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