural climate. A decline in temperatures from the 1940s to the late 1970s gave rise to warnings that industrial pollutants were causing global cooling (2,3). Subsequent warming has been attributed to increased concentrations of atmospheric carbon dioxide produced by burning fossil fuels, and other greenhouse gases (4). Climate models suggest that this trend could accelerate in the coming century, although the contribution of human-induced greenhouse gases to global temperatures is far from clear (4-6).

Discussions of the potential impact of human-induced global warming frequently include malaria, a disease widely perceived as tropical. Articles in the popular and scientific press have predicted that warmer temperatures will result in malaria transmission in Europe and North America (7-12). Such predictions, often based on simple computer models, overlook malaria's history; until recently, malaria was endemic and common in many temperate regions, and major epidemics extended as far north as the Arctic Circle (13). Despite the disappearance of the disease from most of these regions, the indigenous mosquitoes that transmitted it were never eliminated and remain common in some areas. Thus, although temperature is important in the transmission dynamics of malaria, many other variables are of equal or greater importance. This article reviews the history of the disease in a nontropical country—England—during the coldest years of the Little Ice Age.

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The Little Ice Age

The 16th century is the first period for which we have a reliable history of climate and

weather. Private diaries, ships' logs, accounts of military campaigns, and similar sources give descriptions of wind direction, wind speed, cloud formations, and other weather indicators. Precisely dated annals, chronicles, audited accounts, agricultural records, tax ledgers, and other archival material provide indirect information, particularly on extreme weather events, such as droughts, floods, or unusual cold. Additional evidence is available from glacial moraines, lake and ocean sediments, pollen strata, deposits of insects, tree rings, coral structure, radiometric analysis of ice cores, archaeologic sites, and many other sources. All this information can be combined to reconstruct past climates (Figure 1).

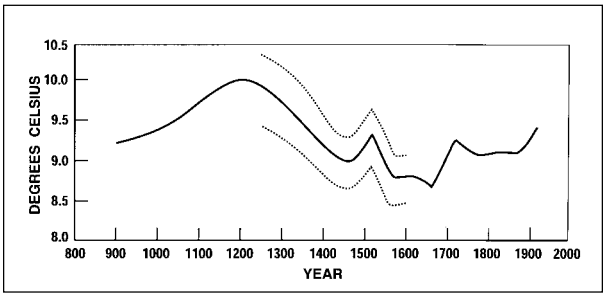


Figure 1. Estimated mean annual temperatures (°C) prevailing in central England since A.D. 800. These are 50-year averages, based on a variety of proxy data (see text). Dotted lines indicate the range of apparent uncertainty of the derived values.
Redrawn from Lamb (1).

The first half of the 16th century appears to have been much warmer than the previous 150 years, which had seen a fairly steady decline in temperatures after the Medieval Warm Period. However, the new genial climate was not to last. In the middle of the century, after a decade or so of exceptionally warm years—warm enough for people to bathe in the Rhine in January—a drastically sharp change occurred. The winter 1564-65 was long and bitterly cold and was the first of many that brought great hardship and strife throughout Europe. Climatologists regard the next 150 to 200 years the climax of the Little Ice Age. On average, temperatures were probably lower than in any similar episode since the end of the last major Ice Age (14).

The impact of this sudden change of climate was dramatic and global (1). Glaciers advanced rapidly in Greenland, Iceland, Scandinavia, and the Alps. The Arctic pack ice extended so far south that there are six records of Eskimos landing their kayaks in Scotland. Large tracts of land, particularly at higher latitudes and altitudes, had to be abandoned. In many years, snowfall was much heavier than recorded before or since, and the snow lay on the ground for many months longer than it does today. Many springs and summers were outstandingly cold and wet, although there was great variability between years and groups of years. Crop practices throughout Europe had to be altered to adapt to the shortened, less reliable growing season, and there were many years of dearth and famine. Violent storms caused massive flooding and loss of life. Some of these resulted in permanent losses of large tracts of land from the Danish, German, and Dutch coasts. The dramatic cooling was captured in the paintings of the Flemish artist Pieter Bruegel (c. 1525-1569), who initiated a new genre by completing at least seven winter landscapes in 2 years (Figure 2).

Climate changes were equally striking in other parts of the world. In Ethiopia and Mauritania, permanent snow was reported on mountain peaks at levels where it does not occur today. Timbuktu, an important city on the trans-Saharan caravan route, was flooded at least 13

Figure 2. Hunters in the Snow by the Flemish painter Pieter Bruegel the Elder (1525–1569). Completed in February 1565, during the first of the many bitter winters of the Little Ice Age. Bruegel painted at least seven such snow scenes, including biblical themes such as Adoration of the Magi (in a snowstorm) and the Census at Bethlehem, and the genre was adopted by other painters of the period. Despite the cold, malaria persisted in northern Europe until the second half of the 20th century. The World Health Organization declared Holland free of the disease in 1970.
Reproduced courtesy of the Kunsthistorisches Museum, Vienna.

times by the Niger River; there are no records of similar flooding before or since. In China, warm weather crops, such as oranges, were abandoned in Kiangsi Province, where they had been grown for centuries. In North America, the early European settlers also reported exceptionally severe winters. For example, in 1607 to 1608 ice persisted on Lake Superior until June (1).

Ague

The identity of diseases described in early literature is often uncertain, but fevers with a periodicity described as tertian or quartan are highly suggestive of malaria. Descriptors in the context of such accounts are often supportive. For example, in temperate regions, epidemic transmission of malaria tended to occur in late summer and autumn, giving rise to the common terms aestivo-autumnal or harvest fever for tertians and quartans.

During the Medieval Warm Period, mention of malarialike illness was common in the European literature from Christian Russia to caliphate Spain: "As one who has the shivering of the quartan so near,/ that he has his nails already pale/ and trembles all, still keeping the shade,/ such I became when those words were uttered." (The Inferno, Dante [1265-1321]).

The English word for malaria was ague, a term that remained in common usage until the 19th century. The Medieval Warm Period was already on the wane when Geoffrey Chaucer (1342-1400) wrote, in the Nun's Priest's Tale, "You are so very choleric of complexion./ Beware the mounting sun and all dejection,/ Nor get yourself with sudden humours hot;/ For if you do, I dare well lay a groat/ That you shall have the tertian fever's pain,/ Or some ague that may well be your bane."

Such mention of agues did not disappear when the coldest years of the Little Ice Age began. In 16th century England, many marshlands were notorious for their ague-stricken populations and remained so well into the 19th century. William Shakespeare (1564-1616), who was born in the autumn of Bruegel's first fierce winter, mentioned ague in eight of his plays. For example, in The Tempest (Act II, Scene II), the slave Caliban curses Prosper, his master: "All the infections that the sun sucks up/ From bogs, fens, flats, on Prosper fall and make him / By inch-meal a disease!" Later, Caliban is terrified by the appearance of Stephano, who, mistaking his trembling and apparent delirium for an attack of malaria, tries to cure the symptoms with alcohol: ". . . (he) hath got, as I take it, an ague . . . he's in his fit now and does not talk after the wisest. He shall taste of my bottle: if he have never drunk wine afore it will go near to remove his fit . . . Open your mouth: this will shake your shaking . . . if all the wine in my bottle will recover him, I will help his ague."

Alcohol and opium were commonly used to suppress the rigors of the first stage of the malarial paroxysm (15). We can assume that Shakespeare's audience was familiar with such details, although the play, one of his last, was probably not presented until 1611, long after the change to a colder climate.

Geographic Distribution: the Marsh Parishes

Five indigenous species of *Anopheles* mosquito are capable of transmitting malaria in England. The most competent, *An. atroparvus*, prefers to breed in brackish water along river estuaries. Contemporary accounts of the distribution of ague in 16th and 17th century England reflect the ecology and distribution of this species. For example, the anaerobic bacterial flora of saline mud produces a strong and distinctive sulfurous odor. This was widely perceived to be the actual cause of agues in salt marsh areas—Shakespeare's "unwholesome fens"—hence the Italian term mala aria (bad air).

The history of malaria in England and its demographic, epidemiologic, and social impact have been described in detail (16). Descriptions by 17th century topographers of the airs and waters surrounding settlements along the River Medway (a tributary of the Thames estuary) alluded to this association (17). Thus, Upchurch, a town on the open estuary: "lies in a most unhealthy situation, close to the marshes . . . the noxious vapours arising from which subject the inhabitants to continued intermittents." In nearby Iwade: " . . . in summer dry weather, the stench of the mud in the ponds and ditches. . . contribute so much to its unwholesomeness, that almost everyone is terrified to live in it . . ." In Burnham, further upstream, the air was: "rather more healthy . . . owing to the marshes being fewer and less offensive than those lower down the river. . ." and finally, the freshwater reaches were "far more healthy . . . instead of the noisesome smells, arising from the salt marshes

. . . the river here is encompassed with a range of pleasant fertile meadows, greatly conducive both to health and pride."

Even vicars of the Church were afraid of living in the estuarine areas. Dobson (15) reviewed several questionnaire surveys conducted by their bishops that asked "Do you reside personally upon your Cure, and in your Parsonage House? If not, where do you reside? What is the reason for your nonresidence?"

Nearly all the vicars of coastal "marsh parishes" answered no to the first question (Dobson lists 28 such parishes from the counties of Essex and Kent), and gave reasons such as "the place is so very Agueish," "frequently taken with agues and fevers," "the Thames having a very foul shore in this parish . . . attacked by so many repeated agues," "so violently afflicted with the worst of agues and languishing so long under it till our constitutions were almost broke," and "so unhealthy a situation as to be absolutely unfit for any curate."

Demographic data give striking justification for the vicars' fears (16) (Figures 3 and 4, Table). Descriptions of the marsh inhabitants resemble those of malaria-endemic area populations in the tropics today. Visitors commented on the swollen bellies of the children and their sallow, sickly faces, suggestive of anemia. An enlarged spleen was called ague cake. Nevertheless, despite the coldness of the climate, there is no indication that there was any major change in these death rates during the 17th century.

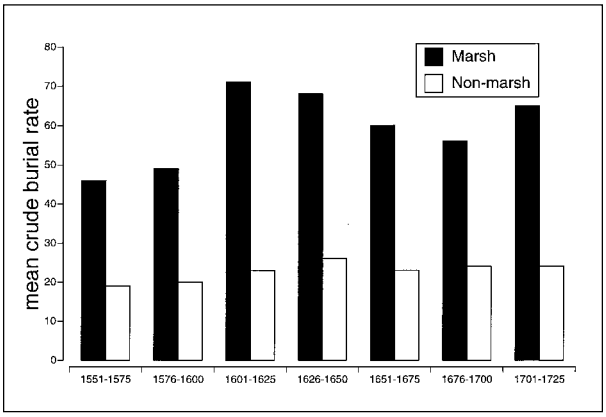


Figure 3. Average crude burial rates in Kent and Essex parishes, 1551–1750.
Drawn from Dobson (15).

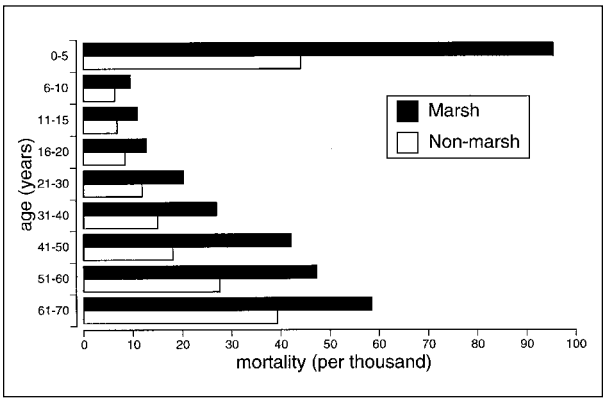


Figure 4. Age-specific death rates (per 1,000) in Essex parishes, c. 1800.
Drawn from Dobson (15).

Table. Deaths per 1,000 per year in late 18th century England (17) and 20th century Nigeria (39)

Essex, England			Garki, Nigeria	
Age	Nonmarsh	Marsh[a]	Age	Savanna
5	44	95.3	1-4	154
6-10	6.3	9.4	5-8	15
11-15	6.8	10.8	9-18	10
16-20	8.4	12.7	19-28	6

[a]The high death rates in the marsh parishes are attributable to malaria and were actually lower than in the previous century during the peak of the Little Ice Age. Moreover, the Essex data are skewed because many of the marsh communities included predominantly men. The rate was similar to that measured in the 1970s by the World Health Organization in a malaria-endemic area of sub-Saharan Africa.

Not all the summers of the 17th century were equally cool. The diarist Samuel Pepys (1633-1703), who himself had ague, wrote that the summers of 1661, 1665, and 1666 were dry and remarkably hot. This may well have enhanced transmission rates, for Thomas Sydenham described an epidemic of tertian and some quartan fevers in 1661, which "was doing frightful mischief" by August. Drought malaria, common in many parts of the world, arises when rivers and ponds are reduced to the smaller pools and puddles that anopheline mosquitoes prefer for breeding. Dobson (15) found a positive correlation between warm, dry summers in 1660 to 1810 and seasonal burial rates in Bradwell-juxta-Mare, a marsh parish in Essex. Macdonald (18) showed a strong relationship between high summer temperatures and cases of ague in Kent in the mid-19th century. High was defined as days when the temperature rose above 16°C, as measured at the Royal Greenwich Observatory.

Such hot weather, though clearly not tropical, could certainly have increased the probability of transmission by shortening the extrinsic incubation period (the time required for the mosquito to become infective after feeding on an infected person). Nevertheless, agues were not restricted to the warmer years.

Clinical Descriptions

Some accounts of intermittent fevers during the Little Ice Age were by physicians whose emphasis on precise methods, detailed observations, and accurate records leave us in no doubt that they were referring to malaria. William Harvey (1578-1657) made careful pre- and postmortem observations of cases of ague in London hospitals (St. Bartholomew's and St. Thomas').[1] His interest was probably sharpened because a persistent ague had caused him to miss much of his final year (1598-99) at the University of Cambridge. In his treatise on the circulation of the blood Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus (1628), he gave a detailed description of the clinical pathologic features of the febrile episodes: "In tertian fever . . . in the first instance . . . the patient (is) short-winded, disposed to sighing, and indisposed to exertion . . . the blood (is) forced into the lungs and rendered thick. It does not pass through them (as I have myself seen in opening the bodies of those who had died in the beginning of the attack), when the pulse is always frequent, small, and occasionally irregular; but the heat increasing . . . and the transit made, the whole body begins to rise in temperature, and the pulse becomes fuller and stronger. The febrile paroxysm is fully formed . . ."

Obstruction of circulation—commonly termed sludging—is mainly seen in *Plasmodium falciparum* infections. It results from complex changes in the consistency of the blood (19) and is manifest in many organs, although most often cited in the context of cerebral malaria. As Harvey noted, this dangerous condition often occurs suddenly, soon after the initial symptoms of a paroxysm.

Thomas Sydenham (1624-1689) is widely regarded as a founder of clinical medicine and epidemiology. His book on fevers (1666), which was later expanded into Observationes Medicae (1676), a standard text for 2 centuries, includes extensive details of agues, with descriptions of the course of paroxysms, the periodicity of tertians versus quartans, and the seasonality of the disease. As with Harvey, there can be no doubt that his descriptions refer to malaria; moreover, he states, "When insects do swarm extraordinarily and when . . . agues (especially quartans) appear early as about midsummer, then autumn proves very sickly." We cannot assume that insects referred to mosquitoes, but the statement that an early appearance of quartans was a premonition of a high incidence of illness (presumably agues?) has the distinct ring of modern epidemiology. Nevertheless, it is clear from these and other sources that the disease remained prevalent, even in the coldest years. For example, Harvey's ague began in the fall of 1597, yet the years 1594 to 1597 had been so cold and wet that wheat harvests were a disaster (1). Similarly, in 1657 to 1658, snow lay on the ground for 102 days—indicating exceptionally cold weather even for the times. The summer crops were a disaster, yet Oliver Cromwell (1599-1658) died of a tertian ague in September 1658, just as another severe winter was setting in.

Malaria Species

P. vivax and *P. falciparum* both have a tertian periodicity, so it is unclear which species was responsible for tertiary ague in England. *P. vivax* can persist for many years as a dormant hypnozoite in the liver, giving rise to occasional clinical relapses (caused by production of the blood stages of the parasite), whereas there is no evidence of hypnozoites in *P. falciparum*. Twentieth-century studies in Russia and Holland showed that some strains of *P. vivax* from the northern hemisphere—given the subspecific name *P. vivax hibernans*—did not produce clinical symptoms until 8-9 months after the infective bite. Both features would clearly

[1]St. Thomas' Hospital (1213), in the Borough of Lambeth, was on the edge of the River Thames, surrounded by tidal marshes. Parliament met in two buildings at a similar site in the Borough of Westminster, directly across the river. Both areas were notoriously malarious. Centuries later, the American Founding Fathers followed British parliamentary procedure in choosing a site for their new nation's capital at the edge of a malarious swamp, later referred to as "A Mud-hole Equal to the Great Serbonian Bog." The Serbonian Bog probably refers to the vast flood plains of the Danube that border northern Serbia and Bulgaria. The Balkan region was the last major stronghold of malaria in Europe. Malaria was finally eliminated there in 1975.

have survival value for *P. vivax* in a temperate climate, enabling it to cope with long winters and episodes of successive cold summers. On the other hand, *P. vivax* is generally a more benign pathogen than *P. falciparum*, but the death rate in the English marsh parishes suggests that infections were far from benign. This may indicate that *P. falciparum* was also present, and Harvey's observations on the consistency of blood in fatal cases support this possibility.

Laboratory studies have shown that tropical strains of *P. falciparum* do not multiply in European mosquitoes. However, European strains did cause major epidemics in Russia and Poland in the 1920s, with high death rates as far north as Archangel, Russia. In Holland, mosquitoes sheltering in the warmth of stables and human habitations continued to feed and transmit malaria throughout the winter and during colder summers. Thus, the dormancy and delayed onset of *P. vivax* were probably not essential for transmission, and it is conceivable that *P. falciparum* was endemic in England during the Little Ice Age. Alternatively, a more virulent strain of *vivax* may have been present. The question may never be resolved because the strains involved are now extinct.

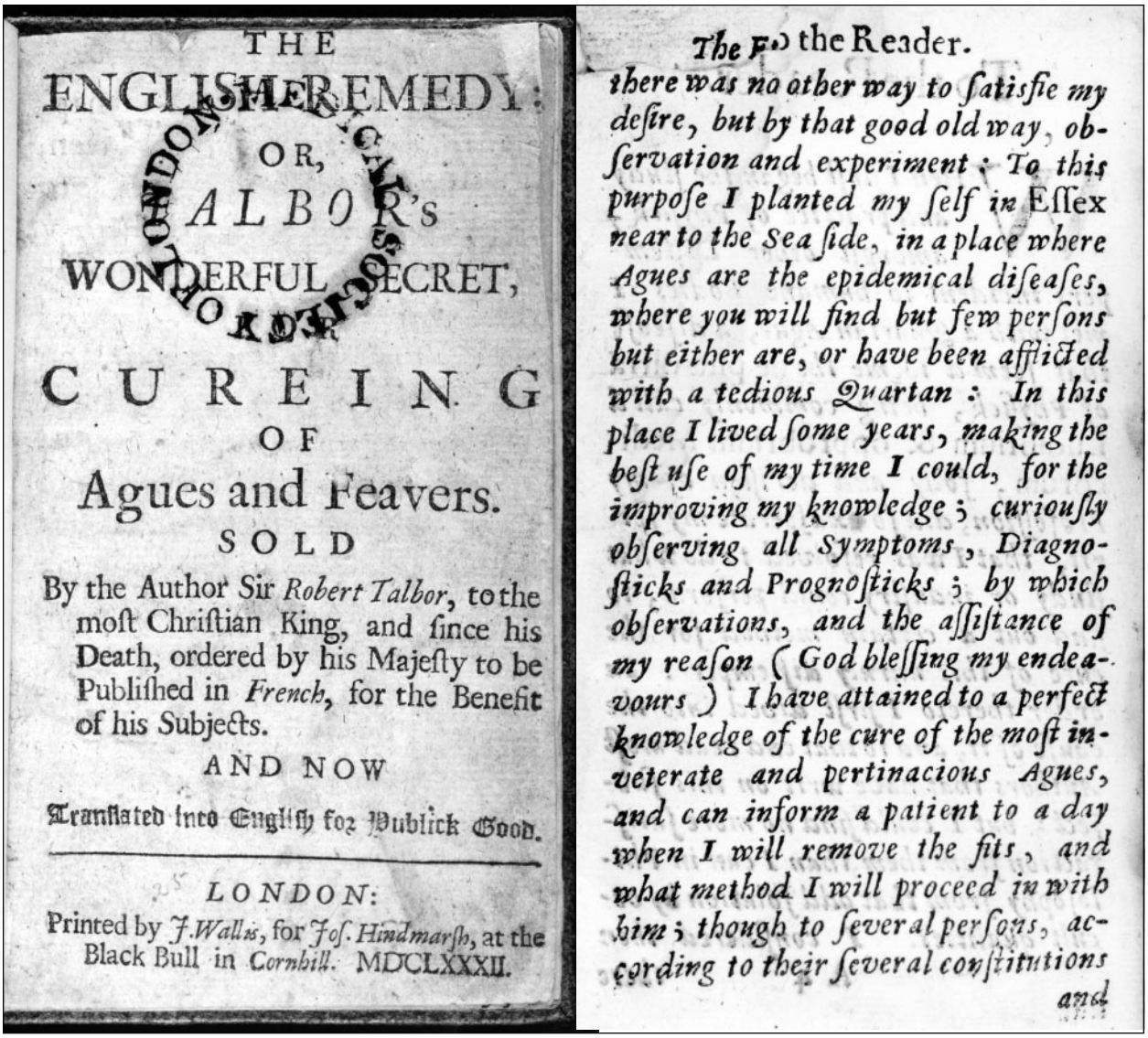
The Cure

The strongest evidence that ague was indeed malaria is the identity of its cure. Until the mid-20th century, the only effective remedy for malaria—at least in western medicine—was an extract of cinchona powder, obtained from the bark of several tree species native to the Andes, in South America. The principal active ingredient in this bark is quinine, a drug that has probably benefited more people than any other in the combat of infectious disease. Quinine is still used today, but few people know that its effective use for malaria therapy was first developed in tests with ague patients living in the salt marshes of Essex, less than 50 km from the center of London. What is more, the field experimentation that led to this English remedy was conducted during the coldest years of the Little Ice Age.

The first prescription of cinchona powder in England is attributed to Robert Brady in 1660. Thomas Sydenham advocated its use in his Methodus curandi febres in 1666. By that time, the "Jesuit's Powder" was already widely known in Europe, but in Protestant England many orthodox physicians were prejudiced against its use—partly because its export from Peru and Bolivia was in the hands of Catholics and its use had not been mentioned in the classical medical texts of Galen and partly because a reliable prescription had not been developed. In the end, popularization of the drug came in a highly unorthodox manner: a relatively untutored man, Robert Talbor, abandoned his apprenticeship to an apothecary to develop a safe dosage and an effective treatment regimen: "I planted myself in Essex near the sea side, in a place where agues are the epidemical diseases, where you will find but few persons but either are, or have been afflicted with a tedious quartan." After several years of study and testing, he developed what we would now call a patent medicine, a secret formulation that was essentially an infusion of cinchona powder in white wine.

In 1672, Talbor popularized his remedy by publishing Pyretologia: a Rational Account of the Cause and Cures of Agues. The success of his treatments became widely known and brought him rapid fame and fortune. Charles II appointed him Physician Royal in 1672. He was knighted in 1678, after he cured the King of an ague (20). Sir Robert Talbor then traveled to France, where he cured the son of Louis XIV. With the additional title of Chevalier Talbot, he became famous throughout Europe, curing Louis XIV, Louisa Maria, Queen of Spain, and hundreds of other royal and aristocratic persons (Figure 5).

Talbor's rise to fame has been told in many publications (21,22), yet none of these mention that the entire story took place in a period when temperatures were probably colder than in any other period in the past 10,000 years. Records for central England from 1670 to 1700 suggest that snow lay on the ground for an average of 20 to 30 days (in some years more than 100 days) as opposed to 2 to 10 days in the present century. In the winter 1683-84, the ground was frozen to more than 1 m. Belts of sea ice 5 km wide were present along the coast in the English Channel and are believed to have been 30 to 40 km wide off the coast of the Netherlands. The average summer growing season was approximately 5 weeks shorter than in the 20th century, and in some years the difference may have been more than 2 months (1). Nevertheless, the fact that the European aristocracy was eager to pour money and honors on an untutored commoner suggests that malaria continued to be a serious problem.



THE
ENGLISH REMEDY:
OR,
TALBOR's
WONDERFUL SECRET,
CUREING
OF
Agues and Feavers.
SOLD
By the Author Sir *Robert Talbor*, to the most Christian King, and since his Death, ordered by his Majesty to be Published in *French*, for the Benefit of his Subjects.
AND NOW
Translated into English for Publick Good.
LONDON:
Printed by *J. Wallis*, for *Jos. Hindmarsh*, at the Black Bull in *Cornhill*. MDCLXXXII.

The [illegible] to the Reader.

there was no other way to satisfie my desire, but by that good old way, observation and experiment: To this purpose I planted my self in Essex *near to the Sea side, in a place where Agues are the epidemical diseases, where you will find but few persons but either are, or have been afflicted with a tedious Quartan: In this place I lived some years, making the best use of my time I could, for the improving my knowledge; curiously observing all Symptoms, Diagnosticks and Prognosticks; by which observations, and the assistance of my reason (God blessing my endeavours) I have attained to a perfect knowledge of the cure of the most inveterate and pertinacious Agues, and can inform a patient to a day when I will remove the fits, and what method I will proceed in with him; though to several persons, according to their several constitutions*

and

Figure 5. The English Remedy: Talbor's Wonderful Secret for Curing of Agues and Feavers (1682). Robert Talbor sold the secrets of his malaria treatment to King Louis XIV for 2,000 guineas, on condition that they would not be published until after his death. In 1682, Talbor's remedy was published in French; the English translation appeared in the same year. Front page of English translation and introductory page in which Talbor describes how he went to Essex, and used "that good old way, observation and experiment . . . by which observations, and the assistance of my reason (God blessing my endeavours) I have attained a perfect knowledge of the cure of . . . Agues."
Reproduced courtesy of Wellcome Institute for the History of Medicine, London.

Defoe and Beyond

Robinson Crusoe was shocked to discover that a footprint in the sand was not his own: "so that I shook with cold, like one in an ague." His creator, Daniel Defoe (1660-1731), had traveled extensively in southern England in 1685 to 1690. In A Tour through the Whole Island of Great Britain (23), he described the depredations of ague in the Dengie marshes of Essex (Figure 6), an area 70 km east of London that remained notorious for the disease until the end of the 18th century (15): ". . . our London men of pleasure . . . go from London on purpose for the pleasure of shooting [the abundant wildfowl] but those gentlemen who . . . go

Figure 6. Map of the county of Essex in 1594. The extensive coastal salt marshes provided high-quality grazing for sheep and cattle but were also a favored habitat for *An. atroparvus*, a highly effective malaria vector. The disease was a major cause of illness and death in the area until the end of the 18th century.
Reproduced courtesy of Essex Record Office.

so far for it, often return with an Essex ague on their backs, which they find a heavier load than the fowls they have shot."

These sportsmen may have been wealthy enough to buy Talbor's remedy, but ordinary people suffered greatly in these unhealthy marshes. They risked living there because, as the land was excellent for rearing high-quality sheep and cattle, landlords were willing to pay high wages: " . . . for it being a place where every body cannot live, those that venture it, will have encouragement, and indeed it is but reasonable they should."

However, as in the malarious tropics today, the nonimmune were in great danger. Thus Defoe observed: "a strange decay of the [female] sex here . . . it was very frequent to meet with men that had had from five to six, to fourteen or fifteen wives . . . the reason . . . was this; that they [the men] being bred in the marshes themselves, and seasoned to the place, did pretty well with it; but that they always went into the hilly country . . . for a wife: that when they took the young lasses out of the wholesome and fresh air, they were healthy, fresh and clear, and well; but when they came out of their native aire into the marshes . . . they presently changed their complexion, got an ague or two, and seldom held it above half a year, or a year at most; and then . . . [the men] would go to the uplands again, and fetch another; so that marrying of wives was reckoned a kind of good farm to them."

During the 18th century, temperatures began to return to pre-mid-16th century levels. However, the wide variability of the Little Ice Age continued for at least 150 years. In the 1770s, much as today, alarm that the climate was becoming increasingly erratic prompted new emphasis on the recording of weather variables.

There are numerous accounts of malaria in all the northern European countries in the 18th and early 19th centuries. The wealth of records in this period confirms that the disease was common at many coastal sites in England and in some parts of Scotland, with occasional transmission as far north as Inverness (57°20'). The northern limit (24) was roughly along the 15°C July isotherm—not the 15°C winter isotherm, as stated by some authors (8,10). Thus, there was endemic transmission in southern Sweden and Finland, with occasional devastating epidemics that extended to the northern end of the Gulf of Bothnia, close to the Arctic Circle (25,26). In North America transmission occurred throughout most of the United States (27) and in some parts of Canada (28).

In 1827, John Macculloch (29) wrote "We may take the average of life among ourselves, in round numbers, at fifty with sufficient safety for this purpose. In Holland it is twenty-five; the half of human life is cut off at one blow, and the executioner is malaria, for there is no other cause for the superior mortality of that country."

The same was probably true of the coastal marshes of England, although the incidence appears to have decreased over the century. After the 1880s, transmission dropped precipitously, and the disease became relatively rare, except during a short period following World War I. This decline cannot be attributed to climate change, for it occurred during a warming phase, when temperatures were already much higher than in the Little Ice Age. Factors contributing to the decline include the following: Mosquito habitat was eliminated by improved drainage and extensive land reclamation. Root crops, such as turnips and mangel-wurzels, were introduced as winter fodder, enabling farmers to maintain much greater numbers of animals throughout the year; this diverted *An. atroparvus,* a zoophilic species, from feeding on humans. Rural populations declined as manual labor was replaced by machinery, further reducing the availability of humans as hosts for both mosquitoes and parasites. New building materials and improved methods of construction made houses more mosquito-proof, especially in winter, thus reducing the risk for contact with mosquitoes. Greater access to medical care and a rapid drop in the cost of quinine reduced the survival rate of the malaria parasite in its human host.

A similar decline occurred in the more prosperous countries of Europe—Norway, Sweden, Denmark, Germany, Holland, Belgium, France, and northern Italy. However, malaria maintained a much firmer grip on Eastern Europe—Finland, Poland and Russia, and the countries bordering the Black Sea and the eastern Mediterranean. It was not until the advent of DDT, after World War II, that a concerted attempt could be made to eradicate the disease from the entire continent (30). At the same time, the Communicable Disease Center (forerunner of the Centers for Disease Control and Prevention) was set up in Atlanta to eliminate malaria from the United States, where it was still endemic in 36 states (27), including Washington, Oregon, Idaho, Montana, North Dakota, Minnesota, Wisconsin, Iowa, Illinois, Michigan, Indiana, Ohio, New York, Pennsylvania, and New Jersey.

Conclusion

In 1975, the World Health Organization declared that Europe was free of malaria. The last indigenous case in England had been in the 1950s and in Bruegel's Holland in 1961. Results were equally spectacular on other continents. By 1977, 83% of the world's population was living in regions from which malaria had been eradicated or control activities were in progress. The only areas in which the control effort was limited were those of high endemic stability, particularly sub-Saharan Africa.

However, this momentum could not be sustained, and the goal of worldwide eradication was abandoned. Today, the disease is again common in many parts of Central America, the northern half of South America, much of tropical and subtropical Asia, some Mediterranean countries and many of the republics once part of the Union of Soviet Socialist Republics. This rapid recrudescence has been attributed to population increase, forest clearance, irrigation and other agricultural activities, ecologic change, movement of people, urbanization, deterioration of public health services, resistance to insecticides

and antimalarial drugs, deterioration of vector control operations, and disruptions from war, civil strife, and natural disasters. Claims that malaria resurgence is due to climate change ignore these realities and disregard history. For example, the many statements that recent climate change has caused malaria to ascend to new altitudes (10,31,32) are contradicted by records of its distribution in 1880 to 1945 (33-35).

With the return of malaria, there has been an exponential rise in international travel. Tens of thousands of cases are imported into Europe and North America each year. As was anticipated 30 years ago (36), a few of these cases give rise to autochthonous transmission by indigenous mosquitoes.

In much of Western Europe, the likelihood that malaria will become reestablished is probably small (37). However, in countries (e.g., in the Balkans) where malaria control has ceased but the probability of transmission remains high, reintroduction could threaten the public health. Indigenous transmission associated with imported cases has recently been reported in Kazakhstan, Kyrgyzstan, Turkmenistan, Uzbekistan, Bulgaria, the Republic of Moldova, Romania, Italy, and Corsica, and the malaria-free status of Europe may be in jeopardy (38). Public concern should focus on ways to deal with the realities of malaria transmission, rather than on the weather.

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Could a Tuberculosis Epidemic Occur in London as It Did in New York?

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In early 1999, more than 160 senior physicians, public health officials, and nurses met to discuss London's tuberculosis (TB) control program. The program was examined against the public health response of New York City's Bureau of Tuberculosis Control during a 1988 to 1992 epidemic. This article outlines TB epidemiology and control in New York City 10 years ago and in London today to assess whether the kind of epidemic that occurred in New York could occur in London.

Worldwide, tuberculosis (TB) kills more people than any other infection (1). Prevalence of the disease has been increasing in many countries since the mid-1980s, and more people are ill with TB now than at any other time in history (1). Although most cases occur in the developing world, some major urban areas in industrialized countries have also had a resurgence of the disease (1).

In the late 1980s and early 1990s, New York City had a major epidemic of TB, with rates tripling in 15 years and outbreaks of multidrug-resistant TB in many hospitals. Massive reinvestment in TB services to control the epidemic (2,3) resulted in a 59% decrease in cases, from a peak of >3,700 in 1992 to <1,000 in 1998. The incidence of multidrug-resistant TB decreased by 91% over the same period (4).

In the United Kingdom, TB rates began to increase in 1988 but have since leveled off (5), except in London, where the increase has been much more dramatic and has not subsided (6). This article compares the current epidemiology and control of TB in London with the situation in New York City in the late 1980s. Urgent action is needed to strengthen TB control in London if an epidemic like that in New York City is to be avoided.

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Epidemiology of TB in the Two Cities

The increase in TB notifications in London is similar to that seen during the first 10 years of the epidemic in New York City (Figure) (7). As in New York, rates of disease differ considerably in different parts of the city, with the highest rates in areas of low socioeconomic conditions and large immigrant populations (2,3,6). In New York, the highest rates were in central Harlem (79 per 100,000 in 1980 to 170 in 1989) (2). In London, the highest rates are in Newham, Tower Hamlets, and Brent (77-79 per 100,000 population) (6). Rates in several London boroughs have increased two- to threefold in 10 years (6). In both cities, the increase in case reports has

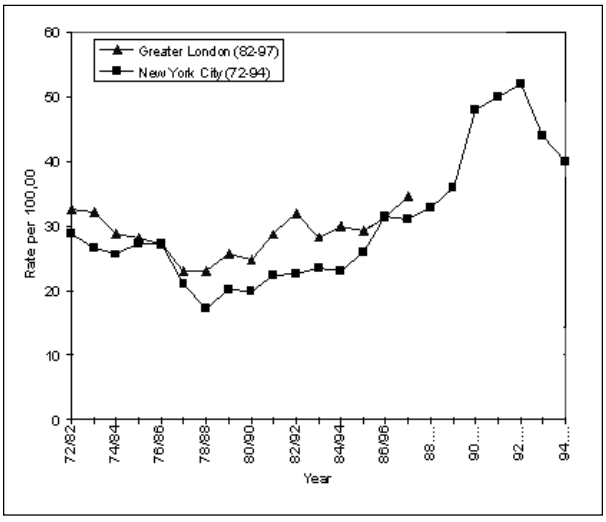

Figure. Tuberculosis rates in London, 1982–1997, compared with those in New York, 1972–1994.

been mainly among young adults (15-24 years of age) (3,6).

In both cities, most cases are in nonwhite residents. In New York City in 1994, 50% of TB patients were classified as black, non-Hispanic; 26% as Hispanic; 12% as white, non-Hispanic; and 12% as other/unknown (8). In London in 1993, 40% were classified under Indian Subcontinent, 31% white, and 29% "other nonwhite" (9). From 1988 to 1993, London rates increased most in the "other nonwhite" category (mainly black Africans) but also increased among whites and nonwhites who were born in the United Kingdom. (The rate among those of Indian Subcontinent background who were born in the United Kingdom rose from 12 per 100,000 in 1988 to 41 per 100,000 in 1993 [9]). Therefore, importation of disease is not the only factor causing the increase in London.

In London (as in New York at the beginning of the epidemic), data on patient origins are not routinely collected. Preliminary data suggest that at least 55% of cases are among foreign-born persons (H. Maguire, pers. comm.). In New York in 1993-94, approximately 28% of TB cases were among immigrants (3). Thus, in both cities a substantial proportion of cases is among immigrants, but this disparity is greater in London.

Early in the epidemic in New York, 38% of persons with culture-confirmed cases of TB were known to be HIV infected (2). Data on HIV infection in TB patients are not routinely collected in the United Kingdom, but a study in 1993 estimated that at least 7% of TB cases in London were among HIV-positive persons (10). A more recent study of 157 patients starting treatment for TB at an inner-city London hospital in 1996 and 1997 showed that 25% were coinfected with HIV (44% Europeans, 49% Africans, and 3% Asians). These data suggest that, in some ethnic groups in some areas of London, HIV infection is strongly affecting the epidemiology of TB (11).

Data on drug resistance were not routinely collected early in the epidemic in New York City. In the early 1990s, 19% of patients with culture-confirmed TB had multidrug-resistant TB, and in 26% of patients TB was resistant to isoniazid (12). These figures had declined to 4.1% and 4.4% by 1998 (4). In 1994-96, 2.6% of isolates in London were multidrug resistant, compared with 1% in the rest of the country; 8% were resistant to isoniazid, compared with 4.7% in the rest of the country (13). The current levels of drug resistance in London are lower than those during the epidemic in New York. However, data from New York demonstrate that transmission of resistant strains in hospitals and other settings can lead to a rapid increase in rates of resistance (14). Several outbreaks of TB, including two involving multidrug-resistant TB, have already occurred in London, principally affecting HIV-positive patients in teaching hospitals (15-17).

Recent transmission appears to be a substantial problem in both cities. Preliminary results from a molecular study to identify recent transmission of TB in inner London suggest that approximately 27% of cases are clustered (10). In New York City, an analysis of the molecular epidemiologic links of TB isolates obtained in April 1991 showed that, of 344 patients, 126 (37%) belonged to one of 31 clusters, and clustering was more frequent in patients who had multidrug-resistant TB (involved in 53% of clusters), were black (44%), and were homeless (49%) (14).

Both London and New York City have large populations of homeless people (86,000 in New York in 1990 compared with 50,000 in London in 1992) (18,19). In New York City in the 1970s and 1980s, approximately 6% to 7% of homeless persons had active TB (20,21). By 1992, up to 25% of the city's cases of TB occurred in the homeless (22). Fewer data are available for TB rates in London's homeless, but information suggests very high levels of TB. Screening at a shelter in London in 1993 identified active TB in 1.5% of those screened. Another 3.5% had chest X-rays suggestive of active disease, but the patients were lost to follow-up before further investigation could take place (23). At least 5% of TB patients in London have a history of residence in a hostel for the homeless (H. Maguire, pers. comm.). In both cities, poor compliance with treatment and transmission of disease are major problems in the homeless (18,21).

TB transmission in state correctional facilities was a major problem in New York (3). No outbreaks of TB have yet been documented in prisons in London.

Control in London and New York City

The loss of government funding of TB programs in the 1970s and 1980s in New York City made access to treatment more difficult for

poorer sectors of the population (often nonwhite patients) (3), likely contributing to increases in disease in these groups. In the United Kingdom, all patients have free access to National Health Service treatment, but new immigrants, asylum seekers, refugees, and illegal immigrants may have difficulty accessing health services because of cultural and linguistic barriers (24). Some may have difficulty in obtaining free prescriptions because of lack of necessary documents (24). In addition, immigration policies differ: in the United States, immigrants are screened and treated for active disease before entering the country, and skin-test-positive new entrants are usually treated for latent infection (25). In the United Kingdom, a few entrants are screened at airports, with the rest being screened at the local level. In some areas, only a few new entrants are screened, and chemoprophylaxis is rarely used except in children (24,26). Thus, disease prevalence among nonwhite patients may be related to inadequate screening in the United Kingdom.

Treatment completion rates in some areas of New York were low. In 1989, for example, 40% of patients with TB did not complete treatment in the city (27), and in some locales, up to 90% of patients did not complete treatment (2). Early in the epidemic, directly observed therapy was rarely used. In London, the number of patients who complete treatment is not known, and directly observed therapy is rarely used (24). An audit in a London hospital showed that 19% of patients were lost to follow-up before completing treatment. In another 15%, patient records were unavailable (A. Pearson, pers. comm.). A study of TB among the homeless in London found that 43% of suspected cases were lost to follow-up even before the diagnosis could be confirmed (23). Currently, treatment completion rates are not routinely monitored in London.

In the United Kingdom, section 37 of the Public Health (Control of Disease) Act of 1984 allows detention of patients who pose a serious risk for infection to others. In contrast to New York City, where coercion was considered by many to be an important element of TB control (28), this power is rarely used in London (24). Use of detention is difficult to justify when the infrastructure to allow less coercive adherence-support methods (such as directly observed therapy) is not in place (29). Increasing use of incentives and flexible provision of directly observed therapy in New York City has greatly decreased the use of detention (28). Directly observed therapy to improve compliance is rarely used in London, and for the least compliant patients it is almost impossible to provide (24).

Early in the epidemic in New York, there were insufficient measures to prevent spread of TB in hospitals, but by 1997 the city had 400 negative-pressure isolation facilities (30). In 1995 in London, there were 103 negative-pressure isolation facilities, only 17 of which had continuous air-pressure monitoring and 49 were considered adequate for housing patients with infectious multidrug-resistant TB (A. Hayward, unpub. obs.). Guidelines have recently been published to improve infection control in hospitals (31), but adherence will be difficult without an increase in the availability of isolation facilities.

Inappropriate treatment of isoniazid-resistant patients (e.g., failing to use an initial regimen including at least four drugs) is thought to have contributed to the development of multidrug-resistant TB in New York (12), but the explosive rise in rates of multidrug-resistant disease was mainly due to transmission of disease among hospitalized HIV-infected patients (14). It is not known what proportion of patients in London are initially treated with a four-drug regimen. The high rates of isoniazid resistance in London indicate the potential for rates of multidrug-resistant TB to increase if such regimens are not used. Hospital transmission could then lead to further rapid increases.

At the beginning of the epidemic in New York, surveillance did not provide routine data on drug resistance, ethnicity, country of origin, HIV-related TB, or treatment completion rates. The lack of information delayed recognition of the problem, and assumptions that TB was an immigrant problem proved not to be accurate (32). Similarly, surveillance in London has failed to collect routine data. Drug resistance levels have been monitored routinely only since 1994, and information (such as knowledge of previous treatment) that would be of value in interpreting routine data is often not available. An enhanced surveillance system has been launched in the United Kingdom, but many London districts are having difficulties in obtaining the additional information required (24). The new system does not currently collect data on treatment completion rates, but collecting such data is

proposed for the next stage of development (J. Watson, pers. comm.).

During the early part of the TB epidemic in New York City, TB control was underfunded and highly fragmented (3). Underfunding of TB services is also a major barrier to improving TB control in London (24), and the system is also highly fragmented (24). For example, in London in 1993, TB was treated by 250 doctors working in 45 different hospitals (5). In the United Kingdom, district-based "Consultants in Communicable Disease Control" are responsible for control of TB and other infectious diseases in the community. Unlike TB coordinators in the United States, they have no direct authority to design treatment programs or institute directly observed therapy.

Conclusions

The epidemiology and control of TB in London now differ in several ways from those in New York City early in the epidemic: London has a lower proportion of cases in HIV-infected patients, a higher proportion of cases among immigrants, and lower levels of multidrug resistance. However, similarities in epidemiology and control include similar numbers of cases; a similar increase in disease prevalence; very high prevalence in areas of lower socioeconomic conditions and large ethnic populations; concentrations of disease in the homeless, the HIV infected, and immigrants; transmission of multidrug-resistant strains in hospitals; inadequate isolation facilities; unknown treatment completion rates; high levels of loss to follow-up; and no overall coordination among providers of TB services. All these factors suggest that if TB control in London is not improved, the city could experience an epidemic of similar proportions to that in New York.

Although need for action was recognized in New York City in 1987, a clear plan for action was not implemented until 1992. By 1995, the epidemic had cost more than $1 billion (3). London needs to learn from the New York epidemic and take prompt action to improve control by developing solutions based on the local epidemiology of the disease.

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Japanese Encephalitis Immunization in South Korea: Past, Present, and Future

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Japanese encephalitis (JE), once a major public health problem in South Korea, has declined since the 1980s, as a result of improved living conditions, a mosquito eradication program, and a national JE vaccination program, which includes annual booster vaccine for all children ≤ 15 years of age. Increased immunity has greatly reduced illness and death; however, vaccine adverse effects are increasing, and a National Compensation Program for Vaccine Injury was begun in 1995. This article reviews past successes, current problems, and future direction of the JE vaccination program in South Korea.

Epidemiology of Japanese Encephalitis (JE) in South Korea

Historically, JE occurred seasonally in South Korea, causing high rates of illness and death. Although public health records concerning the incidence of the disease before 1933 are not available, outbreaks of "summer encephalitis" were recognized. During summer 1934-35, an outbreak was reported of suspected encephalitis, with acute onset of spiking fever and headache, as well as neurologic abnormalities and mental changes. Although these outbreaks were described as meningococcal meningitis by Korean health authorities, some Japanese workers reported that many of the cases were encephalitis (1,2). In 1946, Sabin et al. (3) isolated JE virus from an American soldier stationed in South Korea. Deuel et al. (4) conducted the first extensive serologic survey by neutralizing test in four areas of South Korea and reported that JE virus was widely disseminated. In 1947, JE was added to the list of first-class emergency diseases in South Korea.

In 1949, when JE became notifiable, 5,548 cases of clinically suspected disease were reported with 2,429 deaths (49% case-fatality rate). The criteria for reporting were high spiking fever, headache, and mental changes with any symptom of central nervous system involvement. Hullinghorst et al. (1), who described the 1949 epidemic in South Korea, reviewed >600 hospital records. Results of bacterial cultures of cerebrospinal fluid (CSF) were reviewed for 567 cases, and the complement fixation and neutralization tests were performed in selected cases. Pathology findings and JE virus isolation from a case, negative results of CSF bacterial cultures, and a relatively uniform clinical picture, including acute high fever, headache, and various neurologic abnormalities, suggested that the outbreak in 1949 was JE (1,5). Thereafter, several outbreaks of thousands of JE cases were reported every 2 to 3 years, and JE was recognized as a recurring public health problem in South Korea (6,7).

In 1954, when the Communicable Disease Control Act (Reg. 308) was enacted, JE was classified as a first-class notifiable communicable disease. The largest recorded JE epidemic, involving 6,897 reported cases, occurred in 1958 (7). In 1963, JE was reclassified as a second-class notifiable disease, along with polio, pertussis, measles, mumps, and other childhood diseases. Since 1964, seroepidemiologic studies with the hemagglutination inhibition test (HI) have been conducted routinely for clinically suspected cases. In 1964, 108 paired and 1,636 acute-phase sera were tested (8). The results indicated that 86.7% of acute-phase sera were negative by the HI test and 54% of paired sera were positive in their second sample. Several thousand cases were reported annually until 1968, with the exception of 1963, when only 19 cases were

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reported; Chang et al. (9) provide evidence for underreporting in that year (Figure 1). Case reports sharply declined to <100 cases in 1969; <1,000 cases were reported annually until 1973. The last large epidemic was recorded in 1982, when 1,197 cases and 40 deaths were reported. This rapid decline in reported cases may be related to the extensive use of insecticides in rice farming, which led to a marked decrease of *Culex tritaeniorhynchus* in rural habitats (10,11).

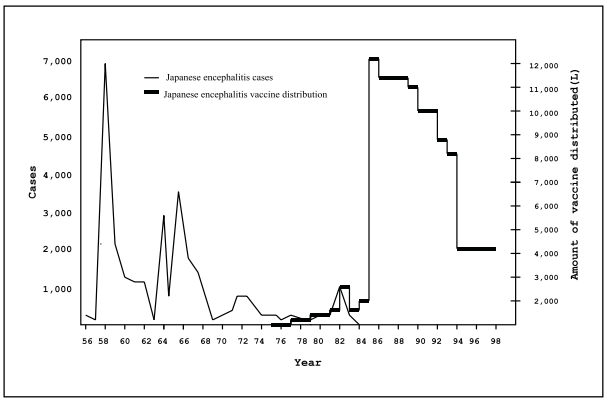

Figure 1. Cases of Japanese encephalitis by liters of vaccine distributed, 1936–1998, South Korea.

The highest incidence of JE was recorded in Seoul and Kyonggi Province during the first officially reported outbreak in 1949 (Figure 2A). In the epidemics during 1965 to 1984, a higher incidence was recorded in the southwestern than in the northeastern provinces (Figure 2B). The southwestern provinces are mainly under rice cultivation with large flooded rice paddies, while the northeastern provinces are mountainous. Vector mosquito populations were more prevalent in the southwest than in the northeast (8), leading to greater risk for enzootic viral transmission in the southwestern provinces. The high incidence in Seoul in the 1949 outbreak may be accounted for by the rapidly increasing susceptible population in the capital and its suburbs since 1945 (7). Approximately 90% of the cases occurred from mid-August to mid-September; more than 80% were among children 3 to 15 years of age (9,10).

Factors affecting the size of JE epidemics include the risk for being infected by the virus when bitten by a vector mosquito and the JE seropositive rate in the population. In 1965, before the national vaccination program was implemented, Chang et al. (9) estimated the

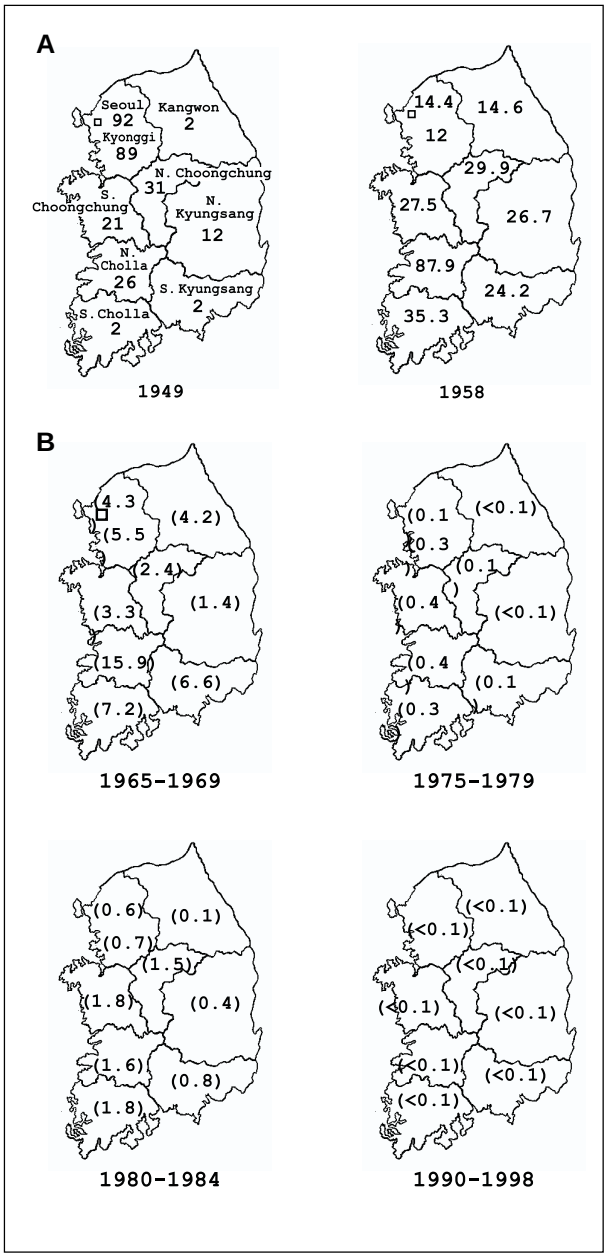

Figure 2. A. Incidence of Japanese encephalitis (JE) per 100,000, by province, South Korea, 1949 and 1958 (7). B. Incidence of JE per 100,000, by province, South Korea, 1965–98 (modified from 8-11,24).

natural seroconversion rate of HI antibody in children <15 years of age at approximately 9%, indicating that the estimated incidence of JE cases during epidemics would have been higher without intervention. Several studies comparing seroprevalence rates before and after the epidemic season indicated that the seropositivity rate was higher in South Choongchung Province (86.4% before the epidemic season vs ~100% after

the epidemic season in 1984) than in Seoul (56% before the epidemic season vs ~72% after the epidemic season in 1987) (Figure 3) (12-15).

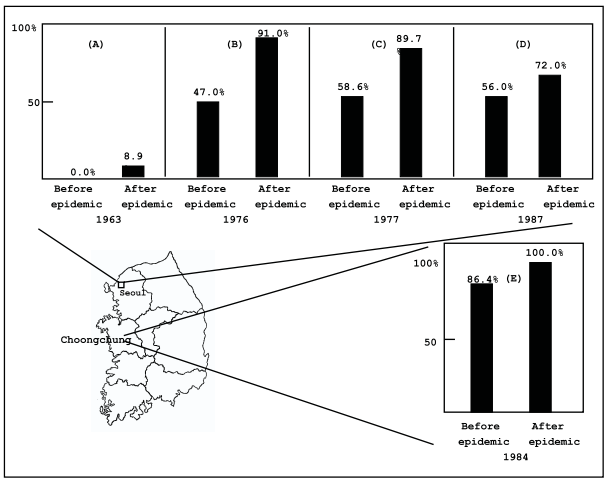

Figure 3. Seropositivity of HI antibody before and after the Japanese encephalitis epidemic. (A), (B), (C) before the implementation of vaccination program; (D), (E) after the implementation of vaccination program (modified from 13-16).

From 1985 to 1998, only 21 cases of JE were serologically confirmed. Factors leading to this decrease include the National Immunization Program (NIP), initiated in 1983, which has conferred protective immunity on children at risk. The national socioeconomic growth for the past 3 decades has allowed improved standards of living and environmental conditions. The modernization of agricultural practices led to changes in the epidemiology of JE.

Epidemic Forecast Program

Since 1975, an epidemic forecast program to alert the public against JE has been implemented by the health authorities every year before the epidemic season (16) (Table 1). To predict the possibility of an outbreak, the health authorities have attempted to isolate the virus from the vector mosquito, *C. tritaeniorhynchus;* estimate population density of *C. tritaeniorhynchus* among captured mosquitoes; and determine JE antibody levels in pigs. Monitoring for *C. tritaeniorhynchus* begins in early May. The population density of *C. tritaeniorhynchus* in mosquitos captured in seven urban and rural areas was 76.1% in 1982, when the most recent epidemic occurred (11). Because outbreaks in humans seem closely related to a rapid rise of HI antibody titers in pigs, the blood of young pigs (<6 months old) is screened weekly at slaughterhouses for immunoglobulin (Ig) M antibody. In areas where epidemics have occurred, vector mosquitos collected are homogenized and injected into suckling mice; JE virus is identified by HI and, recently, by polymerase chain reaction in the brains of mice manifesting JE symptoms.

Table 1. The Japanese encephalitis (JE) epidemic forecast program, South Korea

Year	Date of attention	Date of alarm	Date index case diagnosed	Province[a]
1975	6/24	8/19	8/22	South Cholla
1976	6/25	8/18	9/10	South Cholla
1977	6/20	8/ 9	8/18	Cheju
1978	6/23	8/16	8/22	South Cholla
1979	6/20	8/ 7	8/30	South Cholla
1980	6/16	8/22	8/28	South Cholla
1981	6/26	8/19	8/22	Kyonggi
1982	6/14	7/23	8/ 6	South Cholla
1983	5/24	8/16	8/23	South Cholla
1984	6/5	8/16	-	NA[b]
1985	5/21	8/20	-	NA
1986	5/22	8/25	-	NA
1987	5/21	7/30	8/11	South Cholla
1988	5/30	8/10	9/8	South Cholla
1989	5/25	8/25	-	South Cholla
1990	5/23	8/21	9/26	South Cholla
1991	5/23	7/31	7/29	Kyonggi
1992	5/13	8/13	8/24	South Cholla
1993	6/1	8/10	8/12	Cheju
1994	5/20	8/4	9/6	South Cholla
1995	5/11	8/24	-	South Cholla
1996	5/23	8/13		South Cholla
1997	5/27	6/20		Kyonggi
1998	5/7	8/13	8/6	South Cholla

[a]Province is the place where JE virus was first isolated in that year.
[b]Not available.

When the JE vector mosquito is first detected, health authorities issue an alert that encourages JE vaccination (Table 1). If JE IgM antibody titers increase in pigs or JE virus is isolated, aggressive mosquito control is implemented.

Active JE Immunization in the Spring Mass Immunization Campaign

A small amount of the mouse brain-derived formalin-inactivated Nakayama vaccine from Japan was imported in 1967 (17,18). In the early

1970s, the Nakayama vaccine began to be produced locally. Despite an active campaign with the Nakayama vaccine, the annual number of doses distributed remained under 500,000 in the 1970s, for a vaccine coverage rate of <5% (11). However, the vaccine coverage rate increased to 16.8% in 1981. The most recent epidemics—1,197 reported cases in 1982 and 139 in 1983—prompted a mass immunization program for children at risk. Children 3 to 15 years of age were vaccinated at state public health centers, and simultaneous vaccination in the private sector was encouraged; as a result, the immunization rate increased to 60% in 1983, when a JE epidemic occurred. In 1986, 12 million doses were distributed in the public and private sectors, which was more than the estimated number of children <15 years of age. From 1985 to 1992, almost 10 million doses of Nakayama vaccine were distributed each year, enough to vaccinate 97% of the nation's children (Figure 1).

In 1983, the age of initial dose of JE vaccine was recommended by the health authorities at 3 years of age, except for areas in which the disease had recently become endemic, where it could be given earlier. The series consisted of three doses of primary vaccination, with annual boosters until 15 years of age. Because the vaccination rate could not be maintained above 90% by individual immunization during the spring campaign (May to July), mass immunization at schools and public health centers was used.

Since 1984, when JE vaccine coverage reached almost 90%, only a few sporadic cases occurred in young adults or the elderly. The incidence of JE was reduced from >18.5/10^5 in 1964 to <0.02/10^5 over the past 3 decades. Several factors contributed to the rapid decline. In addition to improved living conditions, public health, the widespread use of agricultural herbicides and insecticides, a decrease in the population density of vector mosquitos by the removal of mosquito habitats, and the modernization of agricultural practices, such as improved sanitary conditions on pig farms in rural areas, may have decreased the risk for human infection. In South Korea, *C. tritaeniorhyncus* usually appears in May and multiplies rapidly in July and August. From 1982 to 1985, mosquitos were collected in seven areas from May to late October. The number of mosquitos gradually decreased, from 11,278 per day (76% *C. tritaeniorhyncus*) in 1982; to 4,334 (59.2%) in 1983; 2,150 (40.3%) in 1984; and 1,212 (34.4%) in 1985 (11). The virus infestation rate of the vector mosquito has been consistently <1% since 1990. Consequently, the risk of being exposed to the vector mosquito and infected by JE virus has decreased.

Another contributing factor is the policy of maintaining high levels of immunity through the expansion of the routine childhood immunization program. An immunization coverage rate >97% has significantly contributed to the prevention of JE. In 1969, a JE vaccine efficacy trial was performed among schoolchildren in southwestern provinces in South Korea; approximately 45,000 children were vaccinated and another 45,000 were controls. Although no epidemic occurred during this period (19,20) because of vaccination, the HI seropositive rate of 11.6% in prevaccination sera increased to 75% in postvaccination sera after two doses of mouse brain-derived Nakayama vaccine. The seropositivity rate by neutralizing antibody test was 99% postvaccination.

At present, visitors to South Korea on business or vacation are considered at low risk for JE; routine vaccination with Nakayama vaccine may not be necessary for these travelers. However, JE immunization is recommended for visitors to disease-endemic areas during transmission season. Vaccine is advised especially for travelers who stay for an extended time (>30 days) or are at high risk for exposure to vector mosquitos through activities or housing.

Vaccine Manufacture and Supply

In 1972, the manufacture of Nakayama JE vaccine was privatized and transferred to the South Korea Green Cross Company. Currently, seven pharmaceutical companies manufacture JE vaccine. Before 1995, when boosters were given annually, the total amount of vaccine needed for the population at risk was 8,500 to 9,000 liters per year. Vaccine production is projected more than 1 year in advance; the amount to be used the next year is usually determined in February. Then the injection of JE virus into mice, recovery of the virus from mouse brain tissue, and production of bulk vaccine begin. Vaccine is ready in October, and after a thorough testing in January of the following year, is distributed in March.

Vaccine Adverse Effects

Although the mass immunization campaign was successful in attaining high rates of vaccination coverage, JE vaccine-related adverse effects may have been overlooked. A reporting system was not available until 1995, so the incidence of JE vaccine-related adverse effects among South Korean children is not known. However, in 1994, six cases of severe systemic illness after JE vaccination were reported (21) (Table 2). Two sudden deaths were attributed to anaphylactic reaction to the vaccine, and four cases of severe neurologic illness, including encephalopathy and acute disseminated encephalomyelitis, were reported. Since 1994, seven cases have been filed with the Advisory Committee of the National Immunization Program, according to the National Compensation Program for Vaccine Injury (Table 2).

Table 2. Cases of adverse reactions to Japanese encephalitis vaccine reported to the National Compensation Program for Vaccine Injury, South Korea, 1994

Case	Age/ sex	Previous vaccination	Vaccine strain	Date of vaccination	Date of onset	Symptoms at onset/ sequelae/ laboratory findings	Outcome
1	5/F	2 doses of JE	Nakayama (Lot 3008)	5/23/94	5/23/94	Anaphylactic shock collapse, cardiac arrest	Death
2	5/M	2 doses of JE	Nakayama (Lot 3008)	5/23/94	5/23/94	Anaphylactic shock collapse, cardiac arrest	Death
3	15/F	8 doses of JE	Nakayama (Unknown)	4/4/94			
		2 doses of HRFS	Hantavax (Unknown)	4/20/94	5/13/94	Headache, auditory hallucination, seizure Brain CT normal MRI normal EEG moderate to severe cortical dysfunction, multifocal generalized polyspike and slow wave on both hemispheres CSF protein (20 mg/dl), glucose (78 mg/dl), WBC (17/mm^3)	Death
4	8/F	>2 doses of JE	Nakayama (Lot 0132-93011)	5/7/94	5/19/94	High fever, seizure Brain CT normal EEG compatible with encephalopathy CSF protein (82 mg/dl), glucose (99 mg/dl), WBC (18/mm^3)	Recovery
5	9/F	>3 doses of JE	Nakayama (Unknown)	4/24/94	5/11/94	High fever, headache, vomiting, seizure Brain CT diffuse brain edema CSF protein (6 mg/dl), glucose (75 mg/dl), WBC (10/mm^3)	Death
6	2/F	none	Nakayama (Unknown)	4/11/94	4/23/94	Urinary incontinence, drooling, high fever, motor weakness, vomiting, cranial nerve palsy (III,IV,VI) diplopia and phtosis, headache, seizure MRI compatible with acute disseminated encephalomyelitis	Mental retardation
7	10/M	>3 doses of JE	Nakayama	4/23/96	5/25/96	Headache, vomiting, seizure, coma	Death

JE, Japanese encephalitis; HFRS, Hemorrhagic fever with renal syndrome; CT, computer-assisted tomography; MRI, magnetic resonance imaging; EEG, electroencephalograph; CSF, cerebrospinal fluid; WBC, white blood cells.

The Future of the Immunization Program

Although the cost-effectiveness of the JE immunization program, including annual boosters, has not been evaluated, JE has been successfully controlled in South Korea for the past 2 decades. Questions remain about the optimum immunization schedule, including appropriate ages for initial vaccination and boosters. The state health authority recommends two doses 4 weeks apart with a booster 1 year later as a primary immunization, and annual boosters thereafter up to 15 years of age. Since the longevity of neutralizing antibody after primary vaccination was not known and seemed short lived, frequent boosters appeared necessary. However, without sound evidence, the total of 14 doses of vaccine until 15 years of age led to concerns about excessive vaccination and increased risk for vaccine adverse effects.

The 1994 report of the cluster of severe adverse systemic effects after JE vaccination prompted medical and social debate (22,23). A causal association between JE vaccine and the cluster of severe adverse effects has not been clearly established; however, empiric evidence, including the timing of events, characteristics of the adverse reactions, susceptibility, and lack of alternative etiologic agents, indicates a causal relationship. Public concern over adverse reactions led to refusal of the vaccination and a consequent decrease in coverage rate. In response, the National Compensation Program for Vaccine Injury was begun in 1995.

During public debate about the JE immunization schedule, the safety and quality of the domestically produced JE vaccines were questioned. The Advisory Committee of the National Immunization Program announced that the annual booster immunization schedule had become impractical because of the inappropriate administration of the vaccine during the mass immunization in the spring, the need to define the long-term immunity in the elderly (> 65 years of age), poor recognition of adverse effects, the need to guarantee the quality of the vaccine manufactured in South Korea, and public reluctance to receive JE vaccination. The committee agreed that the JE immunization program should be improved with regard to the schedule, age of initial vaccination, booster schedule, and vaccine strain. The annual booster schedule was changed to once every 2 years, and single-dose rather than multidose vials were used, as recommended by the South Korean Society of Pediatricians.

In 1996, a seroprevalence study for plaque-reduction neutralizing antibody (PRNT) was carried out to indirectly evaluate the efficacy of booster vaccination in 311 schoolchildren (24). The neutralizing antibody titers were found to decrease gradually as the interval between boosters increased (Figure 4A). The seropositivity rate of 98.1%, 99%, and 95.6% at 6, 18, and 30 months, respectively, had declined to 71.4% by 42 months (Figure 4B).

To replace the inactivated mouse brain-derived vaccine, a clinical trial with a live attenuated SA14-14-2 vaccine, which has been

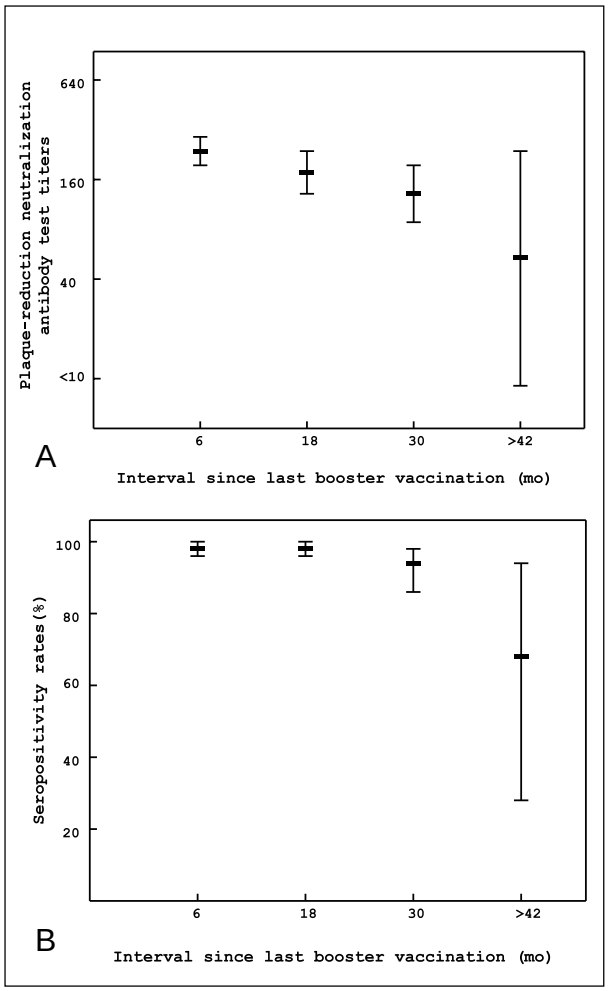

Figure 4. A. Plaque-reduction neutralizing antibody titers (PRNT) according to interval since last booster injection with Nakayama vaccine among 311 South Korean children, 1996. B. PRNT antibody seropositivity rate according to the interval since last booster injection.

used safely and effectively in >100 million children in China since 1988 (25), was conducted in South Korean children in 1997. A preliminary immunogenicity study among South Korean children 1 to 3 years of age indicated a 96% rate of seroconversion in PRNT antibody, with a geometric mean titer (GMT) of 188 after a single primary immunization dose. In children who had been immunized with two or three doses of inactivated Nakayama vaccine, the booster administration of SA14-14-2 vaccine produced an anamnestic response with a GMT of 3,378 in all cases without virus-specific IgM response (Figure 5) (26).

During the last 10 years, JE has occurred more frequently in adults than in children—two-thirds of Korean JE patients were middle-aged adults. Adults are not protected by the current immunization program, probably because of waning immunity. Long-term immunity must be maintained to prevent secondary vaccine failure. A single booster with the live-attenuated SA14-14-2 vaccine might confer long-term immunity for adults and decrease the frequency of vaccination, as well as the risk for vaccine adverse effects.

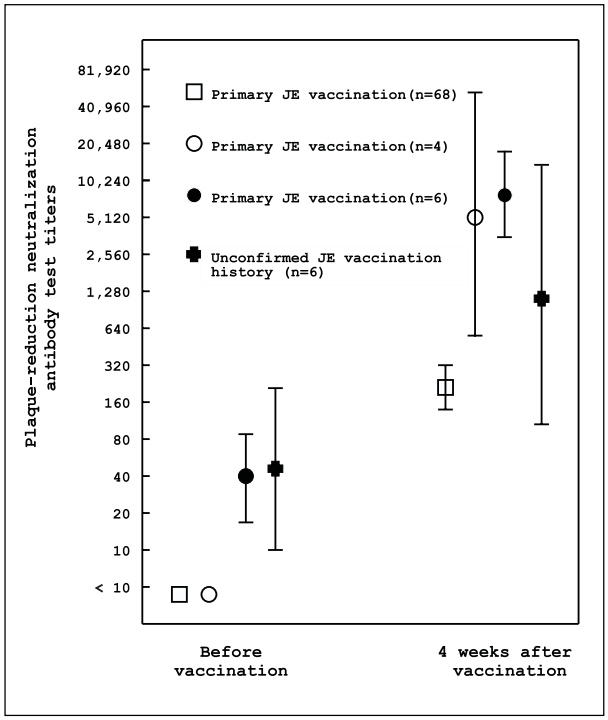

Figure 5. Primary or booster neutralizing antibody responses to one dose of Japanese encephalitis SA14-14-2 vaccine in South Korean children, 1997 (26).

The national immunization program against JE in children should be continued according to established schedules in South Korea; however, the booster schedule should be adjusted. Surveillance for JE and vaccine adverse reactions should be strengthened to better assess the number of cases and reactions associated with immunization. A new, more advanced vaccine, such as the live attenuated SA14-14-2 vaccine, should be adopted and integrated into the pediatric immunization schedule.

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Dr. Sohn is professor of pediatric infectious disease at Yonsei University and a member of the Advisory Committee of the National Immunization Program. He was a member of a working group for the improvement of the national immunization program and of an ad hoc committee for assessing JE vaccine adverse effects. He also participated in the clinical trial of SA14-14-2 vaccine in South Korea.

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Coccidioidomycosis in New York State

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Coccidioidomycosis, a systemic fungal disease caused by *Coccidioides immitis,* is endemic in the southwestern United States and in parts of Mexico and Central and South America. Only sporadic cases have been reported in areas (including New York) where the disease is not endemic. We used hospital discharge records and state mycology laboratory data to investigate the characteristics of *C. immitis* infections among New York State residents. From 1992 to 1997, 161 persons had hospital discharge diagnoses of coccidioidomycosis (ICD9 Code 114.0 - 114.5, 114.9). From 1989 to 1997, 49 cultures from patients were confirmed as *C. immitis*; 26 of these patients had traveled to disease-endemic areas. Fourteen of 16 isolates had multilocus genotypes similar to those of Arizona isolates, which corroborates the travel-related acquisition of the disease. Our results indicate that coccidioidomycosis may be more common in New York residents than previously recognized. Increased awareness among health-care providers should improve timely diagnosis of coccidioidomycosis and prevention of associated illnesses and deaths among patients in nondisease-endemic areas.

Coccidioidomycosis, a systemic disease caused by the dimorphic fungus *Coccidioides immitis,* is endemic in the southwestern United States and parts of Mexico and in Central and South America (1,2). The incidence of this systemic mycosis in disease-endemic areas increased during the 1990s (3-5). An estimated 100,000 infections occur in the United States annually, and 1 in 200 infections progresses to disseminated disease (1,6). Sporadic cases of coccidioidomycosis have been reported among visitors to the Southwest, and one earlier report recognized coccidioidomycosis as a serious travel hazard for visitors to that region (7-9).

C. immitis, a soil fungus, inhabits a unique ecologic niche in the topsoil of the lower Sonoran life zone (10). The infectious propagules are arthroconidia, single-cell fragments of mycelial threads, which become easily airborne to cause inhalation exposure. In the alveoli, arthroconidia undergo dimorphic transition to spherules, which fragment into endospores. When released from the spherule, each endospore can act as a new infectious unit in vivo (1). *C. immitis,* one of the most virulent and infectious fungal pathogens, poses a serious occupational hazard for laboratory personnel, especially in areas where the disease is not endemic and workers are less likely to practice biohazard safety level (BSL)-3 containment, which is required for the handling of this pathogen. The serious biohazard potential of *C. immitis* has led to its inclusion among the biological agents covered under the recently enacted Anti-Terrorist and Effective Death Penalty Act, which regulates interstate transport of infectious materials (11).

The extent and source of *C. immitis* infections have not been thoroughly investigated in areas where the disease is not endemic. In this study we summarize discharge data from persons with coccidioidomycosis hospitalized in New York, a nondisease-endemic area with a single coccidioidomycosis case report (7). In addition, we tested a proposed set of molecular markers for multilocus genotyping of *C. immitis* to determine the geographic origins of fungal isolates obtained in this study.

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The Study

Data on hospitalization for coccidioidomycosis in the state of New York from 1992 to 1997 were obtained from the New York Statewide Planning and Research Cooperative System, which compiles uniform inpatient data from all general acute-care hospitals (all public and private hospitals and hospital-based and free-standing ambulatory surgery facilities).

Patients were selected if their hospitalizations listed a primary or secondary discharge diagnosis of coccidioidomycosis (ICD9 114.0-114.5, 114.9) during 1992 to 1997. Information was collected from hospital discharge data abstracts and uniform billing forms for age at admission, sex, race, ethnicity, county of residence, insurance status, seasonality, disposition of patient, hospital length of stay, and total hospital cost.

Hospital medical records were requested from the 16 patients with positive *C. immitis* cultures available for multilocus genotyping (1994-1997). These records were reviewed for travel histories.

Laboratory Methods

All suspect *C. immitis* isolates were transported and processed with full safety precautions in a BSL-3 facility. The isolates were subcultured on modified Sabouraud's agar and Mycosel agar, morphologic and microscopic features were examined, and cultures were processed for specific nucleic acid detection. The AccuPROBE *C. immitis* culture identification test (Gen-Probe Inc., San Diego, CA) was used to confirm the fungal identity. Before 1993, most culture identifications were confirmed by using a *C. immitis* exoantigen test kit (Scott Laboratories Inc., Fiskeville, RI).

The fungal DNA was extracted from mycelial cultures according to the method of Pan et al. (12). PCR-RFLP was done as described by Burt et al. (13), with minor modifications. We studied five of the 10 loci originally used for each *C. immitis* isolate because analysis showed these loci to be most useful for strain differentiation (13). The loci/restriction enzymes used included a1/*BsrI*, bg/*DdeI*, bl/*DdeI*, bq/*HinFI,* and e1/*BsmI*. Accordingly, California isolates were expected to show polymorphism at e1, and Arizona isolates could be either positive or negative at each locus, while isolates from Texas would be polymorphic only for the a1, bq, and e1 loci.

Findings

Hospital discharge data from 1992 to 1997 in New York showed 181 hospitalizations with coccidioidomycosis as the primary or secondary discharge diagnoses (Table 1). The yearly distribution of hospitalizations was 30 (1992), 28 (1993), 30 (1994), 33 (1995), and 32 (1996), with no discernible seasonal pattern. Coccidioidomycosis was the primary diagnosis in 75 hospitalizations and the secondary diagnosis in 106. The clinical diagnoses according to ICD9 Codes included 105 hospitalizations for pulmonary coccidioidomycosis (114.0, 114.5), one with extrapulmonary coccidioidomycosis (114.1), five with coccidioidal meningitis (114.2), 18 with other forms of coocidioidomycosis (114.3), 19 with chronic pulmonary coccidioidomycosis (114.4), and 33 with unspecified coccidioidomycosis (114.9). The mean length of stay was 16 days, with an average hospital cost of $22,516.00.

One hundred sixty-one patients were hospitalized, of whom five had two hospitalizations, two had three, one had four, and one had nine (Table 1). Thirty-two (20%) patients had HIV, 16 patients (10%) had cancer, and 18 patients (11%) died while hospitalized. Overall,

Table 1. Coccidioidomycosis hospital discharge data, New York State, 1992–1997

Characteristic	N	(%)
Total hospitalizations	181	
Total patients	161	
Male	93	(58)
Female	68	(42)
Race		
White	128	(79)
Black	18	(11)
Asian/Pacific Islander	2	(1)
Other	9	(6)
Unknown	4	(3)
Ethnicity		
Hispanic	4	(2)
Non-hispanic	151	(94)
Unknown	6	(4)
Coccidioidomycosis diagnosis		
Primary	75	(41)
Secondary	106	(59)
Concurrent diagnosis		
HIV	32	(20)
Cancer	16	(10)
Died while in hospital	18	(11)
Mean length of stay (days)	16	
Age range (yrs)	0-93 (median 58)	
Mean hospital charge	$22,516.00	

120 (75%) of these 161 patients were either >54 years of age, had cancer, or were HIV infected.

Mean and median ages for HIV-infected patients were 36 and 33 years, compared with 66 and 64 years for cancer patients and 59 and 63 years for others. Further comparison of HIV-infected patients with patients who had neither cancer nor HIV infection showed differences in percentages with a primary diagnosis of coccidioidomycosis (22% vs 50%), male sex (81% vs 50%), black race (31% vs 5%), and death during hospitalization (31% vs 6%).

Forty-nine *C. immitis* cultures were identified at the state mycology laboratory from 1989 to 1997 (Figure).

Sixteen patient isolates were available for multilocus genotyping. The hospital records of these patients were reviewed to determine the likely source of infection. All had a history of travel to the Southwest, with 12 of 16 patients traveling to Arizona. However, date(s) of travel could not be correlated with onset of the disease. The typing of five alleles allowed unambiguous matches with Arizona genotypes for eight isolates from patients whose histories supported this conclusion (Table 2). It was also possible to type the respective *C. immitis* isolates to Arizona in five instances in which patients gave a history of travel to more than one disease-endemic area (four with travel to Arizona). The Arizona genotypes of three isolates (474-97, 639-97, and 779-97) did not match, as these patients had a history of travel to California, California and Mexico, and California, respectively.

Conclusions

Before 1994, the Centers for Disease Control and Prevention (CDC) definition for coccidioidomycosis relied solely on a physician's clinical diagnosis (5). The current Council of State and Territorial Epidemiologists/CDC surveillance case definition requires the presence of both clinically compatible symptoms and laboratory evidence of infection (5). The laboratory criteria include a positive culture, positive histopathologic results, molecular evidence of *C. immitis,* a positive serologic test, or a positive skin test. The retrospective nature of this study and our

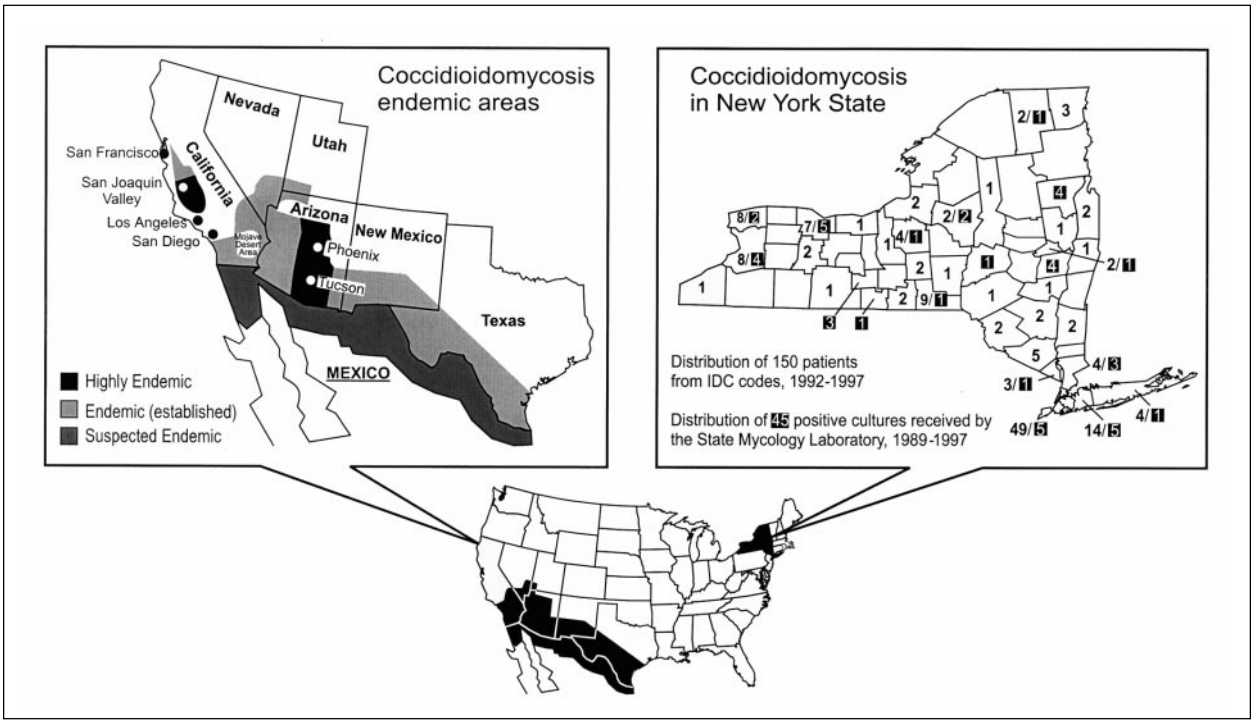

Figure. Coccidioidomycosis in New York, 1989–1997. The figure on the left highlights coccidioidomycosis-endemic areas in the United States (adapted from Kirkland TN, Fierer [2]). The figure on the right depicts New York countywide distribution of 150 out of 161 patients in the discharge records (1992–1997); the highlighted numbers show counties from which 45 of the 49 *Coccidioides immitis* cultures were referred to the state mycology laboratory (1989–1997).

Table 2. Correlation among clinical history, travel, and multilocus genotypes of *Coccidioides immitis* isolates from 16 patients, New York, 1994–1997

Isolate no.	Clinical diagnosis	Treatment[a] outcome	Travel history[b]	Multilocus[c] genotype	Typing pattern
104-94	-	-	AZ	01101	AZ
376-95	Pneumonia	Itra/discharged	AZ	11101	AZ
515-95	Immunocomp.[d]	Amp B, Itra/died	AZ/CA	11100	AZ
750-95	-	-	AZ	11101	AZ
131-96	Pneumonia	Flu/discharged	AZ/TX	11100	AZ
229-96	Immunocomp.	Amp B, Flu, Itra/died	AZ	11101	AZ
366-96	Pneumonia	No treatment/discharged	AZ	11100	AZ
819-96	Immunocomp.	Amp B, Itra/died	AZ/CA	11100	AZ
269-97	Lung mass	No treatment/discharged	AZ	11100	AZ
371-97	Immunocomp.	Amp B,Flu,Itra/discharged	AZ	11100	AZ
474-97	Hydrocephalus	Flu/discharged	CA	11101	AZ
588-97	Pneumonia	Amp B,Flu/discharged	AZ	11101	AZ
639-97	Lung mass	Thoracotomy/discharged	CA/Mexico	11101	AZ
686-97	-	-	Southwest	11100	AZ
779-97	Pneumonia	Itra/discharged	CA	11100	AZ
815-97	URI[e]	Flu/discharged	AZ/CA	11101	AZ

[a]Amp B, Amphotericin B; Flu, Fluconazole; Itra, Itraconazole.
[b]AZ, Arizona; CA, California; TX, Texas.
[c]The presence of polymorphism was scored as 1 and its absence as 0; every isolate was assigned a five-numeral genotype.
[d]Immunocomp, immunocompromised.
[e]URI, upper respiratory infection.

exclusive reliance on hospital discharge summary data precluded detailed evaluation of diagnostic criteria. Nevertheless, the average of 30 hospital discharges per year suggests that coccidioidomycosis may be more common in New York residents than previously recognized. The largest number of positive cases was reported from New York City, but the rate was not increased, and cases were reported throughout the state. Furthermore, while information on travel history was limited, all 16 patients from whom information was obtained had traveled to disease-endemic areas before becoming ill.

Immunocompromised persons, infants, and non-Caucasians are considered at increased risk for coccidioidomycosis (1). The mean age of patients in this series was 54 years, which is in agreement with >40-year age-group most commonly diagnosed in disease-endemic areas (5,6). As expected, a large proportion of patients had other diagnoses consistent with underlying immunosuppression, including a primary diagnosis of HIV in 20% and cancer in 10%. Overall, 75% were 55 years of age, had cancer, or were HIV infected. The availability of *C. immitis* cultures from only 10% of cases precluded any definitive investigations of geographic areas in which New Yorkers are at greater risk for infection.

The discovery of recombination in clinical isolates of *C. immitis* (14) has shown genetic evidence of two sexually distinct taxa in this pathogen, which was previously thought to reproduce asexually (15). A corollary of these investigations was the development of a multilocus genotyping scheme for determining the geographic origin of clinical isolates. Although the discharge data available for this study did not include travel information, we were able to delineate Arizona isolates in a subpopulation of our study for which cultures and medical record travel data were available. Thus, a patient's travel history was correlated with disease acquisition in a particular area by independently demonstrating the geographic pattern of the patient's isolate in 13 (81%) of 16 patients evaluated. However, the typing scheme could not determine the geographic origin of two *C. immitis* isolates, which came from patients thought to have traveled to both California and Mexico. This discrepancy may have resulted from the limited number of alleles used for typing, incomplete travel histories, shortcomings of the typing scheme for California isolates, or the possibility that the two isolates belonged to an outlier group of strains. Further testing is needed on a larger set of isolates from different geographic areas to evaluate this typing scheme.

As a result of this study, laboratories participating in the New York State Clinical Laboratory Evaluation Program (Mycology) were alerted about the hazards posed by *C. immitis*. A safe procedure for initial examination of suspect cultures was recommended. The laboratories were also provided with guidelines for safe transport of *C. immitis* to the state mycology laboratory for confirmation and characterization.

The diagnosis and clinical management of coccidioidomycosis in areas where the disease is not endemic pose unique challenges. The clinical symptoms of the disease mimic those of other infectious diseases, which may result in misdiagnosis and inappropriate treatment (1,16). Additionally, *C. immitis* cultures could be easily confused with a number of nonpathogenic fungi by unsuspecting laboratory personnel, who are at risk for infection. Increased awareness among health-care providers is likely to help in the timely diagnosis of coccidioidomycosis and the prevention of associated illness and death among patients in nondisease-endemic areas.

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Dengue Surveillance in Florida, 1997–98

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Recent dengue outbreaks in the Caribbean and Central and South America and the presence of competent mosquito vectors increase the likelihood of future autochthonous transmission in Florida. During April 1997 to March 1998, a laboratory-based active surveillance program detected 18 cases of dengue involving all four dengue serotypes. All patients reported recent travel to countries with indigenous dengue transmission. These results demonstrate that dengue infections are imported into Florida at a much higher rate than reflected by previous passive surveillance; therefore, the risk for local dengue transmission may be increasing.

Dengue is a mosquito-borne viral disease caused by one of four antigenically distinct dengue flaviviruses: DEN-1, DEN-2, DEN-3, and DEN-4. Primary infection with any serotype may lead to acute illness defined as fever with two or more of the following symptoms: headache, retroorbital pain, myalgia, arthralgia, rash, and hemorrhagic manifestations (1,2). Fever and other symptoms may subside after 3 or 4 days, and the patient may recover completely, or the fever may return with a rash within 1 to 3 days (3). Secondary exposure to the same serotype generally does not produce illness because of pre-existing antibodies. However, secondary exposure to a different serotype may lead to another dengue fever episode, and the patient may be at risk for more serious forms of infection, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (3). Dengue virus infection may also cause a nonspecific febrile illness that can be easily confused with measles or influenza. Therefore, laboratory testing is essential to clinical diagnosis and public health reporting.

Dengue-viremic persons are usually infectious to the mosquito vector 1 day before the onset of the febrile period and remain so for 6-7 days. When a mosquito ingests virus in a blood meal, the virus replicates during an extrinsic incubation period of 8 to 12 days, after which the mosquito remains infective for life (4,5). The life span of *Aedes aegypti*, the primary vector of dengue in the Americas, is usually 21 days, although life span and incubation periods depend on temperature and rainfall (6). Both *A. aegypti* and *A. albopictus,* a recently introduced vector species (7), have been found throughout Florida, and *A. aegypti* breeds year-round in south Florida (8).

The last dengue epidemics in Florida, in the Tampa and Miami areas in 1934-35 (9,10), affected an estimated 15,000 of the 135,000 population of Miami. The last recorded epidemic in the southeastern states was in Louisiana in 1945 (11). Most cases of dengue reported in the United States since the 1940s have been imported; however, indigenous transmission of dengue occurred in Texas in 1986 and 1995 (8,12-14). In response to an outbreak of dengue in Mexico in 1995, the Texas Department of Health initiated an active surveillance program that detected 29 confirmed cases, including seven in persons with no recent history of travel outside Texas (14,15). Thirteen imported dengue cases (0 to 4 cases per year) were reported in Florida from 1985 to 1995 (16).

The recent introduction of DEN-3 in Mexico and Central America is of public health importance because most of the population in the tropical Americas is susceptible to infection with this serotype (17,18). The presence of the vector, the rapid spread of the virus, and increased air travel and immigration contribute to the possibility of future dengue transmission in the

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continental United States (19-21). A serosurvey conducted after the first confirmed dengue outbreak in Peru in 1990 clearly demonstrated earlier undetected dengue transmission (22). Silent transmission of dengue was also demonstrated in 1992 in an area of Taiwan believed free of the disease (23). In both cases, an early warning system based on immunoglobulin (Ig)M antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) laboratory tests was recommended for disease monitoring. Active surveillance, an essential component of an early warning system for detection of dengue, provides information vital to defining epidemiologic aspects of cases and enabling educational and mosquito control efforts (24-27).

Recent outbreaks of dengue in nearby Caribbean and Central and South American countries may increase the likelihood of future autochthonous transmission in Florida (15). Mosquito vectors are widely distributed in the state, and travelers returning from dengue-endemic areas place at risk the resident population, which has minimal (if any) immunity to dengue viruses. Because physicians' awareness of dengue is low and specialized laboratory diagnostic methods are not available locally, low-level dengue transmission may go undetected. Imported dengue may thus be underreported in Florida, which has relied on passive surveillance. We used an educational campaign for county epidemiologists and health-care providers and an active laboratory-based surveillance program that facilitated prompt, accurate diagnosis of dengue to assess the risk for local dengue fever transmission in Florida.

The Study

The first phase of the surveillance program was the design of a dengue information packet for all 67 county health department epidemiologists in Florida, to be distributed to hospital emergency rooms, clinics, health departments, and infectious disease physicians in the county. The letter included information on case reporting, the dengue case definition, specimen requirements and transport instructions, and a dengue case investigation form.

Under cooperative agreements with two Florida commercial clinical laboratories (national reference clinical laboratories), specimens from patients with suspected dengue were forwarded to the state laboratory for free testing. In cases where specimens were tested at commercial laboratories only, dengue antibody-positive results were forwarded to county health departments and to the state laboratory for inclusion in this study. In Florida, dengue testing is offered only by the state laboratory and some commercial clinical laboratories.

Before this study, the hemagglutination inhibition (HAI) assay was the only serologic test for dengue offered at the state laboratory. Laboratory capabilities were enhanced to include testing for IgM antibodies to dengue. Acute- and convalescent-phase serum specimens were tested for dengue antibodies by both HAI assay and MAC-ELISA, using a DEN1-4 serotype cocktail (28-30). Available specimens positive for IgM antibodies to dengue, tested at the Florida state laboratory, were forwarded to CDC's Dengue Branch laboratory for virus isolation, serotyping, and confirmation of serologic results.

Cases were classified as DHF if all the following were present: fever, hemorrhagic tendencies, thrombocytopenia (100,000/mm^3 or less), and evidence of plasma leakage (hematocrit level increased by $\geq$ 20%) or other objective evidence of increased capillary permeability (31). If all the above were present, plus hypotension or pulse pressure $\leq$ 20 mm Hg, the case was classified as DSS.

In this study, a case was classified as presumptive dengue on the basis of serologic evidence of an HAI titer $\geq$ 1:1280, an equivalent IgG titer, or a positive dengue IgM antibody test on a single serum sample. A confirmed dengue case required a fourfold rise in HAI, IgG, or IgM antibody titers between acute- and convalescent-phase serum specimens; isolation of virus; or detection of viral antigen by immunohistochemistry, immunofluorescence, or viral nucleic acid detection. Confirmed or presumptive dengue cases are referred to as laboratory-diagnosed cases.

A case was classified as undetermined if sufficient information was not available on the timing of specimen collection in relation to onset of symptoms or a convalescent-phase serum was not available to demonstrate a fourfold rise in antibody titers. A case was also considered undetermined if the acute-phase serum was negative for antibodies and a convalescent-phase serum was not available.

Epidemiologic data were obtained from dengue case investigation forms that accompanied the patients' specimens. Suspected as well

as confirmed cases of dengue are reportable in Florida (32). County health departments were notified of suspected cases, and a convalescent-phase serum was requested.

We used the Epi Info Software package for data analysis (33). Comparisons were made with historical data on reported cases of dengue (9,16,34-36).

From April 1, 1997, to March 31, 1998, 83 suspected cases of dengue were studied. Commercial clinical laboratories referred specimens for analysis for 36 (43%) of these cases. The rest were referred through county health departments, hospital laboratories, infection control practitioners, or physicians. Recent dengue infection was laboratory diagnosed in 18 (22%) of these cases. Twelve (67%) of the 18 confirmed dengue specimens were referred by commercial clinical laboratories. Virus isolation or polymerase chain reaction of five cases yielded all four dengue serotypes. Dengue was ruled out as the etiologic agent in 24 (29%) cases. The remaining 41 (49%) cases were undetermined because convalescent-phase serum samples were not available (Table 1).

Table 1. Characteristics of 83 suspected cases of dengue investigated in Florida, April 1997–March 1998

Characteristic	Yes	No	Undetermined
History of recent travel to to dengue-endemic area	41 (49%)	14 (17%)	28 (34%)
Fits dengue fever case definition	30 (36%)	8 (10%)	45 (54%)
Flavivirus antibody detected	41 (49%)	42 (51%)	
Convalescent-phase specimen provided	25 (30%)	58 (70%)	
Laboratory confirmation of recent dengue infection	18 (22%)	24 (29%)	41 (49%)

Most (65%) of suspected-dengue patients were male (chi-square goodness of fit test p value = 0.006). Among suspected cases, the mean age was 41 years (1 day to 79 years). Forty-one (49%) initially tested positive for anti-flavivirus antibodies. Convalescent-phase serum was obtained in 25 (30%) of the cases. The average age of patients with confirmed dengue cases was 37 years (8 to 69); 14 (78%) of the 18 patients were male.

Laboratory-diagnosed cases were identified from five counties in central and extreme southeastern Florida (Figure 1). Cases were confirmed in persons residing in the following counties: Dade (8), Hillsborough (4), Orange (3), Palm Beach (2), and Broward (1). Table 2 lists Florida counties with laboratory-diagnosed dengue cases, case travel history, and dengue virus serotypes detected. All 18 laboratory-diagnosed dengue cases were in persons who had

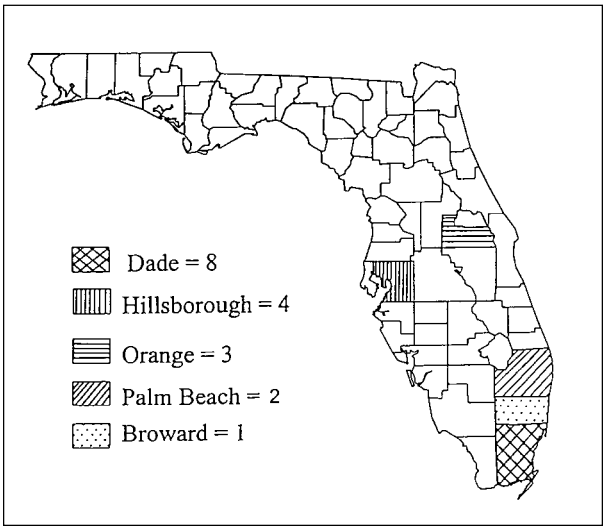

Figure 1. County of residence for 18 laboratory-diagnosed dengue cases detected between April 1997–March 1998.

Table 2. Laboratory-diagnosed dengue cases in Florida by county, area of travel, and serotype, April 1997 and March 1998

County	Area of travel – number of cases (dengue serotype) (n = 18)
Broward	Barbados – 1
Dade	Colombia – 1
	Haiti – 3
	Puerto Rico – 1 (DEN-2)
	Venezuela – 2 (DEN-1)
	Unknown – 1
Hillsborough	Colombia – 1 (DEN-2)
	Nicaragua – 1 (DEN-3)
	Thailand – 1
	Unknown – 1
Orange	Haiti – 2 (DEN-4)
	Puerto Rico – 1
Palm Beach	Haiti – 1
	Puerto Rico – 1

recently traveled to dengue-endemic areas and were therefore classified as imported. We included out-of-state cases in our analysis because the acute phase of their illness occurred while they were in Florida. Current county health department policy dictates that only cases in Florida residents are reported to the state epidemiologist for recording in the weekly and yearly morbidity statistics. Other case reports are forwarded to the county and state of primary residence of the patient.

Hemorrhagic manifestations were reported in 7 (39%) of the 18 confirmed cases; one met the DHF case definition; however, it was not possible to classify the remaining six cases with hemorrhage because information on hemoconcentration and plasma leakage was incomplete. Encephalopathy was present in one case. Antibody titers suggested secondary dengue infections in 10 (56%) of the 18 cases. Only 2 (11%) of the 18 cases appeared to involve primary infections. Laboratory tests necessary to determine infection status (primary vs. secondary) were not available in the other six cases. A woman with acute secondary dengue infection with hemorrhagic manifestations gave birth to a healthy uninfected baby.

Conclusions

During the year of active surveillance, 18 laboratory-diagnosed cases of dengue were detected. On the basis of the previous 10-year mean of 1.3 cases per year (Figure 2), the probability of detecting 18 cases was virtually 0% (Poisson distribution rare event vs. standard test). These cases were identified in Florida counties with high rates of international travel and large immigrant populations, as well as year-round breeding of *A. albopictus* and *A. aegypti* mosquitoes. According to Florida Department of Commerce statistics, of the 6 million international visitors to Florida in 1995, 38.4% traveled from South and Central America, the Caribbean, Mexico, Asia, and other tropical areas (37) in which dengue is endemic.

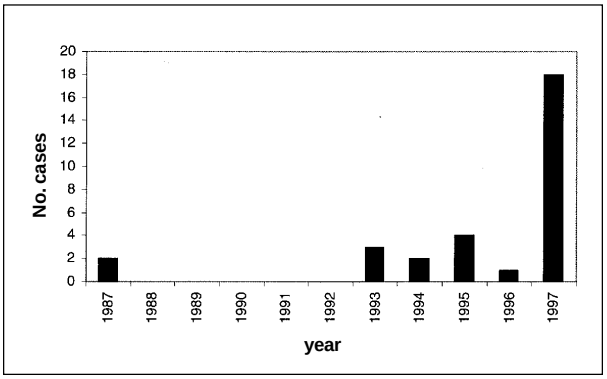

Figure 2. Reported dengue cases in Florida, 1987–1997.
(*1997 = study year April 1, 1997–March 31, 1998)

All four dengue serotypes were detected in five specimens during this study. Improved specimen handling should increase the rate of virus isolation. The serotype of the infecting dengue virus was identified in only five cases for the entire United States in 1995, when 79 laboratory-diagnosed dengue cases were documented (12). In the same year, 22 imported and seven indigenous cases were detected in Texas (15). In 1996, the infecting dengue serotype was identified in 5 of the 43 laboratory-diagnosed cases of imported dengue in the United States (three cases of DEN-1 and two of DEN-2) (38).

This study found multiple problems with routine clinical laboratory confirmation and follow-up of dengue infections: Tests requested by physicians and performed at clinical laboratories were not always optimal for identifying a current dengue infection. Even though the dengue IgM test is the most appropriate assay for determining current infection, it is not routinely performed at commercial laboratories and may not be readily available if requested. Test results are frequently misinterpreted, e.g., a single positive indirect fluorescent antibody test performed at a commercial laboratory may be interpreted as positive for current dengue infection when it only indicates infection with a flavivirus (e.g., dengue, St. Louis encephalitis, Japanese encephalitis) or vaccination (e.g., yellow fever) at an undetermined time in the past. In addition, cases are rarely investigated, and the convalescent-phase serum samples needed for confirmation are rarely requested. When an investigation indicates need for further testing, specimens may have already been discarded. Finally, positive test results are often not forwarded to the county and state epidemiologists in a timely manner. In cases tested only at commercial laboratories, delays of 2 to 4 months before positive cases were reported to the state Bureau of Epidemiology preclude prompt follow-up.

Three of the confirmed dengue cases in this study tested at commercial laboratories had not

been reported to the state epidemiologist by the county health departments because the patients were primary residents of other states, although they became ill while in Florida.

This study indicates that surveillance efforts should be concentrated in densely populated counties with large numbers of international travelers (Dade, Palm Beach, Orange, and Hillsborough), especially during dengue season in the Caribbean (July to November). As a part of the epidemiologic investigation of imported dengue cases, an attempt should be made to identify secondary cases.

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Norwalk-Like Calicivirus Genes in Farm Animals

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Viruses closely related to Norwalk-like viruses (NLVs) were recently found in stored stool samples from two calves (United Kingdom and Germany) and four pigs (Japan), sparking discussions about the potential for zoonotic transmission. To investigate if NLVs are commonly present in farm animals, pooled stool samples from 100 pig farms, 48 chicken farms, 43 dairy cow herds, and 75 veal calf farms from the Netherlands were assayed by reverse transcription-polymerase chain reaction amplification, using primers specific for the detection of NLVs from humans. NLV RNA was detected in 33 (44%) of the specimens from veal calf farms and two (2%) specimens from pig farms. Our data show that NLV infections—until recently thought to be restricted to humans—occur often in calves and sometimes in pigs. While zoonotic transmission has not been proven, these findings suggest that calves and pigs may be reservoir hosts of NLVs.

Caliciviruses infect animals and humans (1). Within the family *Caliciviridae*, four genera have been distinguished: vesivirus, lagovirus, Norwalk-like viruses (NLV), and Sapporo-like viruses (SLV) (2). The genera vesivirus and lagovirus contain a broad range of animal caliciviruses, but viruses in the NLV and SLV genera until recently have been found only in humans. In recent years NLVs, also known as small round-structured viruses, have emerged as a common cause of infectious gastroenteritis in all age groups and the main cause of outbreaks of gastroenteritis in restaurants and institutions such as nursing homes and hospitals (3-5).

Genetically, the human caliciviruses separate into at least three genetic clusters, called genogroups (GG), i.e., GGI and GGII NLV and the GGIII SLV (6). Each of these genogroups comprises genomically and antigenically diverse strains (7,8). This high degree of diversity results in at least 13 distinct genotypes for the NLVs (3,5,8-10). Many types of NLV cocirculate in the general population, causing sporadic cases and outbreaks. However, occasionally epidemics occur in which most outbreaks are caused by a single genotype (e.g., Lordsdale-like virus in the Netherlands in 1996) (5,11). Hypotheses for the mechanisms behind the emergence of epidemic types range from large-scale foodborne transmission of a single strain to introduction from a nonhuman reservoir. An indication for the latter was a recent report from Japan of the finding of NLV-like sequences in stool specimens from pigs (12). Enteric caliciviruses have also been detected in other animal species (calves, dogs, and chickens) (13-16). However, except for canine calicivirus (17), these viruses lack definitive sequence evidence linking them to the *Caliciviridae* (18). Molecular characterization of three calicivirus strains from cattle has recently been reported. The first virus (Tillamook virus; BCV-Bos1), described by Neill et al. (19), caused respiratory symptoms and was phylogenetically related to San Miguel sea lion virus and vesicular exanthema of swine virus, both within the genus vesivirus. Viruses in this genus have a broader host range (20). Two other viruses closely related to GGI NLVs (Jena virus 117/80 and Newbury agent type 2) were detected in feces from newborn calves with diarrhea (21,22). The

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similarity of the porcine and bovine sequences with those of NLVs found in humans suggests that a reservoir for these human pathogens may exist in farm animals.

We studied enteric caliciviruses in recently collected fecal samples from cattle, chicken, and swine in the Netherlands by using polymerase chain reaction (PCR) assays specific for NLVs found in humans. In addition, we analyzed (by sequence analysis) the genetic variation of detected calicivirus strains and compared it with that of prototype GGI and GGII NLV strains.

Materials and Methods

Fecal Specimens

Stool specimens from animals were collected at farms in the Netherlands as part of ongoing surveillance for potential zoonotic microorganisms associated with gastroenteritis in humans. From October 10, 1998, to April 21, 1999, samples were collected from 3- to 9-month-old fattening pigs at 100 pig farms of 22 to 1600 pigs. From January 1 to April 1, 1998, stool samples were collected from 75 veal calf farms of 38 to 930 calves and 43 dairy cattle herds of 25 to 180 cows. The age of the calves was 1 to 52 weeks (average 12 weeks), and of dairy cattle herds 4 to 6 years. From January 6 to April 21, 1998, samples were collected from ≤ 6-week-old chickens at 48 broiler farms, with 5,000 to 70,000 per farm.

Sampling

The sampling strategy allowed monitoring for pathogens in a large number of animals and detection of microorganisms at farm level with a prevalence of 5% and 95% confidence (23). Fecal samples from calves, pigs, and chickens (20 to 60 per farm) were collected from animals housed in one randomly chosen farm building. Dairy cattle of one farm were regarded as a single herd, and stool samples were collected from randomly selected cows. Until tested, fecal samples were stored at -70°C in 15 g/L of Trypton Soya broth (TSB) (Oxoid CM 129) and 10% glycerol.

Molecular Detection of NLVs by RT-PCR

For extraction of viral RNA, stool samples were resuspended in HBBS Hanks (Gibco BRL, Breda, Netherlands) to a final concentration of approximately 10%. These suspensions were centrifuged at 3,000 x g for 20 minutes, and 100 µl was used for RNA extraction by addition of a high-molarity solution of guanidinium isothiocyanate (GuSCN). Bound RNA was washed and eluted as previously described (24). To reduce the risk for contamination, specimens from different species were analyzed separately, and one negative control sample was included for every two stool specimens. A human stool sample positive for NLV by electron microscopy was included as positive control.

We used a single-round reverse transcription (RT)-PCR assay with a broadly reactive primer pair, which had been developed for the detection of NLVs in stool specimens from humans (5). For RT, 5 µl RNA was mixed with 4 µl 50 pmol JV13 primer. The solution was heated to 94°C for 2 minutes, cooled, and 6 µl RT buffer was added. The RT reaction was performed in a final volume of 15 µl, consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 1 mM each of deoxynucleoside triphosphates (dNTPs), and 5 units of avian myeloblastoma virus RT (Boehringer Mannheim, Almere, Netherlands). The mixture was incubated for 1 hour at 42°C, heated for 5 minutes at 94°C to denature the enzyme, and then cooled. Five µl of the RT mixture was added to the PCR mix, containing 10 mM Tris-HCl (pH 9.2), 75 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 2.5 units AmpliTaq (Perkin Elmer, Nieuwerkerk a/d IJssel, Netherlands), and 15 pmol primer JV12. Mineral oil was added, and 40 amplification cycles, 1 minute at 94°C, 1.5 minute at 37°C, and 1 minute at 74°C each, were performed. The amplification products were analyzed by 2% agarose gel electrophoresis and visualized with UV after ethidium bromide staining.

For Southern blots, the RT-PCR products in the agarose gel were denatured by incubating in 0.5 M NaOH for 30 minutes and transferred to a positively charged nylon membrane (Boehringer, Almere, Netherlands) by vacuum blotting (Millipore, Etten-Leur, Netherlands). Hybridization of NLV RT-PCR products was performed as described previously (5).

Sequence and Phylogenetic Analysis

The NLV RT-PCR products of expected size (326 bp) and 21 products of smaller size of the calf herd samples were excised from a 2% agarose gel and purified with a Qiaquick gel extraction kit (Qiagen, Hilden, Germany). Purified RT-PCR products were sequenced with the BigDye Terminator Cycle Sequencing Ready Reaction

Kit (Perkin Elmer Applied Biosystems, Foster City, CA) by using PCR primers. Nucleotide sequences were edited by using SeqEd (V1.03, Applied Biosystems), aligned by Geneworks (V2.5, Intelligenetics, Mountain View, CA), and imported into the Treecon software package (25). The confidence values of the internal nodes were calculated by performing 100 bootstrap analyses. Evolutionary trees for nucleotide sequences were drawn by using the Neighbor-joining method.

Electron Microscopy

Electron microscopy was performed as recommended by Flewett (26) and Doane and Anderson (27), with model Philips 400T (Philips, Eindhoven, Netherlands) at 80 kV. Identification of virus particles was based on morphologic criteria, i.e., the size and characteristic surface morphology (28).

Results

Twenty-five (33%) of 75 veal calf farm samples and 2 (2.0%) of 100 pig farm samples showed visible products of the expected size (326 bp). The gel electrophoresis and hybridization results are shown for eight strains (Figure 1). Hybridization with a set of probes used for detection of NLVs in humans yielded no positive reactions (Figure 1B); therefore, 46 products (25 of the expected size and 21 of a smaller size [Figure 1A, lane 7]) were sequenced. All 25 RT-PCR products of the expected size contained the GLPSG amino acid motif characteristic of viral RNA polymerases (29). The 21 products of smaller size revealed no viral sequences. The NLV-RT-PCR was negative for all pooled specimens from 43 dairy herds and 48 chicken farms.

By phylogenetic analysis, all calf calicivirus sequences formed a tight cluster closely related to NLVs from GGI with the highest similarity with the Newbury calf calicivirus (Figure 2) (22). Nucleotide sequence identities between the calf NLVs and GGI NLVs were 63% to 70% (75% to 77% amino acids). The swine virus sequences clustered as a separate lineage within GGII NLVs, with 69% to 71% nucleotide and 79% to 83% amino acid identity. In addition, the swine sequences strongly resemble (94% nucleotides, 100% amino acids) the swine calicivirus sequences from Japan. Partial polymerase sequences of Bo/NLV/176/1998/NET and Sw/NLV/34/1998/NET have been assigned GenBank accession numbers AF194183 and AF194184, respectively.

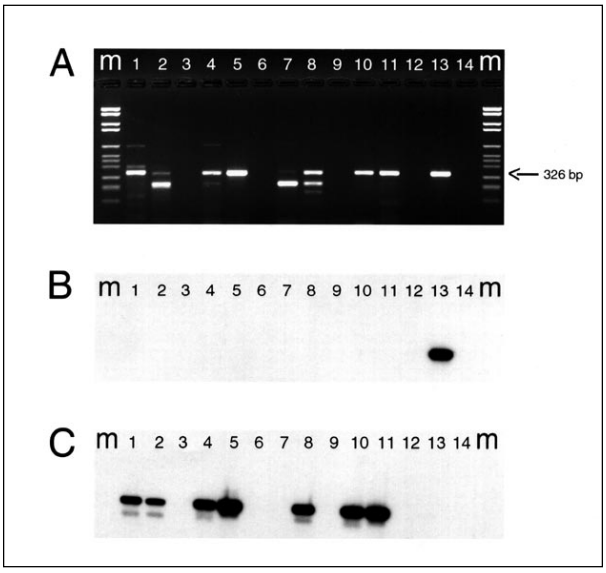

Figure 1. Results of ethidium bromide staining (panel A) and corresponding Southern hybridization (panels B and C) of RT-PCR products of eight calf herd (CH) samples (A) M= molecular mass marker, lane 1: CH124; lane 2: CH125; lane 3: water; lane 4: CH126; lane 5: CH138; lane 6: water; lane 7: CH139; lane 8: CH145; lane 9: water; lane 10: CH156; lane 11: CH176; lane 12: water; lane 13: human NLV positive control (5); lane 14: water. For Southern blot hybridizations, a set of probes used to detect NLVs in humans (B) and calves (C).

Since the calf and pig strains did not hybridize to the probe mixture for detection of NLVs from humans, specific probes were developed based on the consensus sequence of RT-PCR products of 10 calf herd samples and on the pig farm samples, respectively. The probe sequences were: RH1(calf) (5'-GGATGTGGTGCAGGCAA AC-3') and RH2(pig) (5'-TCCGCATCTCTATCGT GG-3'). Hybridizations with the calf probe (RH1) confirmed all RT-PCR-positive calf samples after 15 minutes exposure to an ECL hyperfilm (Figure 1C). Overnight exposure to ECL hyperfilm revealed eight more positives, for 33 (44%) positive calf farm samples.

All veal calf farm samples were screened by electron microscopy for viruses (Table). Particles with NLV morphologic features were found in only one specimen (Figure 3), in which the presence of calicivirus sequence was confirmed by RT-PCR and hybridization (Table).

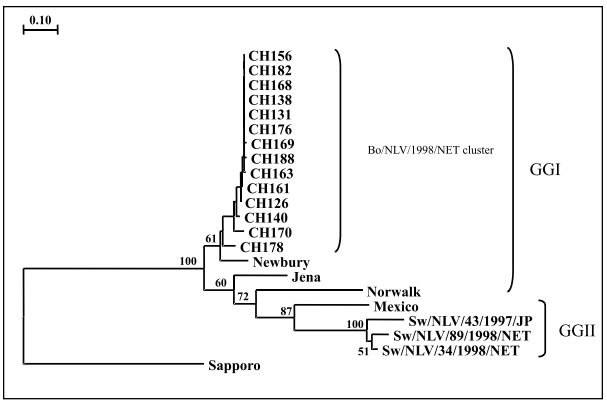

Figure 2. Phylogenetic relationships among human and animal NLVs clustering in genogroup (GG) I and II, based on a 145-bp nucleotide sequence within RNA polymerase gene. Dendrogram includes 14 calf herd (CH) sequences from the bovine NET/98 cluster (CH138, CH156, CH131, CH168, CH182, CH176 [Bo/NLV/176/1998/NET], CH163, CH188, CH169, CH161, CH126, CH140, CH170, and CH178 described in this study), and sequences from Norwalk virus (NV), Mexico virus (MX), Newbury agent (NA), Jena virus (JV), Sw/NLV/43/1997/JP, and the swine sequences described in this study: Sw/NLV/34/1998/NET and Sw/NLV/89/1998/NET. The SLV strain Sapporo (SAP) was included for comparison. Sequences of prototype strains of NLV were obtained from GenBank (Accession numbers: NV [M87661], MX [U22498], Newbury agent [AF097917], Jena virus [AJ011099], Sw/NLV/43/1997/JP [AB009412] and SAP [S77903]). Bootstrap values for each node are given if >50%.

Table. Viruses and virus particles detected by electron microscopy and corresponding Norwalk-like virus (NLV) RT-PCR results (including hybridization with a calf-NLV specific probe) in 16 of the 75 calf herd samples

Sample	RT-PCR[a] results	EM results
CH121[b]	Neg	Adenovirus
CH127	Neg	Parvovirus-like (21 nm) Flavivirus-like particles (34-44 nm)
CH128	Neg	Parvovirus-like (19 nm) Rotavirus
CH141	Neg	BDV-like[c] enveloped spherical particles (47 nm)
CH145	Neg	Parvovirus-like (21 nm)
CH153	Pos	Rotavirus
CH155	Neg	Rotavirus
CH156	Pos	Parvovirus-like (25-27 nm)
CH161	Pos	Rotavirus
CH168	Pos	Coronavirus-like particles
CH172	Neg	Parvovirus-like (21 nm)
CH176	Pos	SRSV[d]
CH178	Pos	Rotavirus
CH185	Pos	Rotavirus
CH186	Neg	Rotavirus
CH187	Neg	Coronavirus-like particles

[a]RT-PCR = reverse transcription-polymerase chain reaction; [b]CH = calf herd; [c]BDV-like: bovine diarrhea viruslike.; [d]SRSV: small round-structured virus or NLV.

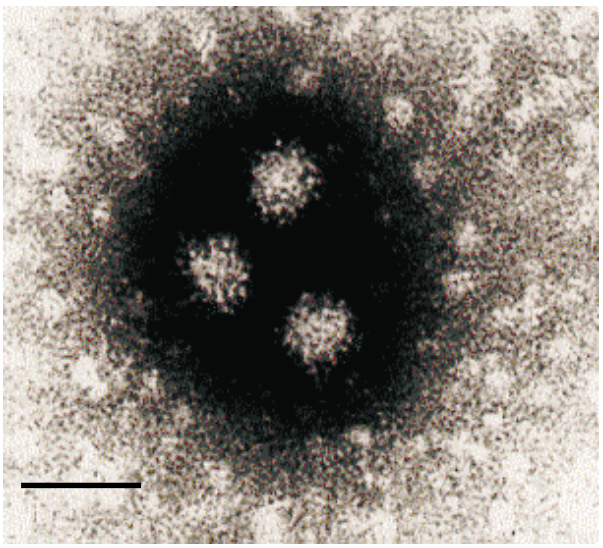

Figure 3. Electron microscopy showing NLV particles in a calf herd sample (CH176), negatively stained with 2% K-PTA, pH 7.0. Bar = 50nm.

Conclusions

Until recently, humans were considered the sole host of NLVs. Recent studies in Japan and the United Kingdom, however, demonstrated calicivirus sequences in the caecum of pigs and in stored calf stool samples containing calicivirus-like particles by electron microscopy (12,21,22). Molecular characterization of calf enteric caliciviruses, named Newbury agent (22) and Jena virus (21), revealed that they were genetically related and more closely associated with GGI NLVs than with any other known calicivirus. In this study, we detected NLV nucleotide sequences in 33 (44%) of 75 of the pooled samples from veal calf farms.

Phylogenetic analysis of the detected sequences showed that they formed a tight cluster with the Newbury sequence (22) and were closely related to NLVs from GGI. Bridger et al. (30) demonstrated that calves can be infected with Newbury calicivirus. The detection of Newbury-like NLVs in pooled veal calf samples of 33 farms from different regions suggests that the species is a normal NLV host. Similarly, the close genetic relationship between calicivirus sequences detected in the pig samples in our study and those from Japan suggests that these viruses commonly infect pigs. The close similarity of porcine and bovine sequences to the NLVs infecting humans indicates the possibility of an animal reservoir for human infection.

However, these findings by no means prove zoonotic transmission. Genetically related viruses are commonly found in different species without resulting in apparent widespread interspecies transmission (e.g., rotavirus). On the other hand, zoonotic transmission cannot be excluded. The genetic distances between the animal and human NLVs are similar to the distances between the GGI and GGII strains, and epidemic spread of some NLV strains (5) led to the widespread—possibly global—emergence of a single predominant strain in the human population in 1995-96 (11). This epidemiologic observation resembles early descriptions of the vesicular exanthema of swine virus (VESV) epidemics (31). These viruses, belonging to another genus within the *Caliciviridae*, the vesiviruses, are known for their broad host range (20). The occasional epidemic spread of VESV was later linked to introduction of caliciviruses from an ocean reservoir (20). Therefore, interspecies transmission of NLVs is possible; the occasional widespread NLV epidemics caused by a single strain may result from introductions of new NLV strains from an animal reservoir.

Reynolds et al. reported electron microscopy detection of caliciviruses in calf diarrhea outbreaks (32), and the bovine caliciviruses Newbury agent (22) and Jena virus (21) were pathogenic for calves under experimental conditions and in field studies (33). In our study, we did not record disease or death on the surveyed farms and therefore cannot determine whether these caliciviruses were associated with disease. Despite repeated attempts, experimental infection of different animal species with the prototype Norwalk virus has not been successful, with the exception of infection in chimpanzees (3). Our findings may lead to the development of an animal model for the NLVs of humans; for example, a pig model would enable studies of (mucosal) immunity following NLV infection (1).

The low prevalence of Norwalk-like calicivirus in swine farms (2 per 100) is in agreement with the findings reported from Japan (12). Electron microscopy confirmed the calicivirus origin of the detected NLV sequences in only one calf sample. This low number of positives by electron microscopy was not surprising, given the greater sensitivity of RT-PCR and the use of pooled specimens for screening. NLVs in humans typically are shed at relatively low levels.

The absence of NLV sequences in all 43 dairy herd specimens may be explained by use of an inappropriate primer pair, which was optimized for detection of NLVs of humans (4) or by an acquired strain-specific immunity in older animals that prevents repeated infections. A third possibility is that levels of shedding in adult animals are very low, precluding virus detection in pooled specimens. The swine farm samples were all from fattening pigs at least 3 months old. We may have found a higher prevalence in recently weaned pigs, as the prevalence of other enteric pathogens is highest in young piglets (34).

Our findings raise important questions about the host range of NLVs. It is unclear if animal NLVs form genetically distinct stable lineages or are part of a common pool of viruses circulating between animals and humans. If calves or swine indeed are reservoirs, the prevalence of calicivirus should be determined in both healthy and diseased cattle and swine and methods to detect such interspecies transmissions at an early stage should be developed, in collaborative research by public health and veterinary sciences.

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Molecular Genetic Evidence of a Novel Morbillivirus in a Long-Finned Pilot Whale (*Globicephalus melas*)

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A long-finned pilot whale with morbilliviral disease was stranded in New Jersey. An immunohistochemical stain demonstrated morbilliviral antigen. Reverse transcriptase-polymerase chain reaction for morbillivirus P and N genes was positive. Novel sequences most closely related to, but distinct from, those of dolphin and porpoise morbilliviruses suggest that this virus may represent a third member of the cetacean morbillivirus group.

In the last 12 years, newly recognized members of the morbillivirus family have caused many deaths among marine mammals, specifically cetaceans and pinnipeds (1). The first recognized marine mammal morbilliviral epizootic occurred in 1987-88 along the Atlantic coast of the United States (2,3). More than half the in-shore population of bottlenose dolphins (*Tursiops truncatus*) may have died. In 1988, thousands of harbor seals (*Phoca vitulina*) (4) and small numbers of harbor porpoises (*Phocoena phocoena*) (5) died in morbilliviral epizootics in northwestern Europe. A separate epizootic claimed thousands of striped dolphins (*Stenella coeruleoalba*) in the western Mediterranean (6). In 1993-94, another bottlenose dolphin epizootic occurred in the Gulf of Mexico (7,8). Recently, morbilliviral infection has been reported in cetaceans in the Pacific (9).

Viruses have been cultured from animals from some of the epizootics, and novel marine mammal morbilliviruses have been recognized. Two cetacean morbilliviruses have been identified and named porpoise morbillivirus (PMV) and dolphin morbillivirus (DMV). PMV was isolated from harbor porpoises that died along the Irish coast. DMV was first identified in striped dolphins from the Mediterranean (10). Since the characterization of these viruses, genetic identification has also been possible by reverse transcriptase-polymerase chain reaction (RT-PCR) and DNA sequence analysis in specimens from epizootics where virus was not cultured (11). These analyses have shown that both cetacean morbilliviruses (DMV and PMV) were present in the first recognized cetacean epizootic in bottlenose dolphins in 1987. While not species-specific in that study, only DMV was recovered in the striped dolphin epizootic in the Mediterranean, and only PMV sequences were noted from bottlenose dolphins in the subsequent Gulf of Mexico epizootic (11). Common dolphins (*Delphinus delphis*) stranded off the California coast from 1995 to 1997 were shown to have morbilliviral infection by virus neutralization and enzyme-linked immunosorbent assay (ELISA), as well as RT-PCR (9). Sequence analysis has shown that these animals were infected with PMV (Taubenberger et al., unpub. data).

We report a case of lethal morbilliviral infection in a long-finned pilot whale (*G. melas*). Sequence analysis of fragments of the morbillivirus phosphoprotein (P) and nucleoprotein (N) genes suggests that this is a novel morbillivirus, phylogenetically related to, but distinct from, the other cetacean morbilliviruses, PMV and DMV.

A female long-finned pilot whale (*G. melas*) was stranded below the deck of a beach home on the Delaware Bay, New Jersey. The whale was inactive and appeared near death. The likelihood

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of recovery was remote, and euthanasia was considered appropriate. The animal was transported by truck to a veterinary diagnostic laboratory, where it died shortly after arrival.

At necropsy, body fat was found to be depleted. The lungs were dark red and congested. Nematodes were observed in the second stomach chamber. Significant histologic lesions were present in many organs. In the lung, alveoli and airways contained numerous neutrophils, histiocytes, and multinucleate cells, interspersed with cellular debris. Multifocal type 2 pneumocyte hyperplasia was observed. Many multinucleate cells and some bronchiolar epithelial cells contained eosinophilic intracytoplasmic and intranuclear inclusion bodies. An immunohistochemical stain demonstrated morbilliviral antigen (8) in bronchiolar epithelium, multinucleate (syncytial) cells, and type 2 pneumocytes (Figure 1). Syncytial cells, often containing eosinophilic intracytoplasmic and intranuclear inclusion bodies, were also observed in spleen, lymph nodes, and liver. Additionally, eosinophilic intracytoplasmic inclusion bodies were present in colonic and gastric epithelia and in transitional epithelium of the kidney. Intracytoplasmic and intranuclear inclusion bodies were seen in glossal epithelium. Other histologic lesions included nonsuppurative encephalomyelitis, necrotizing hepatitis, erosive colitis, necrotizing gastritis, ulcerative glossitis, and ulcerative esophagitis.

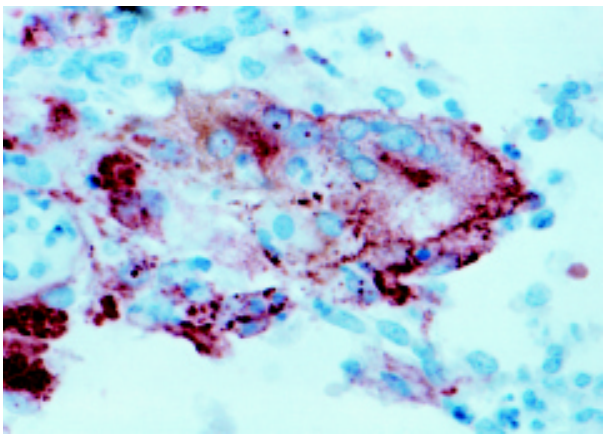

Figure 1. Lung of pilot whale. Positive intracytoplasmic and intranuclear immunoperoxidase staining of morbilliviral antigen in syncytial cell (center) and bronchiolar epithelium (lower left). Avidin-biotin-peroxidase with Harris' hematoxylin counterstain. Original magnification 594x.

RT-PCR was performed on RNA isolated from formalin-fixed, paraffin-embedded lung and brain tissues (7,11). A 378 nucleotide fragment of the P gene (GenBank accession number AF200817 and a 230 nucleotide fragment of the N gene (GenBank accession number AF200818 were amplified and sequenced. In both cases the sequences were novel but related to the previously described cetacean morbillivirus sequences. We have tentatively named this virus "pilot whale morbillivirus" or PWMV.

Sequences of both the N and P gene fragments were analyzed by parsimony and neighbor-joining (Figure 2). Parsimony analyses (12) with Sendai virus as outgroup placed PWMV in the cetacean morbillivirus clade with PMV and DMV, with moderate-to-high bootstrap values (N sequence = 70; P sequence = 100). Neighbor-joining (13) also placed PWMV in the cetacean morbillivirus clade with high bootstrap values (N sequence = 97; P sequence = 100). Thus PWMV is clearly related to, but distinct from, PMV and DMV, and the latter two are more closely related to one another than either is to PWMV (Figure 2).

Both the origin of these cetacean morbilliviruses and the mechanism of spread to different parts of the world remain unknown. Recently, serologic evidence of morbilliviral infection was obtained from 11 of 15 species of odontocete cetaceans of the western Atlantic (14), suggesting that exposure to these viruses has been widespread. Serologic evidence of enzootic infection (with a seroprevalence of 86%) was also obtained from two species of pilot whales (*G. melas* and *G. macrorhynchus*) in the western Atlantic (15). Clinical disease consistent with morbilliviral pneumonia was detected in one *G. melas* calf, although the morbillivirus in that case was not characterized. Duignan et al. (15) speculated that pilot whales may serve as vectors of morbillivirus infection to other odontocete cetaceans.

The results from this case suggest that pilot whales may have their own species-adapted morbillivirus. Given that this is a single case, that no morbilliviral epizootic has been described for pilot whale species, and that seroprevalence is consistent with enzootic morbilliviral infection (15), lethal infection might be rare in these species. Nevertheless, it remains possible for pilot whales to serve as vectors by which

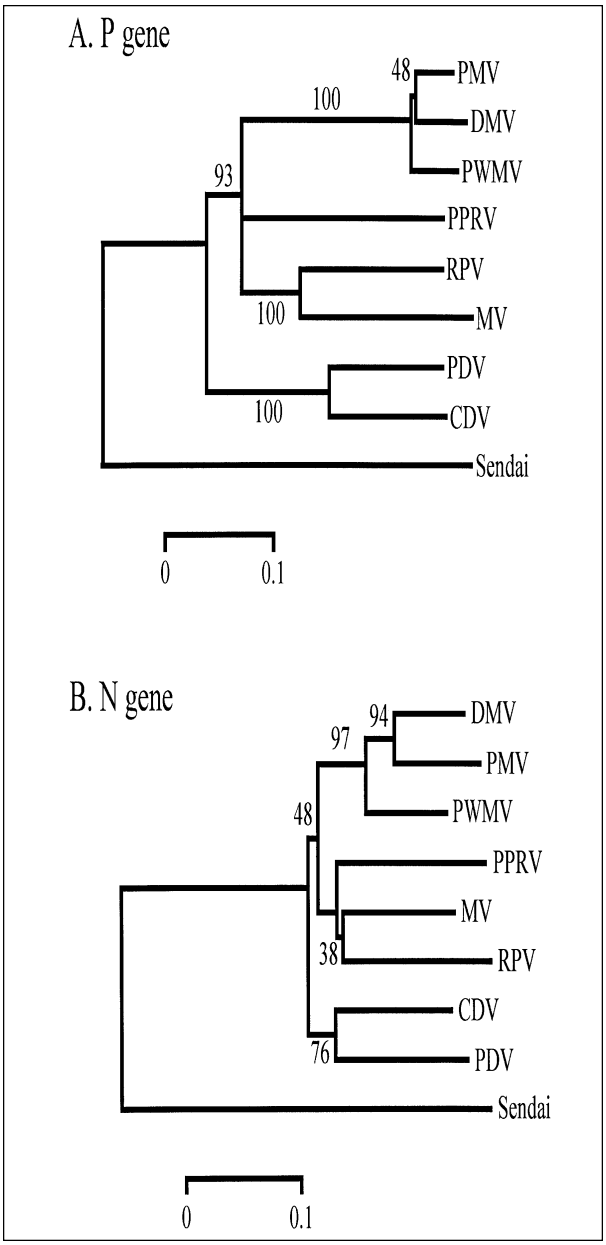

Figure 2. Neighbor-joining analyses of partial P and N gene sequences with branch distances as shown. Analyses were performed with MEGA, version 1.01 (13). For the P gene, a 378 nucleotide fragment was amplified (7,8) using the following primers: 5'-CGGAG ACCGAGTCT-TCATT-3' (forward) and 5'-ATTGGGTTGC ACCACT-TG TC-3' (reverse), corresponding to nucleotides 2190 to 2567 as aligned to the measles virus P gene (Edmonston strain). For the N gene, a 230-nucleotide fragment was amplified using the following primers: 5'-CCHAGRATYGCTGAAATGATHTGTGA-3' (forward) and 5'-AACTTG TTCTGRATWGAGTTYTC-3' (reverse), corresponding to nucleotides 849 to 1078, as aligned to the measles virus N gene (Edmonston strain, GenBank accession number Z66517). RT-PCR and sequence analysis were performed as described (7,8).

immunologically naïve species are exposed to morbillivirus infection. The viruses in these hosts may have undergone species-adaptive changes, reflecting the genetic differences between DMV, PMV, and PWMV. While only partial sequences have been obtained from two of the morbilliviral genes in this case, the fact that it is closer to the root of the cetacean morbillivirus clade (Figure 2) suggests that PWMV resembles the common ancestor of the clade more closely than either DMV or PMV.

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Candida dubliniensis Fungemia: the First Four Cases in North America

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We report the first four North American cases of *Candida dubliniensis* fungemia, including the first isolation of this organism from the bloodstream of an HIV-infected person. All isolates were susceptible in vitro to commonly used antifungal drugs. This report demonstrates that *C. dubliniensis* can cause bloodstream infection; however, the incidence of disease is not known.

In recent years, *Candida* species other than *C. albicans* have emerged as causes of human candidiasis, particularly in HIV-infected and other immunocompromised persons (1). *C. dubliniensis,* a recently described species closely related to *C. albicans* (2), has been implicated as an agent of oral candidiasis in HIV-positive persons (2-5) but has also been recovered from HIV-negative persons with clinical signs of oral candidiasis and from the genital tract of some women with vaginitis (2,4). First isolated from AIDS patients in Dublin, Ireland (2), *C. dubliniensis* has a worldwide distribution (3-6). Most isolates are susceptible to amphotericin B and the azoles, but resistance has been shown in HIV-positive patients on fluconazole for oral candidiasis (7). Its potential to cause deep or disseminated candidiasis is not known, largely because *C. dubliniensis* has rarely been isolated from sterile body sites (6); however, the phenotypic characteristics the organism shares with *C. albicans* (producing germ tubes and chlamydospores) suggest that some *C. dubliniensis* isolates may have been misidentified as *C. albicans*.

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Three cases of *C. dubliniensis* fungemia have been reported from Europe, all in HIV-negative bone marrow transplant recipients with chemotherapy-induced neutropenia (8). We report the first isolation of *C. dubliniensis* from the blood cultures of four patients from the United States. All four patients had multiple underlying conditions and at least one symptom of septicemia (fever, hypotension, or multiple organ system failure) at the time blood cultures were drawn. These cultures were collected from October 1998 to January 1999 through active, population-based laboratory surveillance for candidemia in residents of Connecticut and of Baltimore City or County, Maryland (combined population, 4.8 million). An incident case of candidemia was defined by the first isolation of any *Candida* species from a blood culture from a resident of one of the surveillance areas. Medical records were reviewed to obtain demographic data, clinical data on underlying medical conditions, treatment, and outcome.

Case 1

A 74-year-old black man from Baltimore, with a history of chronic lymphocytic leukemia, chronic obstructive pulmonary disease, coronary artery disease and hypertension, was hospitalized for fatigue and anemia 4 weeks after

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chemotherapy with chlorambucil. He received multiple blood transfusions on day 1 of hospitalization and quickly progressed to multiple organ failure, including renal failure and hepatic and cardiogenic shock, and was transferred to a medical intensive care unit. Multiple indwelling catheters were placed. A peripheral blood culture obtained 3 days after admission grew C. *dubliniensis*. The patient died 1 day after this single positive blood culture was drawn. No autopsy was performed.

Case 2

A 30-year-old black woman from Connecticut with a history of end-stage liver disease was hospitalized for gastrointestinal bleeding and refractory ascites. The patient had a history of intravenous drug use and alcoholism but was not infected with HIV. During hospitalization, she required multiple transfusions; acute renal failure requiring hemodialysis followed. The patient was receiving many medications, including vasopressors, antibiotics, and corticosteroids. Yeasts were isolated from peripheral blood cultures collected on days 11, 15, and 17 of hospitalization; four of the isolates were later identified as *C. dubliniensis*. The patient had a triple-lumen catheter placed on the day before the initial isolation of the organism. She was treated with intravenous fluconazole 200 mg/day for 5 days, starting 6 days after the first yeast isolation. A blood culture collected on day 20 was negative, and the patient died after 24 days of hospitalization. No autopsy was performed.

Case 3

The third patient was a 39-year-old black man from Baltimore who was admitted for complications of end-stage liver disease, including acute renal failure and ascites, and diffuse lymphadenopathy of unknown etiology. He also had a history of diabetes mellitus. A week before hospitalization, the patient had been discharged from another hospital, where he had been admitted because of pancreatitis and treated for *Escherichia coli* bacteremia and renal insufficiency. The patient had a peripheral intravenous catheter and a central venous catheter placed during this hospitalization. On day 2, yeasts were recovered from a peripheral blood culture; these were later identified as *C. dubliniensis*. By the time the result of the blood culture was reported, the patient's clinical status had deteriorated because of worsening respiratory distress. He was treated with fluconazole 400 mg/day for 3 days but died 5 days after the blood culture was obtained. No autopsy was performed.

Case 4

The fourth case occurred in a 37-year-old white woman from Baltimore, who had a history of intravenous drug use, chronic deep vein thrombosis, and valvular heart disease. She was also HIV-infected ($CD4^+$ lymphocyte count was 779 cells/ml). She was hospitalized because of fever and chills, and blood cultures on day 1 of admission grew group A streptococci, for which she was treated with various antibiotics. On day 7, fever developed and peripheral blood cultures grew *C. dubliniensis* and *C. glabrata*. She was treated with oral fluconazole 400 mg/day for 2 weeks and was discharged to a skilled-nursing facility 1 day after being started on fluconazole.

Microbiologic Results

All seven isolates were originally identified as *C. albicans* on the basis of their phenotypic characteristics. They were reexamined at the Fungus Reference Laboratory, Centers for Disease Control and Prevention, and reidentified as *C. dubliniensis* on the basis of biochemical and morphologic criteria (9). The identification was confirmed by reactivity of DNA with a polymerase chain reaction (PCR)-enzyme immunoassay (EIA) probe specific for this species (10) and by PCR amplification of a region containing the novel *C. dubliniensis* group I intron in the large ribosomal subunit (11).

Broth microdilution MICs were determined according to National Committee for Clinical Laboratory Standards document M27-A guidelines (12). All isolates were susceptible to commonly used antifungal agents. MICs of amphotericin B were 0.25 (one patient) to 0.5 µg/ml (three patients); MICs of itraconazole were from <0.015 (two patients) to 0.03 µg/ml (two patients): and MICs of fluconazole and flucytosine were <0.125 µg/ml for all isolates.

Conclusions

The incidence of candidemia due to *C. dubliniensis* is not known, largely because of the difficulty in readily distinguishing this species from the morphologically similar *C. albicans*. However, in laboratory-based

surveillance conducted in 1992-93 in two sites in the United States (population 5.8 million), we did not find *C. dubliniensis* as an agent of candidemia, even with the DNA-based identification method used in this study (13). More recently, three cases of *C. dubliniensis* fungemia have been reported from Europe in patients with chemotherapy-induced immunosuppression and bone marrow transplantation (8). The four cases described here are the first reported in the United States.

The demonstration that *C. dubliniensis* has the potential to cause bloodstream infection provides information central to our understanding of its clinical relevance and pathogenic potential. As in the earlier European report (8), the patients in our study had multiple serious medical conditions. Two of the four patients had end-stage liver disease, which is a known risk factor for bloodstream infections with organisms that are part of the normal gastrointestinal flora because of breakdown of the normal mucosal barrier (14). This strongly suggests that the gastrointestinal tract was the source of the *C. dubliniensis* in these patients. Odds et al. (6) have reported the reidentification as *C. dubliniensis* of a number of *C. albicans* isolates that were obtained from fecal surveillance cultures in hematologic patients.

The isolation of *C. dubliniensis* from mucosal sites in HIV-infected persons has been widely reported (3,5). Although not severely immunocompromised, our fourth patient was HIV-positive, which makes hers the first reported case of bloodstream infection with this organism in an HIV-infected person. The fact that *C. dubliniensis* is able to cause invasive disease in these patients is of clinical interest. However, it may be more significant that *C. glabrata*, a recognized pathogen, was also isolated from blood cultures in our patient.

As our population-based surveillance for candidemia continues, we will be able to estimate more accurately the incidence of candidemia due to *C. dubliniensis* and define more clearly its clinical importance, epidemiologic characteristics, and outcome. The specific proportional impact of *C. dubliniensis* candidemia on outcome is difficult to assess in these patients, all of whom had multiple underlying conditions. The organisms isolated from our patients were all fully susceptible to amphotericin B, flucytosine, fluconazole, and itraconazole. However, resistance has been shown to occur in HIV-positive patients given fluconazole treatment for oral infection with *C. dubliniensis* (7). As our knowledge about this emerging pathogenic yeast increases and diagnostic tests are developed, prevention and better management of the disease will become possible.

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Integronlike Structures in *Campylobacter* spp. of Human and Animal Origin

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Resistance to antimicrobial agents used to treat severe *Campylobacter* spp. gastroenteritis is increasing worldwide. We assessed the antimicrobial resistance patterns of *Campylobacter* spp. isolates of human and animal origin. More than half (n = 32) were resistant to sulphonamide, a feature known to be associated with the presence of integrons. Analysis of these integrons will further our understanding of *Campylobacter* spp. epidemiology.

Campylobacter spp. are isolated from animals and birds and from the environment, particularly surface water. Poultry have been implicated as a major source of sporadic infection (1). Thermophilic *Campylobacter* spp., particularly *Campylobacter jejuni* and *C. coli,* are recognized as one of the etiologic agents of acute diarrheal disease in humans worldwide (2,3). Antimicrobial chemotherapy is usually reserved for patients with advanced infection or patients prone to relapse. Erythromycin, fluoroquinolones, and tetracycline are the antimicrobial drugs of choice.

Bacterial resistance to antimicrobial agents, which is increasing worldwide, is frequently caused by the acquisition of new genes rather than by mutation (4,5). An efficient means of acquiring new genes is by mobile genetic elements such as resistance (R)-plasmids and transposons. Recently, a novel class of naturally occurring mobile genetic elements, integrons, have been described as vehicles for the acquisition of antimicrobial resistance genes (5). Horizontal and vertical transfer can occur readily, as shown by the widespread acquisition of these gene cassettes among the *Enterobacteriaceae* and *Pseudomonas* spp. Integrons comprise two conserved structural regions (5'CS and 3'CS) flanking an internal variable region containing one or more site-specific recombined gene cassettes. While most known cassette-associated genes located distal to the 5'CS region encode resistance to antimicrobial drugs, some cassettes may include one or more open reading frames whose product(s) and corresponding function(s) remain to be defined (5). In the 3'CS downstream of the gene cassette are two genes, one of which encodes resistance to quaternary ammonia compounds (*qacE*Δ1), while the other is the sulphonamide resistance determinant (*sul1*). Antimicrobial resistance among *Campylobacter* spp. to drugs used in the treatment of human infection is increasing (6-8). This article reports the results of an investigation of a collection of Irish thermophilic *Campylobacter* spp. cultured from clinical cases of gastroenteritis and from porcine and poultry sources. We studied a representative sample of 55 isolates (47 *C. jejuni* and eight *C. coli* isolated between 1996 and 1998), cultured from intestinal tissue of animals at slaughter and from human fecal samples.

Antimicrobial agent susceptibility tests were performed by the agar diffusion method on IsoSensitest agar (Difco, Dublin, Ireland) with 5% horse blood (9). Cultures were prepared by inoculating colonies from a fresh, pure, 24-hour culture into sterile distilled water to give an inoculum turbidity equivalent to a 0.5 McFarland

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turbidity standard. The McFarland standard was prepared by adding 0.5 ml 0.048 M $BaCl_2$ to 99.5 ml 0.18 M H_2SO_4 with constant stirring. Samples were swabbed evenly onto agar plates and allowed to dry. Twelve antimicrobial agents were tested on disks. Antimicrobial drugs tested, together with their abbreviations and corresponding concentrations in parentheses, included ampicillin (Ap, 10 µg/disc), chloramphenicol (C, 10 µg/disc), ciprofloxacin (Cp, 5 µg/disc), colistin (Ct, 25 µg/disc), erythromycin (E, 5 µg/disc), gentamicin (G, 10 µg/disc), nalidixic acid (Na, 30 µg/disc), spectinomycin (Sp, 10 µg/disc), streptomycin (S, 25 µg/disc), sulphafurazole (Su, 100 µg/disc), tetracycline (T, 10 µg/disc), and trimethoprim (Tm, 1.25 µg/disc). The plates containing the antibiotic disks were incubated at 37°C under microaerophilic conditions for 24 hours. Inhibition zone sizes were recorded according to the guidelines of the National Committee for Clinical Laboratory Standards (10). Resistance profiles were further confirmed by E-Test (AB Biodisc, Solna, Sweden).

Briefly, 17% of all isolates were resistant to ampicillin, 3.8% to chloramphenicol, 1.9% to ciprofloxacin, 7.5% to colistin, 11.3% to erythromycin, 1.9% to gentamicin, 17% to nalidixic acid, 77.4% to spectinomycin, 20.8% to streptomycin, 62.3% to sulphonamide, and 24.5% to tetracycline. Many of the isolates tested (n = 42, 77%) were resistant to three or more antimicrobial agents with part of the R-type, including SSpTm among others. Two strains, *C. jejuni* CIT-H17 (R-type: ApCtENaSSpSuTTm) and *C. coli* CIT-V6 (R-type: CCpENaSSpTTm), were particularly resistant (Table 1); both were resistant to nalidixic acid, and the latter was also resistant to ciprofloxacin. In reviewing the R-types in the sample, the presence of sulphonamide resistance (in 62.3% of the sample) suggested that integron-like structures may exist in *Campylobacter* spp.

To test the latter hypothesis, genomic DNA was purified from all isolates (11). Using the oligonucleotide primers Int 1 F 5'-GGC ATC CAA GCA CGA AG-3'and Int 1 B 5'-AAG CAG ACT TGA CCT GA-3' designed to anneal to the 5'CS and 3'CS flanking regions (12) of integrons, we tested the *Campylobacter* spp. genome by polymerase chain reaction (PCR) for putative gene cassettes. *Escherichia coli* containing the characterized plasmids R100.1 and R751 (13) together with CIT-F 100, a *Salmonella enterica* serotype Typhimurium DT104 strain cultured from a contaminated food source (14), were included as controls. Gene cassettes of 1.0-kb and 800 bp, respectively, from *E. coli* (data not shown) and 1.0- and 1.1-kb (Figure 1a, lane 2),

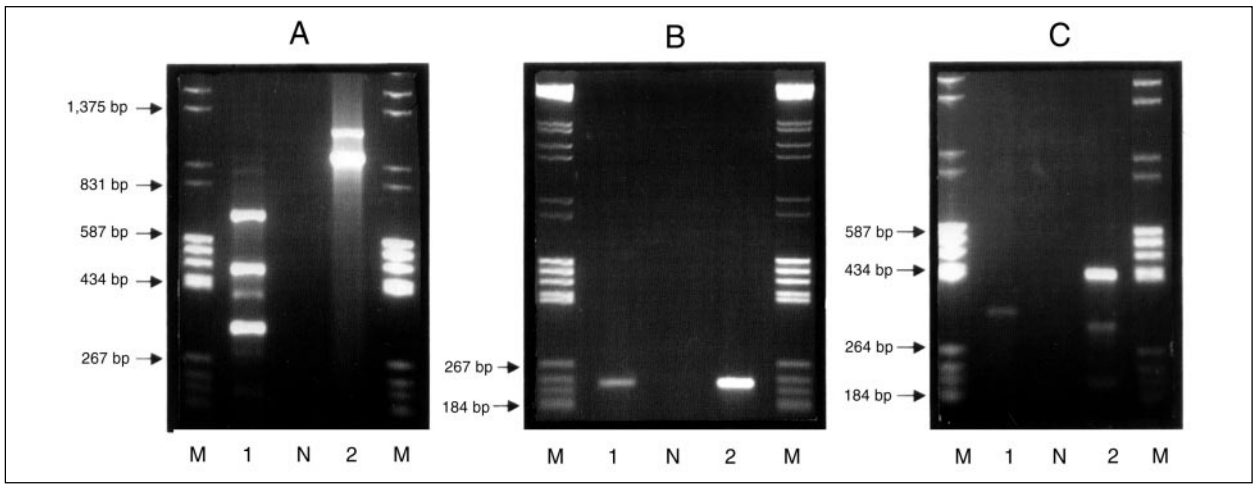

Figure 1. Agarose (1.5%) gel electrophoresis in 1x TAE buffer containing 0.5 µg/ml ethidium bromide. Purified genomic DNA was used as the template in a PCR reaction with the primers Int 1 F 5'-GGC ATC CAA GCA CGA AG-3' and Int 1 B 5'-AAG CAG ACT TGA CCT GA-3' (12). Lane M contains an equal mixture of molecular weight markers grades III and V (Boehringer Mannheim, Germany), Lane N is the negative containing all reaction components with the exception of template DNA, Lane 1, *Campylobacter coli* CIT-H 6 (IP-I); Lane 2, *Salmonella* Typhimurium DT104 CIT-F 100 (IP-C). As in A above except that the primers used were *qacE*?1 F 5'-AT GCA ATA GTT GGC GAA GT-3'and *qacE*?1 B 5'-CAA GCT TTT GCC CAT GAA GC-3' (13). As in A above using primers *sul1* F 5'-CTT CGA TGA GAG CCG GCG GC-3' and *sul1* B 5'-GCA AGG CGG AAA CCC GCG CC-3' (13).

from *Salmonella* Typhimurium were detected after amplification. These amplicon profiles were designated as integron pattern (IP)-groups A, B, and C, respectively (Table). After amplification and conventional agarose gel analysis of all *Campylobacter* spp. isolates in the study population, DNA amplicons of 230 bp to 1.47 kb were detected. A total of 22 gene cassette structures were identified (Figure 2). The most commonly occurring amplified gene cassette pattern was designated IP-group I, consisting of four DNA fragments of 350 bp to 700 bp (Figure 2, lane 1 and Figure 1a, lane 1). This gene cassette pattern was present in both poultry and clinically derived *C. jejuni*, accounting for 38% of strains. IP-group II (Figure 2, lane 2) accounted for 7% of all *C. jejuni* isolates only. The IP-groups III (Figure 2, lane 3), XIV (Figure 2, lane 14), XVI (Figure 2, lane 16), and XX1 (Figure 2, lane 21) each accounted for 6% of the collection, with IP-groups XIV and XVI being unique to *C. coli*. All other IP-groups (Table; Figure 2) were represented by single isolates. A 350-bp amplified DNA fragment was common to all isolates, with the exception of the poultry-derived *C. coli* CIT-P2 and a clinical isolate

Table. Isolates of *Campylobacter coli* and *C. jejuni* from which gene cassettelike structures were amplified

Isolate No.	Year[a]	R-type	IP-profile	Isolate No.	Year[a]	R-type	IP-profile
Campylobacter jejuni				*C. jejuni*, cont'd			
CIT-H6	1997	SpSuTm	I	CIT-H1	1996	SpSuTm	VIII
CIT-H7	1996	SpTm	I	CIT-P1	1997	SpSuTm	IX
CIT-H8	1997	ApSpTm	I	CIT-H2	1996	SpSuTm	X
CIT-H9	1997	SpTm	I	CIT-H3	1997	SuTTm	XI
CIT-H10	1997	Tm	I	CIT-H4	1996	SpSuTm	XII
CIT-H12	1997	NaSpTm	I	CIT-H5	1997	SuTm	XIII
CIT-H14	1997	SpTm	I	*C. coli*			
CIT-H15	1996	SpTm	I	CIT-P3	1996	EGSSpSuTm	XIV
CIT-H16	1997	SpTTm	I	CIT-V3	1998	ESpTTm	XIV
CIT-H22	1996	SpSuTm	I	CIT-V6	1998	CCpENaSSpTTm	XIV
CIT-H25	1997	SSpTm	I	CIT-V1	1998	SpSuTm	XV
CIT-H26	1997	ApSpTm	I	CIT-V4	1998	SSpTTm	XVI
CIT-P4	1997	TTm	I	CIT-V5	1998	ESSuTTm	XVI
CIT-P5	1997	SpSuTm	I	CIT-V2	1998	ESSpSuTTm	XVI
CIT-H30	1997	SuTTm	I	*C. jejuni*			
CIT-H31	1996	SpSuTm	I	CIT-H23	1996	SpSuTm	XVII
CIT-P10	1997	SpTm	I	CIT-H28	1997	CtTm	XVIII
CIT-P13	1996	NaTm	I	*C. coli*			
CIT-P14	1996	SuTTm	I	CIT-P2	1997	SpSuTm	XIX
CIT-P15	1996	SpSuTTm	I	*C. jejuni*			
CIT-P16	1996	ApSpSuTTm	I	CIT-H19	1997	SpSuTm	XX
CIT-H29	1997	SuTm	II	CIT-H17	1996	ApCtENaSSpSuTTm	XI
CIT-P7	1997	NaSuTm	II	CIT-H21	1997	SpSuTm	XXI
CIT-P8	1997	SpSuTm	II	CIT-H32	1996	SSpSuTm	XXI
CIT-P9	1997	/	II	CIT-H18	1997	ApCtSSpSuTm	XXII
CIT-P6	1997	SpSuTm	III	CIT-H20	1996	ApSpTm	XXII
CIT-P11	1996	NaSpTm	III	Control strains[b]			
CIT-P17	1997	ESpSuTm	III	*Escherichia coli*			
CIT-H11	1997	ApSpSuTm	IV	[R100.1]	/	/	A
CIT-H13	1997	ApCSpTm	IV	*E. coli*			
CIT-P12	1996	CtNaSpSuTTm	V	[R751]	/	/	B
CIT-H24	1997	ApNaTm	VI	*Salmonella* Typhimurium			
CIT-H27	1997	NaSSpSuTm	VII	CIT-F 100	1998	ACSSuT	C

[a]Year of isolation.
[b]*E. coli* and *Salmonella enterica* serotype Typhimurium control strains. The former carried plasmids R100.1 and R752, respectively, provided by D. Sandvang (13). *S.* Typhimurium DT104 [CIT-F 100] was previously characterized by M. Daly et al. (14).
H, hospital isolate; P, poultry isolate; V, veterinary isolate; /, not available or not determined. Antimicrobial agents: Ap, ampicillin; C, chloramphenicol; Cp, ciprofloxacin; Ct, colistin; E, erythromycin; G, gentamicin; Na, nalidixic acid; S, streptomycin; Sp, spectinomycin; Su, sulphafurazole; T, tetracycline; Tm, trimethoprim.

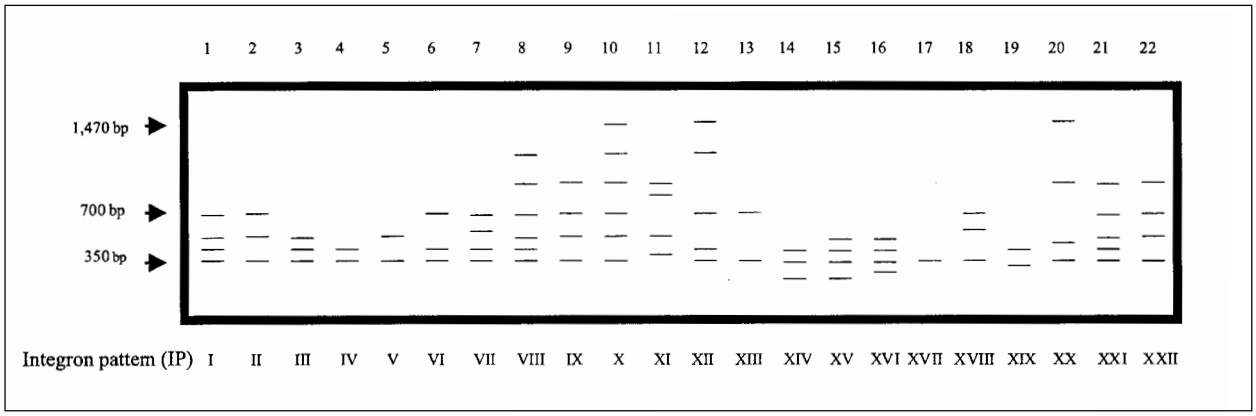

Figure 2. Schematic representation of all amplified gene cassettes from *Campylobacter* spp. in the study population. Roman numerals refer to the designated integron pattern (IP)-type assigned to each pattern.

C. jejuni CIT-H3. Amplicons of 230 and 250 bp were conserved among *C. coli* isolates only.

Three putative gene cassettes of 243, 388, and 466 bp were cloned after amplification by using the Int 1 F and Int 1 B primers (4,12) as described above. All were sequenced by automated methods. Sequencing data showed a short, imperfect inverted repeat element at the 3' end of the cloned fragments which represented the 59 base element (5'-GTTRR-3'). This is the target for site-specific recombination involved in the insertion and excision of gene cassettes (4,5,15). Isolates were also tested for the 5' CS encoded integrase (*int*) and the 3' CS encoded *qacE* 1 and *sul1* genes by PCR. A DNA fragment of 225 bp was detected after amplification and agarose gel analysis (Figure 1b, lane 1) using primers *qacE*?1 F 5'-ATC GCA ATA GTT GGC GAA GT-3', and *qacE*?1 B 5'-CAA GCT TTT GCC CAT GAA GC-3' (13). The latter fragment corresponded with a similar sized amplicon in *S.* Typhimurium (Figure 1b, lane 2). The 3'-CS region of integrons, known to contain a *sul*1 gene, was similarly tested with the primers *sul1* F 5'-CTT CGA TGA GAG CCG GCG GC-3' and *sul1* B 5'-GCA AGG CGG AAA CCC GCG CC-3' (13). When compared with an *S.* Typhimurium DT104 amplicon of 436 bp (Figure 1c, lane 2) (13), the *Campylobacter* spp. *sul*1-primer derived DNA fragment (Figure 1c, lane 1) appears smaller at approximately 360 bp. Nevertheless, the latter amplicon was consistently amplified from all *Campylobacter* spp. Smaller *sul1* primer generated DNA fragments were also detected in *S.* Typhimurium after PCR and gel analysis (14). These may derive from the partial *sul1* genes recently located in a 14-kb gene cluster on the chromosome of *S.* Typhimurium (16). On probing the *Campylobacter* spp. *sul1*-primer derived amplicon (Figure 1c, lane 1) with the digoxigenin-labeled 436 bp *S.* Typhimurium *sul1* DNA amplicon (Figure 1c, lane 2), no hybridizing signal was detected (data not shown). This result suggests that the *Campylobacter* spp. *sul1* gene is different when compared with *S.* Typhimurium. To investigate the 5'-CS region, primers (int1 F [Tn21]): 5'-GAA GAC GGC TGC ACT GAA CG-3' and int1 R [Tn*21*]: 5'-AAA ACC GCC ACT GCG CCG TTA-3') were designed to amplify a 1.2-kb DNA fragment from the integrase gene of Tn*21* and were tested against *Campylobacter* spp. and *S.* Typhimurium (as a control) (Table). The predicted amplicon was detected in the latter, together with two smaller amplimers of 270 bp and 450 bp. These latter PCR products (270 bp and 450 bp) were also identified in *Campylobacter* spp. (data not shown), consistent with a deleted form of a class 1 integrase gene in these isolates.

The DNA sequences from the amplified cassettes (of 243 bp, 388 bp, and 463 bp) above were also searched by using the BLAST search tool (17). GenBank accession numbers were assigned as follows: AF155357 (243-bp gene cassette); AF155356 (388-bp gene cassette), and AF152561 (463-bp gene cassette). The former amplicon contains two open reading frames. No corresponding sequences were identified in the database. The 388-bp amplicon also contained two open reading frames and did not match any sequences when subjected to a BLAST search of

the current databases. Finally, the larger 463-bp amplicon contained two incomplete open reading frames. BLAST searches using the latter sequence identified glycyl-tRNA synthetase from the genome of *Helicobacter pylori* matching 102 (85%) of 119 nucleotides. Further characterization of other gene cassettes is in progress, focusing on amplicons of 700 bp and greater.

Drug selection may promote recombinational events between *Campylobacter* spp., *Enterobacteriaceae* and other gram-negative organisms (15). A common habitat for these organisms is the human and animal gastrointestinal tract. Modern animal husbandry promotes the use of large animal housing facilities, thereby ensuring genetic interconnection between large populations of bacteria. *Campylobacter* spp. have a natural ability for transformation (18), and in shared animal reservoirs, interspecies transfer of DNA, including antimicrobial resistance encoding genes and other unrelated genes, may occur by strategies analogous to site-specific recombination (19,20). Our findings may indicate a novel mechanism by which unrelated DNA becomes incorporated into cells (21). Detailed characterization of these integronlike structures is an essential step in understanding the role(s) of these novel genetic elements. The existence of these structures may have interesting implications regarding the diversity of the *Campylobacter* spp. genome and the evolution of this species. Together with the corresponding DNA fingerprint profile (Lucey B., Fanning S., manuscript in preparation) the variation in genetic content and structure of these determinants may be used as a potential tool in elucidating the epidemiology of these pathogens (22,23).

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Burkholderia pseudomallei Traced to Water Treatment Plant in Australia

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Burkholderia pseudomallei was isolated from environmental specimens 1 year after an outbreak of acute melioidosis in a remote coastal community in northwestern Australia. *B. pseudomallei* was isolated from a water storage tank and from spray formed in a pH-raising aerator unit. Pulsed-field gel electrophoresis confirmed the aerator and storage tank isolates were identical to the outbreak strain, WKo97.

Melioidosis is endemic in northern Australia. Since the first description of the disease from far north Queensland in 1962 (1), melioidosis has spread west and south. A small focus of endemic infection was identified in western Australia in 1967 (2). Cases are reported throughout the year but peak during the rainy season (3). An unusually large number of cases was diagnosed in the Northern Territory during the near-record rainfall of 1990-91 (4). A cluster of acute melioidosis cases in a remote, coastal community (population 300) in western Australia during the dry season in late 1997 was, therefore, unexpected (5). Five cases of acute septicemic or pneumonic melioidosis were diagnosed during a 6-week period. All five patients lived in the same remote community and had recognized risk factors for acute melioidosis (e.g., diabetes or renal failure). *Burkholderia pseudomallei* was isolated from all patients; three died. Late-onset septicemia was diagnosed in a resident of this community 6 months later. This infection was preceded by a febrile episode during the presumed exposure period and soft tissue infection 1 month later.

All six isolates were indistinguishable by pulsed-field gel electrophoresis (PFGE). Environmental sampling during the outbreak investigation identified the potable water supply as a possible source of *B. pseudomallei*. Epidemiologic investigations implicated acid bore water, chlorination failure, recent replacement of water pipes, and climate.

Twelve months later, after maintenance work on the water supply was completed, microbiologic samples were collected again. Isolation of *B. pseudomallei* prompted a more detailed environmental investigation with emphasis on the water treatment plant and bore water. The water supply to the community comes from a subterranean source through a group of capped and sealed bore holes. These are fed by an underground common bore main into the water treatment compound, where the bore water is passed through an aerator tower, then stored in two ground-level tanks before chlorination. After passing through a gas chlorinator, water is fed through another underground main pipe to the community, is pumped up into a high-level tank, and flows into the underground domestic reticulation system. Outlets in family dwellings and community buildings are plumbed faucets, showerheads, and water closets. Potable water is supplied to the community at a rate of approximately 500 kL per day.

Preliminary Bacteriology

We collected a 5-L water specimen from the previously culture-positive yard tap and another 5-L specimen from the sample access point before the gas chlorinator unit, after flame-sterilizing the outlet and letting water run for a prolonged period. A clump of wild grass above the bore line entering the compound, near the place where an excavator punctured the pipeline, was dug up with its roots intact. Water specimens were

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filtered, and the filter membrane was cultured in Ashdown broth and subcultured onto Ashdown agar. A 5-g sample of root soil was suspended in sterile saline and processed as for water specimens. Suspect colonies were identified by standard laboratory methods and confirmed by polymerase chain reaction (PCR) amplification of *B. pseudomallei* DNA sequences (6). *B. pseudomallei* was isolated from all three tested specimens, although the species was recovered only from the tap water after enrichment with trypticase soy broth and prolonged incubation (2 weeks). Sediment from the bottom of the water tank and a 5-L specimen of tank contents were cultured. Both were positive for *B. pseudomallei*.

Bacteriology of the Water Treatment Plant

Sampling of the water supply system progressively further upstream drew our attention to an aerator in the treatment plant that during the initial outbreak investigation had been temporarily removed for renovation. The aerator is installed to raise water pH (acid due to dissolved carbon dioxide) by spraying groundwater through a series of open 1 m x 1 m wire mesh trays located in a frame on an elevated platform. A series of water samples were collected in the community, immediately after chlorination, before chlorination, from the ground tank, the aerator, bore lines, and all four capped bores. Aerator spray was collected from the top and lower down the tower. Biofilm was scraped from the sides of the aerator trays. Water and scum from the aerator sump were also sampled. The pH was measured in the spray generated at each level of the aerator. Roots and soil were collected from a shrub (*Acacia coleii*) growing around the stop valve at the entry to the treatment plant complex, in case roots had penetrated the pipeline and soil had entered the water supply. All water specimens were filtered and spread onto Ashdown and trypticase soy broth media. Isolates were identified as described above. *B. pseudomallei* was isolated from the spray at the top and bottom of the aerator in abundant growth on direct culture plates, without any prior enrichment (Table), and from soil around the acacia roots. Despite the use of selective media, the growth of various environmental bacterial and fungal species was so abundant in the lower aerator trays and sump that the presence of *B. pseudomallei* could not be excluded, although all cultures were negative.

Table. Results of 1999 environmental samples from outbreak vicinity

Sample	*Burkholderia pseudomallei*	WKo97
All bores	-	
Bore line root soil	+	-
Acacia root soil	+	-
Aerator		
Upper spray	+	+
Lower spray	+	+
Tray biofilm	-	
Sump water	-	
Sump biofilm	-	
Storage tank		
Surface water	-	
Deep water	+	+
Sediment	+	+
Prechlorinator	-	
Postchlorinator	-	
High-level tank	-	
Domestic tap water	-	
Backyard tap	+	-
Storage tank repeat		
Deep water	-	

All samples from the bore lines and bore field upstream of the aerator were negative for *B. pseudomallei*. Chlorine levels were maintained above 1 ppm.

Molecular Epidemiology

Environmental and clinical isolates of *B. pseudomallei* were typed by PFGE of an XbaI restriction digest of bacterial chromosomal DNA (Figure). After completion of these studies, environmental isolates from parts of the potable water supply, nearby soil, and two distant locations (250 and 1,000 km away) were run simultaneously with human and animal clinical isolates. As two gels were required, clinical isolates were run on one gel with a duplicate of the initial tap water isolate, and environmental isolates were run with a duplicated digest from the fifth case in the cluster. Soil isolates were distinct from the outbreak strain, but the aerator spray and ground tank isolates were identical to the outbreak strain, WKo97 (Figure; Table).

Biocontainment Measures

Confirmation that a specific device in the water treatment plant had become heavily contaminated with *B. pseudomallei* prompted biocontainment questions. Arrangements were

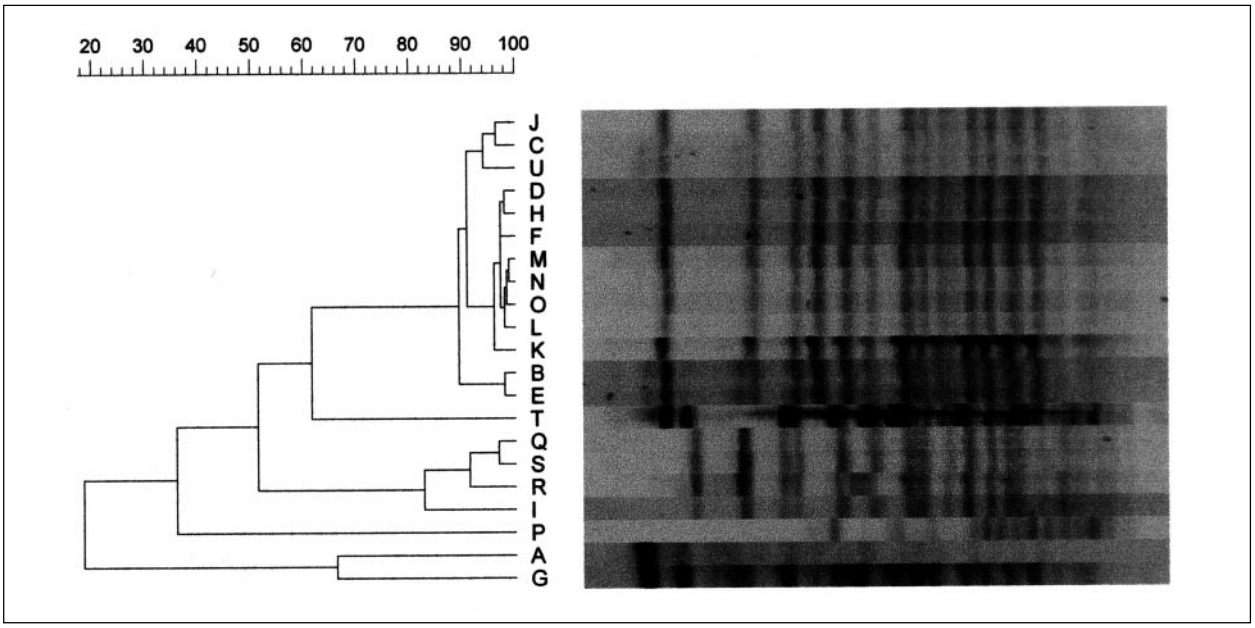

Figure. Results of pulsed-field gel electrophoresis (PFGE) of clinical and environmental isolates of *Burkholderia pseudomallei* from western Australia. Molecular typing was performed by electrophoresis of twice-digested 18h XbaI digests of chromosomal DNA from each isolate with a pulse time and ramp of 5.5 to 52 sec from 20h at 200V. Lanes correspond to the following isolates: A,G initial and recurrent infection separated by >12 months in epidemiologically unrelated case in outbreak community; B,C,D,E,F blood culture isolates from each patient in case cluster; H,K duplicates of same isolate from backyard tap water collected during initial investigation; I soil isolate from distant location; J duplicate of clinical isolate corresponding to lane F; L isolate obtained from prechlorination water specimen collected during initial investigation; M ground-level water tank prior to chlorinator; N spray from uppermost aerator tray; O spray from lowermost aerator tray; P root soil around bore main; Q,R,S domesticated animal isolates from same distant location as isolate I; T from potable water at distant location; U late-onset infection in visitor to outbreak community during exposure period. Relatedness of PFGE patterns was measured with Molecular Analyst software (version 1.6, Biorad, Hercules, CA) and showed 90% or better agreement between all clinical isolates in the cluster and most isolates from the potable water supply. Epidemiologically unrelated clinical isolates and nonwater and distant environmental isolates showed no clustering with the above.

made to isolate the aerator from the water inflow, dismantle it, and remove its parts for incineration. Before being dismantled, the aerator was drained and soaked in concentrated hypochlorite, then wrapped in a polythene sheet. Workers wore submicron particle filter masks, protective overalls, and heavy-duty gloves. The ground-level tank was drained, treated with concentrated hypochlorite solution, and cleaned before refilling. Samples collected from the ground-level tank 2 months after these measures had been completed contained no detectable *B. pseudomallei*.

Conclusions

In our investigation of the persistence of *B. pseudomallei* in the potable water supply, we traced the source of contamination upstream to the water treatment plant and identified the aerator as a possible source. If it had been connected during the initial outbreak investigation and had been sampled then, the aerator might have been identified as a potential source 1 year earlier. Moreover, failure to fully digest the prechlorination isolate in this and a second reference laboratory led to the erroneous belief that the prechlorination point result was unrelated to the case-cluster. We cannot prove beyond doubt that the aerator was the primary source of contamination without contemporaneous bacteriologic evidence. It is unlikely the aerator became contaminated later as a result of retrograde flow, especially from a point downstream to the chlorinator unit. There are thus several possible sources of contamination: inflowing water from a leak in the bore pipeline,

rupture of the pipeline during renovation work, dust or vegetation blown through the mesh walls of the aerator by wind, or direct contact with contaminated soil during refurbishment of the dismantled aerator. The 1997 outbreak may have occurred because a shower of bacteria was released from the aerator into the potable water supply during renovation work on the aerator at a time when chlorine levels were at or near zero. The aerator unit had only a modest effect on pH but clearly provided optimal conditions for multiplication of aquatic bacteria with an aerobic metabolism (7). Whether or not the aerator became contaminated more recently than the outbreak, its ability to act as a persistent source or amplifier of the outbreak strain of *B. pseudomallei* justified its removal.

Aerators are used to correct acidic, ferric, or unusually warm potable groundwater supplies in many remote communities in northern Australia and represent a potential melioidosis risk. Deliberate or natural aeration of a contaminated water supply could amplify *B. pseudomallei* growth. In northeastern Thailand, where melioidosis is endemic and the groundwater is unusually acidic (8), natural or artificial aeration of the potable water supply should be considered as an additional contributory factor.

Measures taken after the initial western Australian outbreak investigation appear to have prevented further cases, despite the presence of multiple *B. pseudomallei* types in soil. Initial water engineering measures have cleared detectable *B. pseudomallei* from the water treatment plant.

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Molecular Typing of Multidrug-Resistant *Salmonella* Blockley Outbreak Isolates from Greece

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During 1998, a marked increase (35 cases) in human gastroenteritis due to *Salmonella* Blockley, a serotype rarely isolated from humans in the Western Hemisphere, was noted in Greece. The two dominant multidrug-resistance phenotypes (23 of the 29 isolates studied) were associated with two distinct DNA fingerprints, obtained by pulsed-field gel electrophoresis of genomic DNA.

Salmonella Blockley is rarely isolated in the Western Hemisphere. According to Enter-net, the international network for surveillance of *Salmonella* and verocytotoxin-producing *Escherichia coli* infections, *S.* Blockley represented 0.6% of all *Salmonella* serotypes isolated in Europe during the first quarter of 1998, a full 100-fold lower than the dominant serotype, *S.* Enteritidis (67.1%) (1). However, *S.* Blockley is among the five most frequently isolated serotypes from both avian and human sources in Japan (2,3), Malaysia (4,5), and Thailand (6). A single foodborne outbreak in the United States (7) and sporadic human infections in Europe associated with travel to the Far East (8), animal infection (9) or carriage (10,11), and environmental isolates have also been reported (12,13).

Regardless of the frequency of *S.* Blockley isolation, its rates of resistance to antibiotics have been high. Among Spanish salmonellae isolated from natural water reservoirs, *S.* Blockley and *S.* Typhimurium had the highest rates of multidrug resistance (12). Comparing 1980-1989 with 1990-1994, researchers from Tokyo noted an increase in the number of *S.* Blockley isolates resistant to one or more antibiotics, from 92.0% to 98.2% for imported cases and from 57.4% to 88.7% for domestic cases (3,14). In Thailand, isolates from human or other sources also had high rates of resistance to streptomycin, tetracycline, kanamycin, and chloramphenicol and lower rates to ampicillin and trimethoprim/sulfamethoxazole (15).

Nevertheless, few attempts at typing *S.* Blockley isolates with molecular methods have been described, and these have been limited to the characterization of plasmid content (2,16).

During the second and third quarters of 1998, Enter-net reported higher numbers of *S.* Blockley isolates than during the same period of the previous year in several European countries (1). The epidemiologic investigations conducted in Germany, England and Wales, and Greece did not confirm a source for this increase (17-19).

In this study, we characterized the Greek outbreak isolates further, both with respect to their antibiotic resistance phenotypes and DNA fingerprints obtained by pulsed-field gel electrophoresis of genomic DNA.

The Study

The study sample consisted of 28 of 35 *S.* Blockley strains isolated from May to December 1998 (19), one strain from February 1999, and four epidemiologically unrelated

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control strains: one from 1996 and three from 1997. All isolates were from human cases of enteritis. Identification was performed by the API 20E system (BioMerieux S.A., Marcy l'Etoile, France) and serotyping with commercially obtained antisera (BioMerieux) (20).

Susceptibility to kanamycin, streptomycin, ampicillin, amoxicillin/clavulanic acid, cefepime, tetracycline, chloramphenicol, trimethoprim/sulfamethoxazole, gentamicin, nalidixic acid, and ciprofloxacin was tested by a disk diffusion assay according to National Committee for Clinical Laboratory Standards guidelines (21). Genomic DNA was prepared and digested with *Xba*I (New England Biolabs) (22). Chi-square tests or Fisher exact tests were used to calculate two-tailed probabilities.

S. Blockley accounted for seven of the 13,199 salmonella isolates identified in Greece from 1976 to 1997. However, 35 gastroenteritis cases due to this serotype were reported from May to December 1998 (19). Twenty-nine *S.* Blockley strains isolated from fecal specimens of patients with gastroenteritis during May 1998 to February 1999, along with four epidemiologically unrelated clinical isolates from 1996 and 1997, were therefore studied for susceptibility to antibiotics. The 1998 outbreak isolates were scattered throughout Greece; *S.* Blockley was isolated later, starting in August 1998, in northern Greece.

All isolates were susceptible to trimethoprim/sulfamethoxazole, ampicillin, amoxicillin/clavulanic acid, gentamicin, and ciprofloxacin (Table). High resistance rates were observed to tetracycline (100%), streptomycin and kanamycin (90%), chloramphenicol (83%), and nalidixic acid (52%). Six resistance phenotypes could be distinguished (Table) with the two major phenotypes of outbreak isolates being resistant to kanamycin, streptomycin, tetracycline, and chloramphenicol (ATC) or kanamycin, streptomycin, tetracycline, chloramphenicol, and nalidixic acid (ATCN). Most (76%) strains isolated after August 24, 1998, were nalidixic acid-resistant (resistance phenotypes ATCN, TCN, ATN), unlike strains isolated up to August 17, 1998 (17%) ($1.29 < RR = 3.03 < 7.11$, $p = 0.005$).

When pulsed-field gel electrophoresis was used to obtain DNA fingerprints for these isolates (Figure 1), all belonged to the same type, A, although eight subtypes, A1-A8, could be distinguished on the basis of one to three DNA

Table. Resistance phenotypes for isolates of *Salmonella* Blockley in Greece

PFGE[a] subtype (n)	Resistance phenotype[b] (n)	Time span	Locations[c]
A1 (1)	AT (1)	May 3, 1998	1
A2 (12)	ATC (9), TCN (1), AN (1), AT (1)	1997; May 16-Oct 13, 1998	6
A3 (3)	ATC (1), ATN (1), TCN (1)	Aug 24-Oct 27, 1998	3
A4 (12)	ATCN (8), ATN (2), ATC (1), TCN (1)	Aug 17, 1998-Feb 23, 1999	6
A5 (1)	ATCN (1)	Sep 29, 1998	1
A6 (1)	AT (1)	1996	-
A7 (1)	ATCN (1)	1997	-
A8 (2)	ATC (1), AN (1)	1997; Aug 10, 1998	1

[a]PFGE, pulsed-field gel electrophoresis.
[b]A, kanamycin and streptomycin; T, tetracycline; C, chloramphenicol; N, nalidixic acid.
[c]Number of locations of isolation during the outbreak.

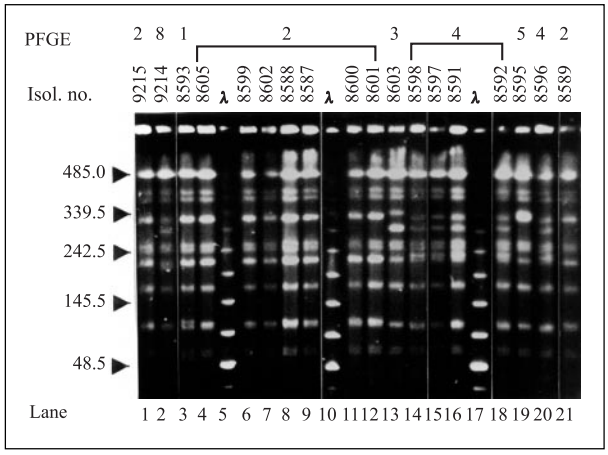

Figure 1. Sample pulsed-field gel electrophoresis (PFGE) gel of representative *Salmonella* Blockley isolates, indicating common and unique DNA fingerprints. Electrophoresis was through 1% agarose/0.5 x TBE, in a CHEF DRIII apparatus (BioRad Laboratories), at 14°C with a 120° switch angle and a run time of 20 hours, with a linear ramp of switching times from 5 to 32 seconds. Gels were stained with 0.5 mg/L ethidium bromide and documented under UV illumination by the EasyWin32 system (HeroLab, Germany). Images were assessed visually, and different PFGE subtypes (A1, A2, ...) were assigned to isolates with electrophoretic patterns differing by one to three DNA fragments (23). Gel images were also processed by the GelCompar software (Applied Maths, Kortrijk, Belgium), and on the basis of PFGE pattern similarities, a dendrogram was constructed by using the Dice coefficient and clustering by the unweighted pair group method, which uses arithmetic averages (UPGMA) with a 2% tolerance in band position difference.
Isol. no.: isolate number. PFGE: subtypes of PFGE type A. The sizes, in kb, of lambda phage DNA concatamers (New England Biolabs) are shown to the left of the gel. All lanes are from the same gel.

fragment differences (Figures 1, 2). Two of the four isolates from previous years belonged to unique subtypes A6 and A7; the other two belonged to subtypes A2 and A8, shared by outbreak isolates (Table). In contrast, 93% of the 1998 outbreak strains yielded PFGE patterns common to two or more isolates. Indeed, most outbreak isolates were grouped in subtypes A2 and A4, consisting of 11 and 12 isolates, respectively (Table).

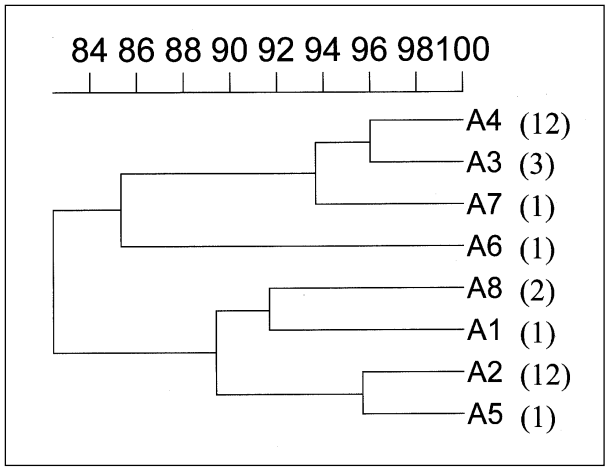

Figure 2. Dendrogram of similarity among the observed pulsed-field gel electrophoresis patterns. A percentage scale of similarity is indicated at the top. Numbers in parentheses refer to the number of isolates with the indicated pulsed-field gel electrophoresis pattern.

Pulsed-field gel electrophoresis subtypes were associated with resistance phenotypes. Most resistance phenotype ATC isolates belonged to subtype A2 (3.03. <RR = 11.86 <46.5, p = 0.0000098), while most resistance phenotype ATCN isolates belonged to A4 (2.76 <RR = 7.11 <18.30, p = 0.0000416). In addition, most isolates in the two major subtypes appearing before August 17, 1998, belonged to the A2 group, while most isolates appearing after August 24, 1998, belonged to the A4 group (1.47 <RR = 9.82 <65.45, p = 0.0006). Finally, unlike A4, the earlier A2 subtype was not isolated in northern Greece.

Conclusions

Our results indicate that PFGE is useful in distinguishing epidemiologically related *S.* Blockley isolates since two of the four nonoutbreak isolates displayed unique PFGE patterns, A7 and A8, while PFGE patterns A2 and A4 grouped most of the 29 outbreak isolates (11 and 12, respectively).

These two chromosomal fingerprints, differing by two DNA fragments, were associated with two distinct resistance phenotypes. The resistance phenotype of A4 isolates, ATCN, was identical to the earlier resistance phenotype of A2 isolates, ATC, except for the resistance to nalidixic acid. Nevertheless, these two PFGE/antibiotic resistance types, A2/ATC and A4/ATCN, displayed a clear distribution both in time and space.

The data may, therefore, indicate two main sources for the outbreak. Alternatively, and perhaps more likely, these two closely related types may together constitute the outbreak clone, evolved with time to acquire resistance to nalidixic acid. Resistance may well have originated in the food source, since several antibiotic classes are used as feed supplements in animal rearing and aquaculture in Greece: sulfonamides (trimethoprim/sulfathiazine), tetracyclines (oxytetracycline), and quinolones (oxolinic acid). However, as in other European countries (17,18), the epidemiologic investigation did not locate a common source to account for the wide geographic spread of cases (19). Although travel was not mentioned in the Greek patients' questionnaire responses, the possibility that the source was an imported food cannot be ruled out. The association with smoked eel of Italian origin in the German outbreak has not been microbiologically confirmed (17). The only other previous European report of a human outbreak attributed to *S.* Blockley, probably from vegetables contaminated by this organism, which was prevalent in irrigation water in the Spanish region of Granada, is anecdotal (13). A documented *S.* Blockley enteritis epidemic in a U.S. hospital in 1966 was attributed to contaminated ice cream; however, this was also not microbiologically confirmed (7).

While this serotype may remain important in Europe, its high rates of resistance to kanamycin, streptomycin, tetracycline, and chloramphenicol, which were in agreement with studies from the Far East (3) and Spain (12), are cause for concern. Unlike the Far Eastern strains, no resistance to ß-lactam antibiotics or cotrimoxazole was observed in our study. The

two dominant resistant phenotypes of *S.* Blockley from natural polluted waters in Spain were sulfonamides, streptomycin, and tetracycline; and neomycin, streptomycin, kanamycin, tetracycline, and chloramphenicol (12), as in the Greek strains, except for the absence of resistance to nalidixic acid.

In agreement with differences in animal reservoirs and transmission routes and therefore the mechanism of resistance acquisition among different *Salmonella* serotypes, the main patterns of resistance observed in *S.* Blockley were distinct from those predominating in the two major serotypes from isolates of both human and animal food origin in Greece. In *S.* Enteritidis, the most frequent resistance phenotype was resistance to ampicillin (24), while in *S.* Typhimurium, the most frequent resistant phenotype was resistance to sulfonamides and streptomycin (A. Markogiannakis, P.T. Tassios, N.J. Legakis, unpub. obs.). Furthermore, the considerably high rate of resistance to nalidixic acid is equally unprecedented in both the Far Eastern and Spanish *S.* Blockley isolates and in other salmonella serotypes from Greece. Since resistance to nalidixic acid can be a precursor of resistance to fluoroquinolones, one of the two drug classes of choice for invasive salmonella disease, this feature of these *S.* Blockley strains is particularly disturbing. *S.* Blockley, previously a prevalent serotype in the Far East but rare elsewhere, nevertheless posed a public health problem in several European countries. The source of the European outbreaks, however, remains unclear. Given the increased international commerce in food, a collaborative study would be useful in identifying potential similarities between the recent European strains and established strains from the Far East.

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Risk Factors for Carriage of *Neisseria meningitidis* during an Outbreak in Wales

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In a school outbreak of meningococcal disease in Wales, we compared risk factors for the carriage of *Neisseria meningitidis* B15 P1.16 with carriage of any meningococci. Students had throat swabs and completed a questionnaire. Sixty (7.9%) carried meningococci; risk for carriage was higher in those >14 years of age.

Outbreaks of meningococcal disease, although rare, may have become more common in the United Kingdom, particularly among teenagers and young adults (1-3). In an investigation of a school-based outbreak in north Wales, extensive nasopharyngeal swabbing and subtyping allowed comparison of risk factors for carriage of the epidemic strain of *Neisseria meningitidis* B15 P1.16 and carriage of other meningococci.

On consecutive days in May 1996, two cases of meningococcal disease were reported in a single year group (year 11, ages 15 to 16 years) in a large (760 students) secondary school. One case was confirmed as due to *N. meningitidis* group B, type 15 P1.16. The second involved characteristic clinical symptoms, although blood culture and polymerase chain reaction (PCR) of serum were negative. In addition, five cases of meningococcal disease from the surrounding areas (total population 8,000) had been reported in the preceding 11 months. Three of these cases were in students of the school. One had been confirmed as *N. meningitidis* B15 P1.16 and one as serogroup C. The observed incidence of notified disease in England and Wales for 1995 was 3.7 per 100,000 and of culture-confirmed disease 2.9 per 100,000 total population (4). We conducted an investigation to determine the prevalence of *N. meningitidis* B15 P1.16 carriage in the school and examine the associated risk factors.

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The Study

Throat swabs from students and staff were spread onto 5% Columbia blood agar containing polymyxin 25,000 units/L and vancomycin 3 mg/L. Primary incubation was conducted at 37°C for 48 hours in 10% CO_2. Plates showing preliminary growth were sent to the Meningococcal Reference Unit at Manchester Public Health Laboratory for further examination and serotyping. Rifampicin was given to students in year 11, which included all those subsequently found to be carriers of the epidemic strain.

Epidemiology

All students from whom a throat swab was taken were asked to complete a questionnaire about personal and household details, lifestyle and social behavior (including travel), and health. A household density ratio was calculated from the ratio of number of household members to the number of rooms in the house. Socioeconomic background was determined by occupation of the head of the household, according to the Office of Population Census and Surveys classification of occupations (5). As stress has been proposed as a risk factor for meningococcal disease (6), we asked about stressful events in the month before the diagnosis of the index cases (e.g., a death in the family, household move, or bad news).

Univariate analysis of risk factors for meningococcal carriage was performed by using Epi-Info (7); the chi-square test was used for statistical significance. Multivariate analysis of carriage of *N. meningitidis* was performed with

SAS (SAS Institute Inc., Cary, NC); variables from univariate analysis were entered into a forward stepwise logistic regression, and conditional odds ratios and 95% confidence intervals were calculated for the resulting significant variables.

Swabs were taken from 744 (97.8%) of 760 pupils at the school. No pupil had received prior antimicrobial chemoprophylaxis. *N. meningitidis* was cultured from throat swabs of 60 (7.9%) students; 33 (55%) were in year 11. Of 17 group B isolates, 12 were type B15P1.16 (Table 1). Of 626 students (83.4%) who completed questionnaires, *N. meningitidis* was isolated from 53 students (8.5%) (Table 1).

The rate of meningococcal carriage was significantly higher in students >14 years of age (Table 2). The proportion of carriers also increased with year in school (chi-square for linear trend 44.3; $p < 0.001$). Although having a stressful event within the previous 3 months was not associated with carriage, specifically receiving bad news was.

Table 1. Subtyping of *Neisseria meningitidis* isolates in a Welsh secondary school

		Student group	
Subtype	Overall	Questionnaire completed	Rate of carriage (%)
B	17[a]	14	2.2
29E	7	6	0.9
C	4	4	0.5
Y	4	4	0.5
Z	2	0	0.3
W135	1	1	0.1
Nontypable	26	24	3.5
Total	61[a]	53	8.1

[a]Including isolate from the single *N. meningitidis*-positive teacher.

Students who reported that they had smoked cigarettes or lived with a smoker were more likely to carry meningococci (Table 3). Students in the same two classerooms and the same year as the index patients were more likely to carry *N. meningitidis*. Attendance at an informal party held by year 11 students 10 days before onset of illness was associated with carriage (Table 3). This informal gathering had no list of invitees; therefore, the number of those who attended but did not have throat swabs taken is not known.

Being in a sports team or regular attendance at youth clubs, Sunday schools, cubs, scouts, brownies, or guides was not associated with carriage, nor was recent travel.

Students who had been in regular contact with one of the patients were more likely to be carriers (Table 4). On multivariate analysis, having more than two smokers in the household, being in the same year in school as the index patients, and having received bad news in the preceding 3 months remained associated with meningococcal carriage (Table 5).

For carriage of the epidemic strain, *N. meningitidis* B15 P1.16, four factors were associated: being in year 11, being older than 14, having attended the end-of-year party, and being male. Because carriage of the epidemic strain was confined to year 11 students (who were >14 years of age), multivariate analysis was not

Table 2. Personal, family, and household factors and meningococcal carriage

	Exposed[a]		Nonexposed			Exposed		Nonexposed		
Variable	Meningococcal carrier	Non-carrier	Meningococcal carrier	Non-carrier	Odds ratio (95% CI)	B15 P1.16 carrier	B15 P1.16 non-carrier	B15 P1.16 carrier	B15 P1.16 non-carrier	Odds ratio (95% CI)
Age >14	40	201	13	372	5.69[b] (2.87-11.49)	11	230	0	384	Undef[b]
Male sex[c]	31	267	22	304	1.60 (0.88-2.95)	9	289	2	324	5.04[b] (1.01-34.07)
Low socioeconomic conditions	21	256	32	317	0.81 (0.67-2.29)	3	274	8	341	0.47 (0.10-1.95)
Shared bedroom[c]	13	106	40	466	1.43 (0.69-2.90)	4	115	7	499	2.48 (0.60-9.62)
Household ratio >0.5	39	382	14	191	1.39 (0.71-2.76)	7	414	4	201	0.85 (0.22-3.53)
≥One child <5 yrs old in same household	1	41	52	532	0.25 (0.01-1.74)	11	573	0	42	Undef
Pet animal[c]	33	408	20	161	0.65 (0.35-1.22)	6	435	5	176	0.49 (0.13-1.88)

[a]Exposure to index patient.
[b]$p < 0.05$.
[c]Data missing.

Table 3. Risk factors for meningococcal carriage in a Welsh secondary school

Variable	Exposed[a] Meningococcal carrier	Exposed[a] Non-carrier	Nonexposed Meningococcal carrier	Nonexposed Non-carrier	Odds ratio (95% CI)	Exposed B15 P1.16 carrier	Exposed B15 P1.16 non-carrier	Nonexposed B15 P1.16 carrier	Nonexposed B15 P1.16 non-carrier	Odds ratio (95% CI)
Year 11[a]	33	96	20	477	8.20[b] (4.34-15.56)	11	118	0	497	Undef[b]
Same classes as index cases[c]	10	42	43	530	2.93[b] (1.28-6.58)	1	121	10	494	0.41 (0.02-3.13)
Smoker	21	136	32	437	2.11[b] (1.12-3.94)	3	154	8	461	1.12 (0.23-4.71)
Other smoker in household	26	202	27	371	1.77[b] (0.99-3.24)	4	224	7	391	1.00 (0.24-3.84)
Student smoker and other in household	12	70	41	503	2.10[b] (1.00-4.41)	2	80	9	535	1.49 (0.00-7.54)
>2 smokers in household[c]	9	37	44	535	2.96[b] (1.24-6.89)	1	45	10	567	1.26 (0.00-12.81)
Football team[c]	6	88	47	415	0.60 (0.22-1.54)	3	91	8	454	1.87 (0.38-8.02)
Rugby team[c]	3	29	50	466	0.96 (0.22-3.51)	1	31	10	506	1.63 (0.00-13.27)
Hockey team[c]	3	29	50	467	0.97 (0.22-3.52)	1	31	10	507	1.64 (0.00-13.30)
Netball team[c]	1	54	52	452	0.16 (0.01-1.13)	0	55	11	493	0.00 (0.00-4.46)
Any sport	10	172	43	401	0.54 (0.25-1.16)	3	179	8	436	0.91 (0.19-3.83)
Regular youth club[c]	21	180	32	341	1.24 (0.67-2.31)	3	198	8	365	0.69 (0.14-2.93)
Regular disco[b]	30	279	23	248	1.16 (0.63-2.14)	4	305	7	264	0.49 (0.12-1.92)
Attendance at party[c]	23	111	29	461	3.29[b,c] (1.75-6.18)	8	126	3	487	10.31[b] (2.42-50.34)
Cubs/brownies/ scouts/guides	1	42	52	531	0.24 (0.01-1.72)	0	43	11	572	0.00 0.00-0.00
Life event	18	146	35	427	1.50 (0.79-2.86)	2	162	9	453	0.62 (0.00-3.15)
Death in family[c]	4	56	49	516	0.75 (0.22-2.30)	0	60	11	554	0.00 (0.00-4.58)
Change of house[c]	5	33	48	539	1.70 (0.55-4.89)	0	38	11	576	0.00 (0.00-7.63)
Received bad news[c]	15	79	38	493	2.46* (1.22-4.91)	2	92	9	522	1.26 (0.00-6.46)
Travel[c]	6	107	47	464	0.55 (0.21-1.40)	1	112	10	501	0.45 (0.02-3.48)

[a]Same school year as index case.
[b]$p < 0.05$.
[c]Data missing.

Table 4. Medical factors associated with meningococcal carriage in a Welsh secondary school

Variable	Exposed[a] Meningococcal carrier	Exposed[a] Non-carrier	Nonexposed Meningococcal carrier	Nonexposed Non-carrier	Odds ratio (95% CI)	Exposed B15 P1.16 carrier	Exposed B15 P1.16 non-carrier	Nonexposed B15 P1.16 carrier	Nonexposed B15 P1.16 non-carrier	Odds ratio (95%CI)
Recent illness	43	482	10	91	0.81 (0.37-1.80)	7	518	4	97	0.33 (0.08-1.37)
Recent injury	10	127	43	446	0.82 (0.37-1.75)	0	137	11	478	0.00 (0.00-1.71)
History of tonsillectomy/ adenoidectomy[a]	8	60	45	511	1.51 (0.62-3.56)	3	65	8	548	3.16 (0.64-13.68)
Close contact with case of meningococcal disease[a]	12	72	40	496	2.07[b] (0.99-4.34)	3	81	8	528	2.44 (0.50-10.51)

[a]Data missing.
[b]$p < 0.05$.

Table 5. Factors remaining significant for meningitidis carriage in final model

Variable	Odds ratio (95% CI) unadjusted	Odds ratio (95% CI) adjusted[a]
Year 11[b]	8.20 (4.34-15.56)	8.61 (4.66-15.91)
>2 smokers in household	2.96 (1.24-6.89)	2.99 (1.25-7.15)
Received bad news	2.46 (1.22-4.91)	2.67 (1.32-5.40)

[a]adjusted for other significant variables.
[b]same school year as index case.

performed. Within year 11, no single factor was associated with carriage of *N. meningitidis* B15 P1.16.

Conclusions

In February 1995, we investigated risk factors for the carriage of any meningococci among the contacts of three ill students at another Welsh secondary school (8). These index patients were students in different school years, and 2 (1.7%) of 119 contacts carried the epidemic strain (type B2b P1.10). In the current outbreak, we identified 11 (1.5%) of 744 students with the epidemic strain (type B15 P1.16). In contrast, outbreaks of disease associated with serogroup C disease are typically accompanied by lower rates of carriage in populations at risk. However, outbreaks of group C disease involving higher carriage rates are occasionally described. In an outbreak at an agricultural college in England, carriage of the epidemic strain of serogroup C organisms among students and staff was 6.2% (9). In the current outbreak, the fact that all carriers of the epidemic strain were in the same school year as the two index patients enabled us to examine risk factors for carriage of the epidemic strain. Risk factors for the carriage of any meningococci may differ. For example, in an outbreak of six cases among first-year students at Southampton University (United Kingdom) in 1997, 0.9% of students surveyed carried serogroup C strains; however, no first year students were carriers (10).

Better knowledge of risk factors for carriage of epidemic meningococci may help identify close contacts who are candidates for antimicrobial therapy to eliminate nasal carriage and prevent spread of disease (11). Carriers of an epidemic strain with the potential to infect others may be missed, and a number of people may receive unnecessary antibiotics, which has implications for the spread of antibiotic resistance.

Carriage of any meningococci was associated in the univariate analysis with the well-described risk factors of increasing age and smoking (active and passive) (12-14). Smoking may predispose to colonization by inhibiting bronchial ciliary action (12). We also found increasing age and active smoking to be associated with carriage in our previous study (8). In the current study, receipt of bad news was associated. No obvious biological mechanism exists to explain what may be a chance finding, although recent stress has been described as a risk factor for meningococcal disease (6). Being in the same year as the index patients and attending the end-of-year party were risk factors for carriage of any strain of meningococci in this outbreak, mainly because these were the only two risk factors associated with carriage of the epidemic strain. These two factors reflect the kinds of social contacts among teenagers and young adults that may permit spread of meningococci. In a review of 22 school-based clusters between 1989 and 1993, the patients in nine of the clusters had contact through extracurricular activities; these activities in four clusters were parties or dances (15). Patronage of a particular bar was implicated in the university outbreak in Illinois (16), and a particular discotheque in an outbreak among eight adults (five of whom were teenagers) in Corrientes, Argentina (17). Such social settings differ from the residential settings, of outbreaks among military recruits and prisoners, where overcrowding and proximity of beds may permit transmission (18).

Attendance at the party may have been the critical factor in carriage of the epidemic strain among year 11 students. However, another hypothesis may account for our observations. Young adults who socialize frequently at discotheques and parties may, particularly if they smoke, be at higher risk for carriage of meningococci of all types. This increased long-term risk for disease may have a protective effect against a virulent outbreak strain (19). In contrast, those who participate infrequently in social events such as the end-of-year party may be at higher risk. This hypothesis may explain some of the risk for first-year university students. In addition to longitudinal studies,

combining the results of surveys during outbreaks may help provide a more scientific basis for the management of future outbreaks.

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Salmonellosis in the Republic of Georgia: Using Molecular Typing to Identify the Outbreak-Causing Strain

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In May 1998, three large outbreaks of salmonellosis, affecting 91 persons, were identified in the Republic of Georgia. Eighteen *Salmonella* Typhimurium strains were characterized by arbitrary primed polymerase chain reaction and pulsed-field gel electrophoresis; the results suggested that all cases were part of a single outbreak caused by a distinct clonal strain.

Salmonella species, which cause a variety of clinical manifestations from mild gastroenteritis to septicemia, are one of the leading causes of foodborne illness worldwide (1-2). Approximately 50% of cases of human disease caused by salmonellae are produced by *S.* Enteritidis and *S.* Typhimurium (3). *S.* Typhimurium is of particular concern because of the recent emergence of a highly antibiotic-resistant strain (resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) designated as definitive type (DT) 104 (4). In approximately 75% of human *Salmonella* cases, the bacterium is acquired from meat, poultry, or eggs (2,3).

The Study

In May 1998, three apparently distinct outbreaks of salmonellosis were identified in the Republic of Georgia. Symptoms in all three included acute diarrhea (100% of cases), severe stomach cramps (35%), nausea and vomiting (22%), and fever (97%). The first outbreak affected children attending a birthday party in Kojori, a suburb near Tbilisi, Georgia. Ten of 14 children attending the party came down with acute diarrhea and were hospitalized within 3 days. *S.* Typhimurium was isolated from stool samples of four patients.

The second outbreak, 4 days later, affected guests at a wedding reception in the village of Asureti (40 kilometers east of Tbilisi). Fifty of approximately 100 guests had diarrhea, and 18 were hospitalized. Salmonellosis was confirmed by the isolation of *S.* Typhimurium from seven of the hospitalized patients.

The third outbreak occurred in Tbilisi approximately 14 days after the first outbreak. Thirty-one of 50 guests at a birthday party were hospitalized for acute diarrhea within 3 days. Salmonellosis was culture-confirmed in 14 cases (i.e., *S.* Typhimurium was isolated from 14 of the patients). All persons with diarrhea in all three outbreaks took various doses of antibiotics (ciprofloxacin or ceftriaxone) when they first became ill.

All 59 hospitalized persons were interviewed to determine a possible common source of infection. In addition, 32 persons who had diarrhea but were not hospitalized and 71 healthy guests were interviewed. Eggs were

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implicated in the first outbreak; the eggs were used to prepare uncooked icing for a homemade cake. In the second outbreak, eating chicken served during the wedding banquet was associated with illness. In the third outbreak, chicken from a farmers' market in Tbilisi was implicated. No food samples were available for bacteriologic analysis.

Stool samples from the 59 hospitalized patients were examined for *Salmonella*, *Shigella*, and *Yersinia* by standard techniques. *Salmonella* were isolated from stool samples of 25 (42%) of the patients; all 25 isolates were identified as *S.* Typhimurium strains by the API20E test system, and 18 of them were randomly chosen for subsequent analysis. No other pathogen was isolated.

Arbitrary primed polymerase chain reaction (AP-PCR) was performed by using an RAPD kit (Amersham Pharmacia Biotech, Piscataway, NJ). All typing was done with primer #6 of the kit (5'- CCC GTC AGC A - 3'), which gave distinctive, reproducible patterns with three or more major bands. Bacterial DNA for AP-PCR was obtained, and amplification was performed (5). After the amplification cycles, the samples were incubated at 72°C for 5 minutes and analyzed by electrophoresis in 2% agarose gel in TAE buffer.

The rapid pulse-field gel electrophoresis (PFGE) procedure developed for typing *Escherichia coli* O157H7 strains was used for PFGE typing of the outbreak strains (6). The strains were analyzed, in separate experiments, by digesting DNA with *Xba* I, *Avr* II, and *Spe* I restriction enzymes, and *Xba* I-digested *S.* Newport strain amO1144 was used as the reference strain in all experiments (Figure 1).

A distinct pattern was observed in all 18 strains when they were analyzed by AP-PCR (data not shown), which suggests close genetic relatedness. This finding was confirmed by PFGE typing, i.e., *Xba* I-, *Avr* II-; *Spe* I- macrorestriction patterns generated by PFGE showed that all strains tested had an identical PFGE pattern. The pattern was distinct from those obtained for 18 other *S.* Typhimurium strains isolated in the Republic of Georgia at about the same time, which were grouped into five PFGE types distinct from that of the outbreak-causing strain.

The national molecular subtyping network, PulseNet, includes a database of the PFGE patterns of *E. coli* O157 and *Salmonella* group B and D strains isolated in the United States. We screened local databases of two public health laboratories in Washington and Maryland that participate in PulseNet, and we used the Internet to compare the PFGE pattern of the Georgian outbreak strain with *Salmonella* patterns obtained in these two laboratories. At least three strains among >300 *S.* Typhimurium isolates (grouped into approximately 50 PFGE types) in the two databases were almost identical to the Georgian isolate. However, the PFGE patterns of the strains differed slightly when their DNA was digested with *Avr* II before PFGE. The patterns obtained by AP-PCR and PFGE (*Xba* I- and *Spe* I-digests) were almost indistinguishable; however, when DNA of the same strains was analyzed by PFGE after digestion with *Avr* II, differences were detected. For example, the PFGE pattern of strain 00354 (a Seattle isolate) had a large fragment not present in the Georgian outbreak strain and had lost one small fragment (Figure 2, lane 5). In addition, the PFGE pattern of strain 01587 (also

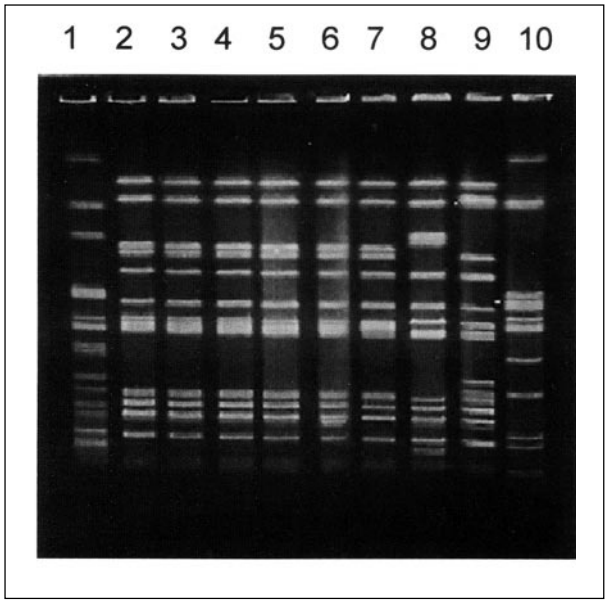

Figure 1. Pulse-field gel electrophoresis patterns of *Xba* I-digested DNA of *Salmonella* Typhimurium strains. Lane 1, *Xba* I-digested *S.* Newport control strain am01144; lanes 2 through 4, *S.* Typhimurium strains isolated during the first, second, and third outbreaks in Georgia, respectively; lane 5, strain 00354 (Washington isolate); lane 6, strain 01587 (Washington isolate); lane 7, 9294-99 (Maryland isolate); lanes 8, 9, and 10, genetically unrelated control *S.* Typhimurium strains isolated in Maryland, Washington, and the Republic of Georgia, respectively.

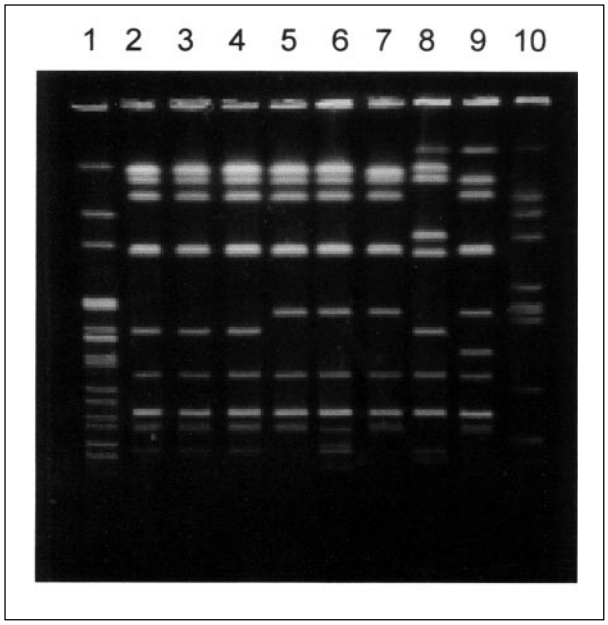

Figure 2. Pulse-field gel electrophoresis patterns of *Avr* II-digested DNA of *Salmonella* Typhimurium strains. Lane 1, *S.* Newport control strain am01144 (*Xba* I-digest); lanes 2 through 4, *S.* Typhimurium strains isolated during the first, second, and third outbreaks in Georgia, respectively; lane 5, strain 00354 (Washington isolate); lane 6, strain 01587 (Washington isolate); lane 7, 9294-99 (Maryland isolate); lanes 8, 9, and 10, genetically unrelated control *S.* Typhimurium strains isolated in Maryland, Washington, and the Republic of Georgia, respectively.

from Seattle) lacked one fragment present in the Georgian outbreak strain and had two other small fragments (Figure 2, lane 6). These differences may result from a single genetic event in the bacterial DNA and are associated with a spontaneous point mutation resulting in either creation or loss of a restriction site. Therefore, the Seattle and Maryland isolates were classified as closely related (7,8) to the Georgian outbreak strain. In Washington, the strain was associated with severe diarrhea like that of the Georgian patients, which suggests that the strain was strongly virulent. (Clinical data for the Maryland isolate were not available.) Detailed evaluation of the pathogenic potential of the outbreak strain (and the closely related U.S. isolates) will require determination of the possible links between unusually severe cases of salmonellosis and isolation of *S.* Typhimurium strains having closely related PFGE patterns, and testing the strain for virulence in laboratory animals.

The outbreak-causing *S.* Typhimurium strain was resistant to ampicillin, chloramphenicol, streptomycin, and tetracycline, but was susceptible to cephalosporins and sulfonamides. Two of the above closely related strains (00354 and 9294-99) had an antibiotic-susceptibility pattern similar to that of the Georgian outbreak strain. The only difference in the pattern for the third closely related strain (01587) was that it was susceptible to chloramphenicol.

Conclusions

Epidemiologically, the outbreaks had no obvious connection. Patients were hospitalized in two different hospitals in two different cities and were not associated with more than one outbreak. In addition, no common source of infection could be identified. Thus, the three outbreaks were initially thought to be separate. However, the observation that they occurred within a short period (2 weeks between the first and third outbreaks), were caused by the same serotype of *Salmonella*, were associated with severe diarrhea, and were limited geographically to a 80-kilometer radius raised the possibility that the outbreaks may have been related. Therefore, we used molecular typing techniques to characterize the *Salmonella* strains isolated from the patients in each outbreak. Our observations suggest that what appeared to be three distinct outbreaks of salmonellosis were, in reality, parts of one large outbreak caused by a distinct clonal strain of *S.* Typhimurium. The common source of infection and the transmission route for the outbreak-causing *Salmonella* strain are not known.

The clinical diagnosis of salmonellosis was confirmed by isolating *S.* Typhimurium from 25 (42%) of the hospitalized patients. All the patients had treated themselves with ciprofloxacin and ceftriaxone (readily available without prescription in Georgia) before hospitalization, which may have contributed to our inability to isolate *Salmonella* (or other potential pathogens) from most of them. Fifty-nine (65%) of 91 persons diagnosed as having salmonellosis had diarrhea severe enough to require hospitalization. The hospitalization rate of these patients was approximately seven times higher than the usual hospitalization rate (<10%) for *Salmonella* cases in Georgia and approximately three times higher than the average hospitalization rate reported for *Salmonella* patients in the United

States (9). Although some persons with diarrhea may have requested hospitalization after hearing that other ill guests were hospitalized, the clinical reports indicate that the high hospitalization rate reflects a severe manifestation of the disease. Since the AP-PCR and PFGE typing results suggested that a single clone was the causative agent in all cases, the question of its possibly increased virulence arose. It has recently been reported (J.G. Morris, et al., unpub. data) that some *Salmonella* serotypes/strains cause more severe illness. The recent emergence of *S.* Typhimurium DT104 strains having increased virulence (4), in addition to being multidrug resistant, also highlights the possibility of supervirulent strains emerging worldwide.

S. Typhimurium, in contrast to *S.* Enteritidis, which is a highly clonal organism (5), is a fairly diverse serotype (10). Therefore, detection of closely related *S.* Typhimurium strains in geographically distinct loci (the Republic of Georgia, and the East Coast and West Coast of the United States) may signal worldwide spread or emergence of closely related clonal groups of *Salmonella* having increased virulence. This possibility may be confirmed by worldwide (or nationwide) standardization of molecular typing protocols and further strengthening of data-sharing capabilities between laboratories involved in the molecular typing of pathogenic microorganisms. An example demonstrating the potential value of such a database is our finding, during screening of the two participating PulseNet laboratories, that two seemingly unrelated strains (Seattle isolate 00354 and Maryland strain 9294-99) were clonal. As described above, this observation was confirmed by both antibiotic-susceptibility testing and side-by-side molecular typing of the strains in question. An epidemiologic link between the two isolates is being investigated.

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First Report of Q Fever in Oman

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Although serologic evidence suggests the presence of Q fever in humans and animals in Saudi Arabia and the United Arab Emirates, acute Q fever has not been reported on the Arabian Peninsula. We report the first two cases of acute Q fever in Oman.

Acute Q fever has not been reported on the Arabian Peninsula; however, serologic studies have documented *Coxiella burnetii* in eastern Saudi Arabia. In Riyadh, in the late 1960s, 8.4% of residents >5 years of age were seropositive (1). Veterinary studies in Saudi Arabia have shown that Q fever occurs in livestock (2) and in Arabian oryx (*Oryx leucoryx*) at the National Wildlife Research Centre at Taif (3). In Abu Dhabi, United Arab Emirates (UAE), the disease was reported in racing camels (4).

Acute Q fever is usually a nonspecific febrile illness, often with atypical pneumonia or transient hepatitis. Seroconversion without symptoms is common, especially in children (5). As a rule, it is self-limiting and resolves without treatment, but some untreated cases may progress to chronic Q fever (endocarditis, granulomatous hepatitis, osteomyelitis, interstitial pulmonary fibrosis) (6). Pericarditis is rare (7), mostly reported from France (6,8) and Spain (9), and sporadically worldwide (10).

In acute Q fever, diagnosis is supported by a fourfold increase in antibody titers to *C. burnetii* in acute- and convalescent-phase serum samples by complement-fixation test (CFT) or indirect immunofluorescence assay (IF). The IF is more sensitive and specific than CFT in chronic Q fever. In the National Reference Center in France, the diagnosis of acute infection is supported by IgG phase II $\geq$ 1:200, IgM phase II $\geq$ 1:50; and of chronic disease, IgG phase I $\geq$ 1:800 (11). Diagnosis can also be made by identifying *C. burnetii* in tissue culture or its antigens by polymerase chain reaction (PCR) in biopsy specimens. We report the first two human cases of Q fever in Oman.

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Case 1

A 45-year-old Omani woman was hospitalized on December 13, 1997, with a 4-week history of fever and productive cough. The patient's temperature was 39°C, she was jaundiced, and she had signs of right lower lobe pneumonia, which was confirmed by X-ray (Figure 1).

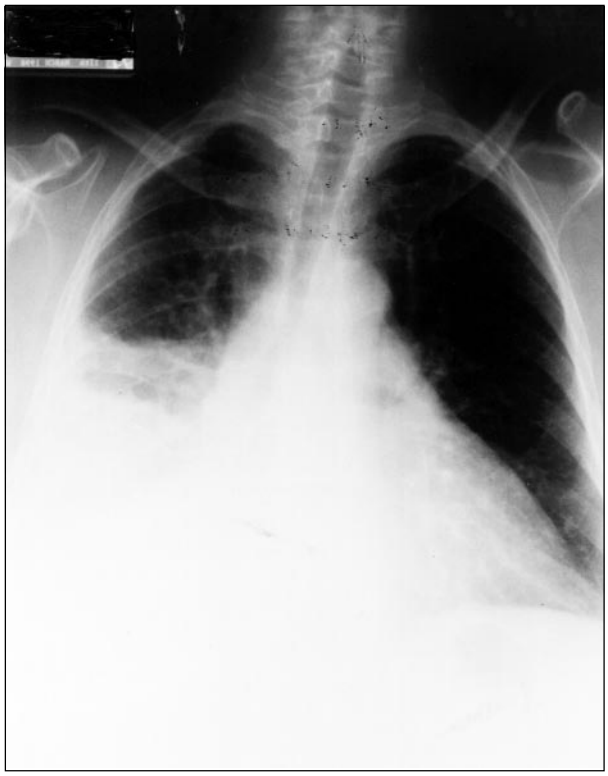

Figure 1. Q-fever pneumonia.

Hemoglobin was 9.5 g/dL, white blood cells 6.6 x 10^9/L (neutrophils 76%, lymphocytes 14.2%), platelets 317 x 10^9/L, and erythrocyte sedimentation rate 101 mm/hour. An electrocardiogram showed nonspecific abnormalities, but an echocardiogram revealed a small pericardial effusion. Liver function tests showed normal serum bilirubin, serum aspartate aminotransferase 111 µ/L (normal range 14-42 µ/L), serum alkaline phosphatase 256 µ/L (normal range 3-210 µ/L), and serum albumen 26 g/L. Community-acquired pneumonia, with pericarditis and hepatitis, was diagnosed, and the patient was treated with intravenous erythromycin, 500 mg 4 times a day.

Since the patient lived on a farm with cattle and goats, Q fever was considered, although *C. burnetii* infection had never been reported in Oman. Sera were sent to England for Q-fever antibody tests, which were not available locally. The patient became afebrile, signs of pneumonia resolved, and she was discharged on December 31, 1997 (before serologic results were received).

On January 12, 1998, the patient had signs of constrictive pericarditis and small bilateral pleural effusions. The results of CFT for Q fever were total antibody titers to phase-II antigen ≥ 1:80 (threshold ≥ 1:10). Phase-I antigen and immunoglobulin (Ig) M tests were not performed, but the results indicated recent Q-fever infection, so doxycycline (100 mg twice a day) was prescribed. Repeat serologic tests (January 12, 1998) revealed total CFT antibody titers to phase-I and phase-II IgG antigens ≥ 1:40 (threshold ≥ 1:10); and IgM and IgA < 1:10 (threshold ≥ 1:10). These results were interpreted as evidence that *C. burnetii* infection was the probable cause of chronic pericarditis.

Echocardiography revealed a grossly thickened pericardium with abnormal septal motion encroaching on the right ventricular cavity in diastole, and the CT scan confirmed a diffusely thickened pericardium (Figure 2). Cardiac catheterization revealed normal left ventricular systolic function, but the central venous pressure was 26 cm. On April 5, at pericardiectomy, the pericardium was 1 cm thick and densely adherent to the heart. Histopathologic examination revealed granulomatous pericarditis without evidence of tuberculosis (Figure 3). The patient recovered well after surgery.

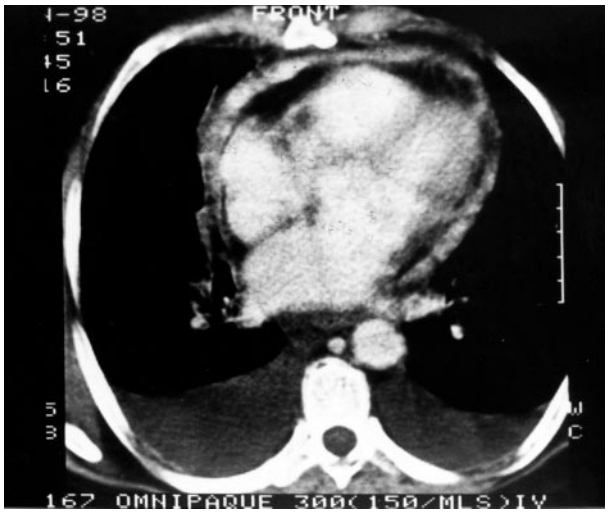

Figure 2. CT scan of heart, showing thickened pericardium.

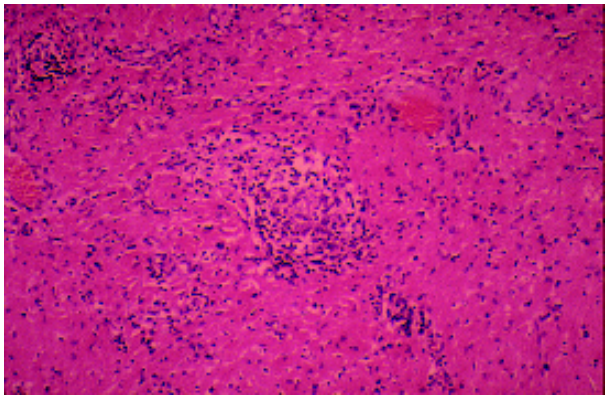

Figure 3. Histologic results of pericardial biopsy, which show granulomatous pericarditis.

Case 2

A 50-year-old Omani man from a rural area was hospitalized on June 2, 1999, with a 3-week history of fever of unknown cause, not responding to cefuroxime. Hematologic examination revealed hemoglobin 13 g/dl, white blood cells 6.7 x 10^9/L (neutrophils 53%, lymphocytes 37%, monocytes 9%), platelets 195 x 10^9/L, and erythrocyte sedimentation rate 2 mm/hour. Signs of bilateral basal pneumonia developed, and Q-fever serologic tests were positive: total antibody titers to phase-II antigen by IF ≥ 1:1280 (threshold ≥ 1:80), and IgM titer ≥ 1:320 (threshold ≥ 1:20). After treatment with doxycycline, the patient's symptoms resolved. Repeated serologic results were total IF antibody

titer 1:2560 and IgM titer 1:160, evidence of the convalescent phase of infection.

Conclusions

In most countries on the Arabian Peninsula, including Oman, most people live in rural areas and are exposed to livestock. In countries where it is endemic, Q fever usually occurs sporadically and may be underdiagnosed and underreported (6,8). In Zimbabwe, where acute Q fever had never been reported, a recent community survey revealed *C. burnetii* antibodies in 37% of humans, 39% of cattle, and 10% of goats (12).

In Case 1, pericardial involvement in the acute stage of Q fever likely progressed to chronic constrictive pericarditis because diagnosis and appropriate treatment were delayed. For acute Q fever, the treatment of choice is doxycycline or orfloxacin, alone or in combination with rifampicin, for 21 days. For treatment of chronic Q fever (e.g., with endocarditis), doxycycline and a quinolone for a minimum of 3 months have been advocated (5-7).

Q fever may be widely endemic but undiagnosed in livestock and domestic animals in Oman. Similarly, the evidence suggests that acute Q fever is prevalent, but undiagnosed (or unreported) in other Arabian Peninsula countries, including Saudi Arabia. Diagnostic serologic tests are not yet available in Oman but will be introduced soon, which will permit us to determine prevalence and distribution of *C. burnetii* in humans and in livestock.

Acknowledgments

The authors thank Didier Raoult, Centre for Rickettsial Studies, University of Marseilles, for PCR testing of the biopsy specimen. The Marcel Merieux Laboratory, Lyon, France, conducted the immunofluorescence tests, and Elizabeth Richens provided sera from Case 1 for immunologic studies.

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The Philosophic Origins of Science and the Evolution of the Two Cultures[1]

Ntinos C. Myrianthopoulos

> "A small but growing number of American philosophers have opened private practices as 'philosopher practitioners' offering a therapy based on the idea that solutions to many personal, moral, and ethical problems can be found not in psychotherapy or Prozac but deep within the 2,500-year-old body of philosophical discourse."

This quotation from the New York Times of March 8, 1998, may have been startling to some and amusing to others—the New Yorker used it as a preamble to a humorous article—but not to anyone, myself included, who has enjoyed the pleasure of delving into the history of philosophy and who appreciates its relevance to the scientific process. What a splendid opportunity, then, to explore the philosophic origins of science and its long and fruitful legacy.

The quest for knowledge is an old preoccupation with roots in prehistory, starting with Adam and Eve, who did not go about it scientifically—and you know what happened to them. Even so, ancient humans continued the quest to understand and study the nature around them: the trees and the animals, the bearing of children, the heavenly bodies; that is, the natural phenomena that today we refer to as the natural sciences.

Among the old civilizations, the Babylonians and Egyptians contributed considerably to these sciences, practiced primitive medicine and surgery, and collected facts about natural history and biology. It was, however, left to the Greeks to enlarge the scope of these collections and formulate from the facts a unified concept of nature and the laws that govern it.

The oldest Greek thinkers were natural philosophers, and it was much later that ethical issues and other problems found a place in Greek thought. Practically all philosophers were teachers; many had their own schools, had to teach several subjects (rhetoric, ethics, poetics, astronomy, physics, biology), and wrote treatises on these subjects, which were surely sometimes used as textbooks. A few of these philosophers were also poets and wrote their own books in verse; Empedocles, for example, wrote two treatises, "On Nature" and "Purifications," in dactylic hexameter. I shall mention only one of these early philosophers, Democritos (approximately 450 BC), for two reasons. The first is that he was the very antithesis of the usual image of a brooding philosopher; he was known by several nicknames, one being "the laughing philosopher" because he was good humored and jolly all the time (γελουσινός). In fact, one of his noteworthy treatises is entitled "On Cheerfulness." The other reason is that to most of us Democritos is known mainly as the father of the atomic theory, which is not quite the case. It was his teacher, Leukippos, who first conceived and formulated the atomic theory, which Democritos immediately espoused and refined. He wrote on almost every field known at the time: physics, psychology, logic, astronomy, the senses, the mind, music, poetics. Aristotle thought highly of Democritos and refers to his work frequently, particularly in his various biological writings.

Aristotle was certainly the greatest of this genre of philosophers and is justly considered one of the greatest thinkers of all time. He was born in 384 B.C. in Stageira, Macedonia, where his father was the physician to the royal court. At age 17, he was sent to Athens to study with Plato in the Academy, where later he also taught. He spent 3 years in Asia Minor in the court of his former student Hermeias, who gave him his niece in marriage. He was then appointed by King Philip of Macedon to be the tutor of his impetuous and brilliant teenaged son Alexander, and after 3 years, he moved back to Athens. Now

[1]Aristotelian lecture given at the 9th International Clinical Genetics Seminar, July 4, 1998, Limassol, Cyprus. Parts of this lecture were published in "Cosmos, 1999," the Journal of the Cosmos Club of Washington, D.C.

a well-to-do man under the protection of Alexander, he founded his own school, the Lyceum (named after the nearby temple of Apollo Lycaeus). The Lyceum served as the prototype of a learned educational institution throughout the world. Here Aristotle taught while taking walks with his students and collaborators and wrote an incredible amount on various and different subjects, including logic, physics, ethics, art poetry, politics, economics, psychology, and biology. He retired to Chalkis, Euboea, where he died at age 62.

To the biologist, Aristotle's work on the "Generation of the Animals" is of special interest because it is the first systematic treatise on animal reproduction and embryology, taxonomy, and evolution. The "Generation of the Animals" is the culmination of Aristotle's zoological works that comprise 10 volumes and include "On the History of Animals," "On the Parts of Animals," and "On the Soul." I shall only touch upon those areas in which he made lasting contributions in the development of the biological sciences that have come down to us.

Aristotle repeatedly pointed out that his predecessors' work and conclusions were often marred by insufficient observation. He himself, after a remarkable analysis of the reproduction of bees, states that he cannot arrive at certain conclusions because "the facts have not yet been sufficiently ascertained. And if at any future time they are ascertained, then credence must be given to the direct evidence rather than to the theories; and to the theories also, provided that the results which they show agree with what is observed." This, indeed, is the principle upon which his work is based. It is also the definition of the scientific method, which was later broadened in scope, especially by Bacon, and by and large constitutes the basis of the scientific method we practice today. Note the subtle yet critical point: Aristotle does not say "the results prove the theory," but "the results agree with the observations." Today, we take this reasoning for granted, that science proceeds and progresses not by proving hypotheses, but by disproving them. If the observations do not agree with a hypothesis, we shelve it; if it does agree with a high enough level of certainty and consistent repetition of the results, we accept it, but we can never prove it.

Up to the time of Aristotle, there had been no serious attempts at classification of animals. Thus, his classification was based almost entirely on his own observations. For animals not found in Greece, he referred to credible observations by others, e.g., Herodotos. In this area also, Aristotle made very important contributions by characterizing and differentiating among a number of systematic categories. In his own words, "Animals may be characterized according to their way of living, their actions, their habits, and their bodily parts." The most important criterion is certainly the parts of the animals, both external and internal: organs of movement, respiration, sense, blood circulation. By combining various qualities, he defined and characterized the groups. Aristotle's two major categories are blooded animals (he refers to red blood only) and bloodless animals.

Under blooded animals: humans, viviparous quadrupeds, oviparous quadrupeds, and footless animals (reptiles, amphibians), birds, and fishes.

Under bloodless animals: malacostraca (soft-shelled, crustacea); malakia (soft, without shell, cephalopods); entoma (insected animals, insects); and ostrakoderma (shell-skinned, testacea). These categories and nomenclature are still used today.

Aristotle also classified animals according to their mode of reproduction, but the most important part of his classification is the final two categories, the genus (γένος) and the species (εἶδος), the latter referring to the individual animal form: horse, dog, lion. This is a farsighted classification, and though it cannot be compared with the Linnaean with its manifold categories, it is certainly a pioneering achievement.

In his work on the reproduction of animals, Aristotle differentiates sexual and asexual reproduction. In sexual reproduction, male and female contribute equally, and in his thorough investigation of the development of animals from egg and embryo, Aristotle points out the phenomenon that we know today as "ontogeny recapitulates phylogeny." He disagreed sharply with the opinion of earlier philosophers that the seed is derived from all parts of the body and thus gives rise to similar individuals. On the contrary, he asserts that the seed goes to all parts of the body to form an individual, an explanation shown to be correct 2,000 years later. In addition, during embryonic development, there is a specific movement or substance in each part of the body which brings about its development as a specific part of the embryo. Today, we call such

substances organizers. Aristotle also recognized congenital malformations as imperfect developmental events in the embryo due to various causes, one being some irregularity in the seminal fluid. He correctly understood the functions of the placenta and the umbilical cord and was an ardent supporter of epigenesis. He made his observations in several animals, and it can be said that he introduced the comparative method to embryology.

Aristotle has been called the first evolutionist. His theory of evolution lies not only in the sphere of discovery, but also in his system of thought, embracing all phenomena of life. Here we find enunciated for the first time a truly complete theory of evolution, subject to natural laws and progressing from the lower to the higher forms of being. Although partly based on metaphysical speculation, the theory has proven fertile ground for future biologists.

Aristotle constantly compares nature and the products of nature with art and the products of art. Like nature, the artist or craftsman works to produce a finished product. Like the artist, nature uses instruments charged with specific modulations to bring these products to fulfillment. The most typical of these products of nature are, of course, living creatures. Nature aims always to produce a finality in the sense of a completely formed individual and that is the Final Cause in each case. "There is," Aristotle says, "more beauty and purpose found in the works of nature than in those of art." And who can disagree?

Although Aristotle was not the last of the era in which the study of nature was in the province of philosophy, by the time of his death, there were already signs of specialization, that is, philosophers began to be concerned mainly with ethics and metaphysics, leaving the other subjects to those more informed about them. This trend continued through the Hellenistic and Roman times. Later, with the increase of knowledge and ease in its dissemination, the establishment of libraries, and invention of the printing press, the graduates of schools of higher education came to be recognized either as scientists, biological or physical, or as artists, poets, writers, painters, or musicians and received different credentials, in our time Bachelor of Arts or Bachelor of Science. In spite of the widening schism, the philosophic origin of the sciences and the arts is acknowledged and maintained today in the award of the highest academic degree, that of Doctor of Philosophy.

In his controversial Rede lecture presented in Cambridge in 1959, C.P. Snow (1905-1980), the British intellectual, first used the phrase "The Two Cultures" to describe the world of the sciences and the world of the arts, which had become culturally isolated. Sir Charles Percy Snow was himself a distinguished physicist, who during World War II made significant contributions to the British and Allied war effort and for his services was elevated to the peerage. He was also an excellent novelist. His magnum opus, "Strangers and Brothers," comprises eleven volumes written over 30 years, in which he recounts a saga of lives, events, and the passage of time, both for individuals and for English society as a whole. As Snow himself described his existence, "There have been plenty of days when I have spent the working hours with scientists and then gone off at night with some literary colleagues. I mean that literally. It was through living with these groups and much more, I think, through moving regularly from one to the other and back again that I got occupied with the problem of what, long before I put it on paper, I christened to myself as 'the two cultures.' For constantly I felt I was moving among two groups—comparable in intelligence, identical in race, not grossly different in social origin, earning about the same incomes, who have almost ceased to communicate at all, who in intellectual, moral and psychological climate had so little in common, that instead of going from Burlington House or South Kensington to Chelsea, one might have crossed an ocean."

Snow, of course, was addressing a situation prevalent in England and Europe in general in the late 1950s, but at that time, conditions on this side of the Atlantic may have been a little better. Nevertheless, the two cultures still exist and combine infrequently in rare and exceptional individuals.

Every era has had such exceptional individuals. The Renaissance produced a sprinkling of them, the towering and awesome figure of that era being Leonardo da Vinci (1452-1519), the Italian painter par excellence, and sculptor, but also architect, engineer, musician, inventor, anatomist, physiologist, geologist, botanist, and everything that you can imagine, and many things you cannot. Indeed, a thumbing through his voluminous diaries, originally

written in "looking-glass writing," will find him discussing, in addition to the subjects that I have already referred to, philosophy, geography, flight physics, mathematics, warfare, sailing, and scores of prophesies on scores of subjects. While in the long service of Ludovic Sforza in Milan, in addition to art, he performed various functions, including tutoring; designing churches, scientific instruments, and war machines; and organizing spectacular pageants. His biological writings constitute about 20% of his total output. They encompass discourses on anatomy, physiology, medicine, optics, and acoustics and are illustrated with exquisite drawings, which he did from his own observations and dissections. He dissected more than 30 bodies of men and women of all ages and made drawings and casts of anatomical structures, most of them at the hospital of Santa Maria Nuova in Florence and at the University of Pavia, where his friend Antonio della Torre held the chair of anatomy. It is impossible to discuss this work at length, but I would like to quote his ruminating on the nervous system: "The frog instantly dies when the spinal cord is pierced. Previous to this it lived without head, without heart, or any bowels or intestines or skin. And here, therefore, it would seem lies the foundation of movement and life."

Leonardo's counterpart in the 20th century is undoubtedly Albert Schweitzer (1875-1965), the Alsatian physician, philosopher, musician, theologian, and great humanitarian. Those of our generation who recognize his name know him as the doctor who founded a hospital in Equatorial Africa. Actually, he came to medicine rather late, and by that time, he was an accomplished musician and organist and had written a monumental biography of J.S. Bach and an analysis of his work, to be followed later with his edition of Bach's organ music, all of which had made him the outstanding authority on Bach. He also attained the rank of professor of theology and director of the Strasbourg Seminary. But he was resolved to become a doctor in French Equatorial Africa, and at age 30 he began his medical studies, meanwhile continuing his other activities. In his mid-30s, he went to Lambarene in Gabon, where he established a primitive hospital, which eventually grew into an extensive medical facility, including a leprosarium. Except for a long period during World War I when he was a prisoner of war, he remained there most of the time to minister to the poor and the sick, with the help of a small, dedicated staff. He traveled frequently to the continent to raise money for the hospital, and once to the United States, in 1949, to address the Goethe Festival in Colorado. On these trips, he was honored in many countries for his work as a scientist, his artistry as an organist and musical scholar, and his contributions as a philosopher. In a three-volume series entitled Philosophy of Civilization, Schweitzer developed his own philosophy, which is summed up in the term Reverence for Life, a universal code of ethics that requires respect for the lives of all other beings. His theological writings include "The Quest of the Historical Jesus," in which he expresses a dissatisfaction with the way that the gospels treat the facts of the life of Jesus, and "The Mysticism of Paul the Apostle." He described his early years at Lambarene in "On the Edge of the Primeval Forest," and in the mid-30s, he wrote his autobiography, "Out of My Life and Thought." Schweitzer was awarded the Nobel Prize for Peace in 1952.

Norman Cousins, the late editor of the now sadly defunct Saturday Review, visited Schweitzer and, in a book about this visit, describes this scene of life in Lambarene, the customary singing of a hymn after dinner: "There was a piano in the dining room, old, dilapidated, unable to hold its tune because of the heat and the moisture. When I saw Dr. Schweitzer sit down at the piano and prop up the hymnbook, I winced. Here was one of history's greatest interpreters of Bach, a man who could fill any concert hall in the world. The best grand piano made would not be too good for him. But he was now about to play a dilapidated upright virtually beyond repair. And he went at it easily and with the dignity that never leaves him."

The Aristotles, the da Vincis, the Goethes, the Jeffersons, the Schweitzers are, indeed, rare individuals, true descendants of those early philosophers of the ancient world. Their works and their writings had, and continue to have, profound influence on life and civilization worldwide and are κτήμα ες αεί, possessions forever. We admire them, stand in awe of them, but they are in a sense remote to us, far beyond us. It is the work, the writings and contributions of individuals in whom the elements of the two cultures are combined in a more modest scale that are understandable and accessible to us, and they are the ones that influence us in our

occupations and interests and enrich our personal lives.

Some of these gifted people have attained a measure of fame, and we can read about them in any encyclopedia or good dictionary; most of them remain unknown, unless we come upon them and their work by accident or acquaintance. One of these, unknown even among biologists, is the British naturalist Langdon W. Smith, who did some excellent biological research and also wrote exquisite poetry. Smith was born in Scotland in 1877 and came to the United States when he was 14. Practically nothing is known about his education, except that in his early 20s he was engaged by the Museum of Natural History in New York to do research and was often invited by scientific societies to lecture. He also wrote articles on scientific subjects for newspapers. He wrote a particularly beautiful poem about evolution titled "A Tadpole and a Fish." A friend of his found this poem, which Smith had carelessly laid aside, and recognized it as something exceptional. He prevailed upon Smith to submit the poem to some of the best papers for an opinion. The first to examine the poem was the editor of the New York Herald, who gave Smith a check for $500, a considerable sum in those times, for the right to publish it.

Smith became ill and returned to England, where he died some months later of tuberculosis. The poem, which was later published under the title "Evolution" in 1909 and was included in anthologies published by the Haldeman-Julius company of Girard, Kansas, in 1922 and 1924, makes a felicitous conclusion to this essay: like the essay, but in a grand context, it takes us back to our roots:

A Tadpole and a Fish

Langdon Smith

I

When you were a tadpole and I was a fish,
In the Paleozoic time,
And side by side in the ebbing tide
We sprawled through the ooze and slime,
Or skittered with many a caudal flip
Through the depths of the Cambrian fen,
My heart was rife with the joy of life
For I loved you even then.

II

Mindless we lived and mindless we loved,
And mindless at last we died;
And deep in a rift of the Caradoc drift
We slumbered side by side.
The world turned on in the lathe of time,
The hot land heaved amain,
Till we caught our breath from the womb of death,
And crept into light again.

III

We were Amphibians, scaled and tailed
And drab as a dead man's hand;
We coiled at ease 'neath the dripping trees,
Or trailed through the mud and the sand,
Croaking and blind with our three-clawed feet
Writing a language dumb,
With never a spark in the empty dark
To hint at a life to come.

IV

Yet happy we lived, and happy we loved,
And happy we died once more;
Our forms were rolled in the clinging mold
Of a Neocomian shore.
The eons came, and the eons fled,
And the sleep that wrapped us fast
Was riven away in a newer day,
And the night of death was past.

V

Then light and swift through the jungle trees
We swung in our airy flights,
Or breathed in the balms of the fronded palms,
In the hush of the moonless nights.
And oh! What beautiful years were these,
When our hearts clung each to each;
When life was filled, and our senses thrilled
In the first faint dawn of speech.

VI

Thus life by life, and love by love,
We passed through the cycles strange,
And breath by breath, and death by death,
We followed the chain of change.
Till there came a time in the law of life
When over the nursing sod
The shadows broke, and the soul awoke
In a strange, dim dream of God.

VII

I was thewed like an Auroch bull,
And tusked like the great Cave Bear;
And you, my sweet, from head to feet,
Were gowned in your glorious hair.
Deep in the gloom of a fireless cave,
When the night fell o'er the plain,
And the moon hung red o'er the river bed,
We mumbled the bones of the slain.

VIII

I flaked a flint to a cutting edge,
And shaped it with brutish craft;
I broke a shank from a woodland dank,
And fitted it, head and haft.
Then I hid me close to the ready tarn,
Where the Mammoth came to drink;—
Through brawn and bone I drave the stone,
And slew him upon the brink.

IX

Loud I howled through the moonlight wastes,
Loud answered our kith and kin;
From west and east to the crimson feast
The clan came trooping in.
O'er joint and gristle and padded hoof,
We fought and clawed and tore,
And cheek by jowl, with many a growl,
We talked the marvel o'er.

X

I carved that fight on a reindeer bone,
With rude and hairy hand,
I pictured his fall on the cavern wall
That men might understand.
For we lived by blood, and the right of might,
Ere human laws were drawn,
And the Age of Sin did not begin
Till our brutal tusks were gone.

XI

And that was a million years ago,
In a time that no man knows;
Yet here tonight in the mellow light,
We sit in Delmonico's;
Your eyes are as deep as the Devon springs,
Your hair is as dark as jet,
Your years are few, your life is new,
Your soul untried; and yet—

XII

Our trail is on the Kimmeridge clay,
And the scarp of the Purbeck flags,
We have left our bones in the Bagshot stones,
And deep in the Coraline crags;
Our love is old, our lives are old,
And death shall come amain;
Should it come today, what man may say
We shall not live again?

XIII

God wrought our souls from the Tremadoc beds
And furnished them wings to fly;
He sowed our spawn in the world's dim dawn,
And I know that it shall not die,
Though cities have sprung above the graves
Where the crook-boned men made war,
And the ox-wain creaks, o'er the buried caves
Where the mummied mammoths are.

XIV

Then as we linger at luncheon here,
O'er many a dainty dish,
Let us drink anew to the time when you
Were a Tadpole and I was a Fish.

Dr. Myrianthopoulos is scientist emeritus, National Institutes of Health, where he conducted research on the genetics of nervous system disorders. He was born in Cyprus, then a British colony. His studies at the University of Athens were interrupted by World War II, during which he served as an officer with the British forces in the Middle East. He completed his doctoral studies at the University of Minnesota. He and his wife Marjorie have three children, each with two X chromosomes.

Isolation of a Dengue Type 1 Virus from a Soldier in West Africa (Côte d'Ivoire)

To the Editor: In Africa, recent reports of epidemic or endemic dengue activity usually come from the eastern part of the continent (1), and the serotype most frequently identified is dengue 2.

We report the isolation of a dengue 1 virus strain from the blood of a young soldier living in Abidjan, the capital of Côte d'Ivoire. This 20-year-old man arrived from France on October 19, 1998. On December 28, 1998, he consulted a physician of his regiment because of headache, diarrhea, and fever (40°C). The results of the laboratory investigations were as follows: platelets (193 10^9 /L), leukocytes (2.2$10^9$ /L); no malaria was found. He was hospitalized for possible arboviral infection, and treatment with paracetamol was prescribed. On December 29, a blood sample was collected; serum and buffy coat were frozen at -20°C for further examination at the Institute of Tropical Medicine of the Military Health Services. On day 3, the patient's temperature dropped to 38°C, then rose to 39.5°C on day 5. All symptoms were resolved on day 6.

On February 15, 1999, the frozen blood was defrosted and the lysed buffy coat was immediately cocultured with C6/36 cells. On day 6, a blind passage was made on the same cells and on Vero. On day 12, no cytopathic effect was observed, but a dengue 1 virus was identified by indirect fluorescent antibody assay (2) with type-specific monoclonal antibodies. This diagnosis was confirmed by reverse transcription-polymerase chain reaction, with a technique slightly modified from Lanciotti (3).

On the first blood specimen, the serologic immunoglobulin (Ig)M assays (M antibody capture enzyme-linked immunosorbent assay [ELISA]) with our antigen screening panel were negative (dengue, West Nile, Chikungunya, Rift Valley fever). The IgG assay (ELISA) against dengue antigen was also negative.

The patient returned to France in February 1999. A second blood sample was collected and tested on April 7, 1999, 3 months after the illness. The serologic assays were positive against the dengue antigens at the following dilutions of the serum: IgM: dengue 1: 1/120,000; dengue 2: 1/40,000; dengue 3: 1/12,000; dengue 4: 1/25,000. IgG: dengue 1: 1/75,000; dengue 2: 1/90,000; dengue 3: 1/60,000; dengue 4: 1/120,000. This seroconversion allowed us to confirm infection of this patient by a dengue virus.

The lack of similar reported cases or epidemics among the local and expatriate populations of Abidjan may indicate poor transmission, recent introduction of the strain, or low virulence of the virus, as previously hypothesized for dengue in West Africa (4). However, the serologic status of the human population needs to be further investigated. Characterization of the isolated viral strain would be of interest for dengue epidemiology. Complete sequencing of the viral RNA is in progress in our laboratory.

Human infection with dengue virus has been rarely reported and studied in West Africa, and the epidemiology of serotype 1 is poorly documented. During the past 35 years, the Pasteur Institute of Dakar confirmed three dengue 1 strains (two from humans in Senegal; one from mosquitoes in Côte d'Ivoire), while during the same period, more than 300 dengue 2 strains were studied, most from mosquitoes (5). In the past 10 years, medical and entomologic surveys showed circulation of dengue 2 virus in Senegal (one isolate in the blood of a French soldier) (6). However, during the 1970s, Nigerian virologists demonstrated circulation of dengue 1 and 2 in their country: more than 50% of the adults living in the savannah had neutralizing antibodies (7). Of 148 blood samples of febrile patients, three viral strains (yellow fever, dengue 1, and Zika) were isolated, all from children (8).

In Africa, outside the epidemics of yellow fever, it is difficult to isolate arboviruses from adult humans. Isolation is often more successful from children or naive expatriates. Accordingly, soldiers participating in international operations constitute a very exposed population. During recent operations in Somalia (1992-93), dengue fever was an important cause of febrile illness among U.S. troops (9). Thirty-nine dengue 2 and three dengue 3 strains were isolated from 96 collected sera.

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Carbapenem-Hydrolyzing Metallo-ß-Lactamase from a Nosocomial Isolate of *Pseudomonas aeruginosa* in France

To the Editor: The carbapenems (meropenem and imipenem), the ß-lactams with the broadest spectrum, are stable to most ß-lactamases (1). Therefore, they are often used as antibiotics of last resort for treating nosocomial infections due to gram-negative bacteria resistant to other ß-lactams. Resistance to carbapenems and susceptibility to other ß-lactams in *Pseudomonas aeruginosa* is common as a result of reduced drug accumulation or increased expression of pump efflux (1).

Several extended-spectrum ß-lactamases have been reported in *P. aeruginosa*, but only two, IMP-1 and VIM-1, possess an extended hydrolysis profile that includes carbapenems (2-5). The chromosome-borne and plasmid-mediated carbapenem-hydrolyzing ß-lactamase, IMP-1, has been described in several gram-negative rods, including *P. aeruginosa*, *P. cepacia, Alcaligenes xylosoxydans,* and *Enterobacteriaceae* isolates in Japan (4,6). Recently, a chromosome-borne carbapenem-hydrolyzing ß-lactamase, VIM-1, was reported from a clinical isolate of *P. aeruginosa* in Italy (5), and uncharacterized carbapenem-hydrolyzing ß-lactamases have been reported in the United Kingdom and Portugal (7,8). The weakly related IMP-1 and VIM-1 (31.4% amino acid identity) are both zinc-dependent (metallo-enzymes) and confer resistance to all ß-lactams except monobactams (3,5).

In 1996, a 39-year-old French woman was hospitalized in Marseille for chronic myelogenous leukemia, pancytopenia, and allogeneic bone marrow transplantation. After a 15-day stay in the transplantation unit, fever developed and imipenem and amikacin were administered. Despite this treatment, the patient died of septic shock syndrome 5 days later. Three-day-old blood cultures grew a carbapenem-resistant *P. aeruginosa* isolate. This *P. aeruginosa* COL-1 isolate was resistant to most ß-lactams, including piperacillin/tazobactam, imipenem, meropenem, ceftazidime, cefepime (minimum inhibitory concentrations [MICs] of 128, 32, 16, 64, 32 mg/L, respectively), amikacin, tobramycin, gentamicin, netilmicin, and ciprofloxacin; however, the isolate was susceptible to aztreonam (MIC determination, genetic techniques and ß-lactamase assays are described elsewhere [9]). A sonicate of crude extract of *P. aeruginosa* COL-1 culture showed strong imipenem and meropenem hydrolysis activity (0.7 mU/mg and 1.9 mU/mg; reference *P. aeruginosa* strain <0.05 mU/mg) by UV spectrophotometry with 0.1 mM of substrate, after incubation in 50 mM phosphate buffer at 30°C. This activity was lost when the enzyme extract was preincubated with 10 mM of edetic acid and was partially restored

by addition of 1 mM $ZnCl_2$, indicating the presence of a metallo-carbapenem hydrolyzing ß-lactamase. Isoelectric focusing revealed two ß-lactamase bands of pI 5.6 and 9. Only the pI 5.6 ß-lactamase band was inhibited if the gel was overlaid with edetic acid before nitrocefin was added as the indicator substrate; the other pI 9 ß-lactamase likely corresponded to a naturally occurring AmpC cephalosporinase. This pI 5.6 value differed from the pI values of the carbapenem-hydrolyzing ß-lactamase previously reported in *P. aeruginosa* (3-5,7,8). Polymerase chain reaction amplification experiments were negative when internal primers were used for the only sequenced carbapenem-hydrolyzing ß-lactamase genes from *P. aeruginosa* encoding IMP-1 and VIM-1 and genomic DNA of *P. aeruginosa* COL-1. Transfer of the carbapenem resistance marker by conjugation to laboratory strains of *P. aeruginosa* or *Escherichia coli* was unsuccessful (9), but transformation by electroporation of a putative plasmid extract from *P. aeruginosa* COL-1 in *E. coli*, followed by selection onto amoxicillin-containing agar plates (9), gave a ca. 45-kb plasmid that produced the carbapenem-hydrolyzing ß-lactamase with a pI value of 5.6. Thus, the carbapenem-hydrolyzing ß-lactamase gene was plasmid-borne.

This case indicates the presence of a novel carbapenem-hydrolyzing ß-lactamase in *P. aeruginosa* in Europe, the first in France; its spread in gram-negative rods, as reported for IMP-1 in Japan, is of concern because, as seen in this case, routine laboratory detection is difficult and therapeutic options are extremely limited.

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Population-Based Study of Invasive *Kingella kingae* Infections

To the Editor: For most of the 3 decades since the first description of *Kingella kingae*, this gram-negative bacillus was considered a rare cause of human disease (1). Since the late 1980s, however, reports of infections by the organism in young children have increased in the United States, Western Europe, and Israel (2-6). The rapid emergence of *K. kingae* as an important cause of pediatric disease does not necessarily imply that the organism is truly a new pathogen. Better isolation techniques and awareness of the bacterium by microbiology laboratories may contribute to the apparent increase (4). Recent studies have demonstrated that primary isolation of *K. kingae* can be substantially improved by injection of synovial fluid and bone exudates into aerobic blood-culture bottles (4). Synovial fluid may inhibit the growth of *K. kingae,* and injection of the clinical specimen

into a 50-mL volume of broth reduces the concentration of inhibitory factors, facilitating isolation of the organism (4).

In 1993, we reported results of routine use of blood-culture bottles for processing cultures of exudates (7) at the Soroka University Medical Center, Beer-Sheva, Israel. From 1988 to 1992, 25 children with invasive *K. kingae* infections, defined as isolation of the organism from blood or normally sterile body fluids, were identified in southern Israel. From 1994 to 1998, 33 additional patients, including 32 children and a 21-year-old adult, were detected in the same area. Twenty-four (63.6%) of the 33 patients were male. Eight (24.2%) cases were diagnosed between January and June and 25 (75.8%) between July and December. Median age of children was 13 months (mean ± SD: 15.0 ± 7.6 months; range 6 to 37 months).

The fact that all children in southern Israel are born and receive inpatient medical services at the Soroka University Medical Center allowed us to calculate the incidence of invasive pediatric *K. kingae* infections in this population. During the 6-year period, the average annual number of births was 10,860. The annual incidence of invasive *K. kingae* infections during the same period was 11.9 per 100,000 in children ≤ 48 months of age, 19.2 per 100,000 in children <24 months of age, and 20.0 per 100,000 in infants <12 months of age.

When medical attention was sought, patients had been ill for a median of 3 days. Symptoms of upper respiratory tract infection were recorded in 12 (36.4%) children, stomatitis in 8 (24.2%), and diarrhea in 4 (12.1%). Occult bacteremia (positive blood culture with no obvious focal infection) was diagnosed in 16 children. In 15 children, *K. kingae* had invaded the bones. Septic arthritis was diagnosed in 11 children, involving the ankle in 4; the knee or wrist in 2 patients each; and the hip, shoulder, or elbow in one patient each. Osteomyelitis was diagnosed in two patients, affecting the femur in one and the tibia in the other. In two additional patients, both with fever and bacteremia, the location of the skeletal infection could not be determined. One limped and had tenderness over the femur, but X-rays and a $Technecium_{99}$-labeled bone scan showed no abnormalities. The other had pain in the heel but no fluid could be aspirated. Bacteremic tracheobronchitis occurred in one child, and endocarditis of the mitral valve was diagnosed in a 21-year-old woman who was receiving immunosuppressive therapy for systemic lupus erythematosus. All 33 patients were treated with ß-lactam drugs and recovered.

Injecting synovial fluid specimens into blood-culture bottles permitted the diagnosis of *K. kingae* in these patients and showed that this organism may be a common cause of invasive pediatric infections. The age distribution of the patients demonstrates that *K. kingae* is a pathogen of young children, especially those between the ages of 6 months and 2 years, among whom the incidence of invasive disease has remained stable since 1988. This age distribution of *K. kingae* infections parallels that for respiratory carriage of the organism. In a surveillance study among 48 children ages 6 to 42 months attending a day-care center in Israel, *K. kingae* was isolated from 109 (17.5%) of 624 throat cultures, and 34 children (70.8%) carried the organism at least once during an 11-month period (8). However, the organism was not detected in healthy infants ages 2 to 4 months attending a well-baby care clinic, which indicates some immunity to colonization and infection by *K. kingae* during the first months of life (8).

When the 1988 to 1993 surveillance data are added to those collected from 1994 to 1998, *K. kingae* infections show a significant seasonal pattern; 44 (75.9%) of 58 cases were diagnosed in the second half of the year ($p = 0.007$). This increase in *K. kingae* infections in winter has also been described in other respiratory pathogens. This finding, as well as the frequent detection of respiratory symptoms in children with invasive *K. kingae* infections, suggests that seasonal viral infections may facilitate the spread of *K. kingae* from the throat, to the bloodstream and bones. In a prospective study, *K. kingae* bacteremia was documented in 4 (13.7%) of 29 young children with culture-proven herpetic gingivostomatitis, confirming the role played by viral infections in the pathogenesis of infections caused by the organism (9).

With few exceptions, isolates of *K. kingae* remain susceptible to antibiotic drugs (10). Our results demonstrate that the prognosis of invasive *K. kingae* infections is generally good and patients respond promptly to appropriate antimicrobial therapy.

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Involving Ornithologists in the Surveillance of Vancomycin-Resistant Enterococci

To the Editor: Because migratory birds cross national or intercontinental borders, they are possible long-range vectors for human pathogens such as viruses, *Borrelia burgdorferi* sensu lato, and enteropathogenic bacteria with antibiotic resistance or virulence factors (1,2). Enterococci are ubiquitous in humans and animals and have a propensity for uptake and transfer of glycopeptide antibiotic resistance (3); therefore, the emergence of glycopeptide-resistant enterococci (GRE) in humans is a public health concern. Low-level vancomycin resistance (genotype *vanC-1-3*) is intrinsic in enterococcal species (e.g., *Enterococcus gallinarum, E. flavescens,* and *E. casseliflavus*) that may normally occur in the intestinal flora of some birds. However, the finding of high levels of GRE in wild birds suggests acquisition from an environmental source.

In March 1998, we obtained fecal samples while banding 318 northbound migrating gulls in Malmö, southern Sweden. Using a selective culture procedure with enrichment broth (bile esculin azide broth, Acumedia, LabFab, Ljusne, Sweden) containing vancomycin (8 µg/ml) and aztreonam (60 µg/ml), we isolated vancomycin-resistant *E. faecalis* from a black-headed gull (*Larus ridibundus*). High-level glycopeptide resistance (>256 µg/ml) was demonstrated by E-test (AB Biodisc, Solna, Sweden), and a *van*A genotype was found by polymerase chain reaction amplification (4). This survey protocol can also be used to detect medium to low levels of glycopeptide resistance. Using the same procedure in a study of 230 sub-Antarctic birds on Bird Island, South Georgia, in 1996, we found four GRE isolates with *vanC1* genotype (MIC 3-8 µg/ml).

Many species of gulls have moved into urban areas, where they commonly feed on human trash and deposit feces. The black-headed gull with GRE described above was banded as a fledgling in Malmö in 1995. Birds of this population spend the winter mainly in Western Europe (5), where they forage at garbage dumps, sewage outlets, and agricultural areas. This bird may have acquired GRE in such an area. *Van*A genotype *E. faecium* and *E. faecalis* have been found in poultry and pigs in the Netherlands and Denmark, where the vancomycin analog avoparcin has been used as a growth promoter (6). Manure from such farms may be a GRE source accessible to wild birds.

We have previously reported the introduction into Sweden of multidrug-resistant *Salmonella* Typhimurium by migratory birds (7). The present report further emphasizes the possibility of migratory birds as long-range vectors of bacteria potentially associated with human disease. The risk to humans for GRE from migratory birds may seem insignificant compared with such risk from hospitalization or from eating meat products from GRE-colonized

animals. However, if the frequency of birds carrying high-level GRE increases and if amplification in a secondary reservoir or spread through polluted water takes place, spread by migratory birds may become a problem. Bacteriologic surveys of birds may provide vital information for assessing the environmental dispersion of GRE from farms and hospitals. In combination with data about migration patterns and reports of banding recoveries from ornithologists, the potential sources of GRE might be deduced.

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Conference Summaries

2nd International Conference on AIDS in India, December 1999

The 2nd International Conference on AIDS in India, which was organized by the Department of Experimental Medicine, Tamil Nadu Dr. M.G.R. Medical University, was held in Chennai, India, on December 5-8, 1999. In addition to the national delegates, the conference was attended by 652 national and international experts.

On December 6, the first AIDS Society of India was formed and inaugurated by Mark Wainberg, president of the International AIDS Society.

HIV/AIDS prevalence is increasing in India, where as many as 8 million people may be infected. Available interventions are not adequate. The conference participants recommended the following prevention measures:

The Chennai Statement

1. Simplified, low-cost medical interventions (including antiviral drugs, which can substantially reduce mother-to-infant HIV transmission, should be implemented.
2. Medicines against HIV and opportunistic infections should be made affordable and accessible, and discrimination against HIV-infected persons in the workplace, health-care industry, and other social contexts should be addressed.
3. Medical professionals should receive special training in the treatment of HIV infection and disease. Formal training leading to postgraduate qualification in HIV medicine will improve the quality and equity of health care for infected persons in various clinical settings. Similarly, formal training toward postgraduate qualification in transfusion medicine will improve blood safety and the use of blood components, and thus the use of donated blood. The Medical Council of India and medical universities around the country should develop such postgraduate training.
4. Folklore, traditions, and other elements of Indian culture should be systematically reviewed and adapted to teaching children and youth about responsible, safe behavior to prepare them for their roles as future parents. Medical systems in India should be rigorously and scientifically evaluated by multicenter studies, and improvements in treating HIV disease should be introduced.
5. India must invest in research to develop vaccines and antiviral and antimicrobial drugs for the prevention and treatment of HIV disease and opportunistic infections.
6. Cooperative efforts should be made within India to collaborate with international coalitions for the prevention, control, and treatment of HIV/AIDS.
7. In all strategic planning, the voice of the people, particularly the voice of infected people, must be taken into account.

We believe that the above recommendations will help control the AIDS epidemic, with all its socioeconomic implications, in India.

N.M. Samuel
AIDS Society of India

Workshop on Micronutrients and Infectious Diseases: Cellular and Molecular Immunomodulatory Mechanisms

Epidemiologic and clinical data, as well as experimental studies, suggest a bidirectional interaction between micronutrients and infectious pathogens. While nutritional deficiencies can alter several aspects of the innate and acquired immune responses to pathogens, some infectious diseases alter the nutritional status of the host and the host's ability to absorb micronutrients. Nutritional deficiencies may also influence pathogen mutations directly, affecting virulence and clinical outcome. These interactions depend on a number of other variables, including the severity of micronutrient deficiency, the age and clinical condition of the host, and a variety of environmental factors.

To address issues pertinent to understanding the role(s) of micronutrients in the control of infectious disease, the National Institute of Allergy and Infectious Diseases organized a 2-day meeting, cosponsored by the Office of Dietary Supplements, National Institutes of

Health. Representatives attended from several other NIH institutes and centers, the U.S. Agency for International Development, the U.S. Department of Agriculture, the U.S. Food and Drug Administration, the Council for Responsible Nutrition, and the World Bank. The objectives of this meeting were to provide a forum for the exchange of ideas between basic scientists, nutritionists, infectious disease specialists, and clinical epidemiologists; to examine the mechanisms involved in the pathogenesis and clinical manifestations of infectious diseases, including the immunology, molecular biology, and potential direct interactions between micronutrients and pathogens; evaluate current strategies for intervention; and summarize research needs and new directions.

The workshop provided information on the effects of micronutrients on innate and adaptive immunity, mucosal immunity, cytokine production, gene expression, and intracellular signaling pathways. In addition, a session focused on the implications of basic research findings for the treatment of disease. The workshop opened with an overview on how dietary characteristics modulate immune responses. Although the field is in its infancy, areas in particular need of research include the role of micronutrients in innate and mucosal immunity, as well as the early phases of development of immune cells. Instances of direct interaction between micronutrients and pathogens were also described, for example, how a benign strain of an infectious agent can become virulent when passaged through micronutrient-deficient mice. Examples of host-pathogen interactions, in which nutrition is an important modulator, provide new opportunities for study, especially as the host's genetic background and the role of specific genes are elucidated. One potential mechanism of immune suppression is the alteration of cytokine responses. Molecular studies using gene knockout mice also provided new information about nutrient transport proteins and their relationship to normal immunity.

At the conclusion of the workshop, a panel of experts, including basic scientists, infectious disease specialists, and clinical epidemiologists, submitted recommendations for future research: the development of a panel of assays that could be used for general screening of a nutrient's impact on immunocompetence; the need to define reproducible molecular and immunologic biomarkers that can be used in human and animal studies; the need to continue basic mechanistic studies on both the role of micronutrients as antioxidants or as regulatory molecules within the immune system and the effects of micronutrient status on the decline of immune function in the elderly; the need to encourage more efficient use of transgenic knock-outs and other appropriate animal models, as well as the use of microarray technology, by investigators in this area of research; the need for investigators to link basic molecular, mechanistic studies with field work in areas of endemic infectious diseases of greatest public health importance; the need for innovative approaches for combining nutritional supplementation and immunotherapy in new forms of intervention; and the need to recruit young investigators with state-of-the-art immunology skills and promote collaborative research with nutrition or infectious disease professionals.

As a result of this workshop, an open LISTSERV has been created for continued interaction among interested parties in the field. The Journal of Infectious Diseases plans to publish the proceedings of the workshop in a supplement in early 2000. For additional information, contact Christopher E. Taylor, telephone: 301-496-5305; e-mail: ct18m@nih.gov; or Elizabeth Higgs, telephone: 301-496-2544; e-mail: eh63a@nih.gov.

Third Annual Conference on Vaccine Research: Basic Science—Product Development—Clinical and Field Studies

Renaissance Hotel, Washington, D.C.
April 30-May 2, 2000

The conference is sponsored by the National Foundation for Infectious Diseases, in collaboration with the Centers for Disease Control and Prevention; National Institute of Allergy and Infectious Diseases, National Institutes for Health; International Society for Vaccines; Albert B. Sabin Vaccine Institute at Georgetown University; World Health Organization; U.S. Department of Agriculture; and the Center for Biologics Evaluation and Research, Food and Drug Administration.

For information, contact Kip Kantelo, National Foundation for Infectious Diseases, 4733 Bethesda Avenue, Suite 750, Bethesda, MD 20814-5228, USA; telephone: 1-301-656-0003 x19; fax: 1-301-907-0878; e-mail: kkantelo@nfid.org; URL: http://www.nfid.org/conferences.

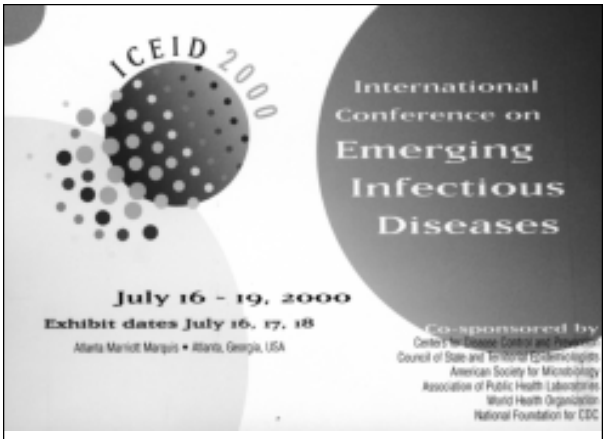
Dangerous Pathogens 2000 Conference

University of Plymouth
United Kingdom
September 4-7, 2000

The Dangerous Pathogens 2000 Conference is organized by the Defence Evaluation and Research Agency (DERA), Porton Down; the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States; and the Society for Applied Microbiology, United Kingdom. The conference will review the ongoing battle between humans and microorganisms, past, present, and future, and identify any gaps in our knowledge that need to be filled to ensure that humans come out on top.

The conference will cover the following themes: The Past, including Plagues of Antiquity (plague, cholera, smallpox, syphilis, influenza), Disease and War, The Role of Sanitation and Clean Water, and The Impact of Science (Koch, Pasteur, Fleming); The Present, including Plagues of the Present (HIV, multidrug-resistant tuberculosis, malaria), Antibiotic-Resistant Organisms, Hospital-Acquired Infections, and Global Vaccine Programs; and The Future, including Emerging Pathogens, Genomics and Bioinformatics, and New Antimicrobial Agents.

For more information about the conference, contact Les Baillie, Principal Scientist, DERA Porton, Porton Down, Salisbury SP4 OJQ, United Kingdom; telephone: 44-1980-613-881; fax: 44-1980-613-284; e-mail: lesbaillie@hotmail.com.



ICEID 2000—Call for Abstracts

Abstract submission to ICEID 2000 is now possible through the Conference Web site, and we strongly encourage you to make use of this electronic method. Not only will you be able to submit your abstract in a finished form (prepared using your own word processing program such as Word or WordPerfect, on PC or Macintosh platforms), but you can check back to learn acceptance status, presentation location, and time information. In addition, it will save you both the costs of postage and the worry of a lost submission.

ICEID 2000 is using the American Society for Microbiology's Web-based Abstract Submission System. If you have used the system to submit to either the 1999 or 2000 ASM General Meetings or the 1999 ICAAC Meeting, you will be able to reenter the system as a returning user.

For more information on ICEID abstract submission, see the ASM Web site: www.asmusa.org. Follow the link to ICEID 2000.

List of Reviewers

Visit our web page at http://www.cdc.gov/ncidod/EID

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Editorial Policy and Call for Articles

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

The journal has an international scope and 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, demography, sociology, and other disciplines. Inquiries about the suitability of proposed articles may be directed to the Editor at 404-371-5329 (tel), 404-371-5449 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features the following types of articles: Perspectives, Synopses, Research Studies, Policy Reviews, and Dispatches. The purpose and requirements of each type of article are described in detail below. To expedite publication of information, we post journal articles on the Internet as soon as they are cleared and edited.

Spanish and French translations of some articles can be accessed through the journal's homepage at www.cdc.gov/eid. Articles by authors from non-English-speaking countries can be made simultaneously available in English and in the author's native language (electronic version of the journal only).

Instructions to Authors

Manuscript Preparation

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

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

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

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

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

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

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

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

Manuscript Submission

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

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

Types of Articles

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

Perspectives: Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases or related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change; human demographics and behavior; technology and industry; economic development and land use; international travel and commerce; and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

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

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

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

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

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

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

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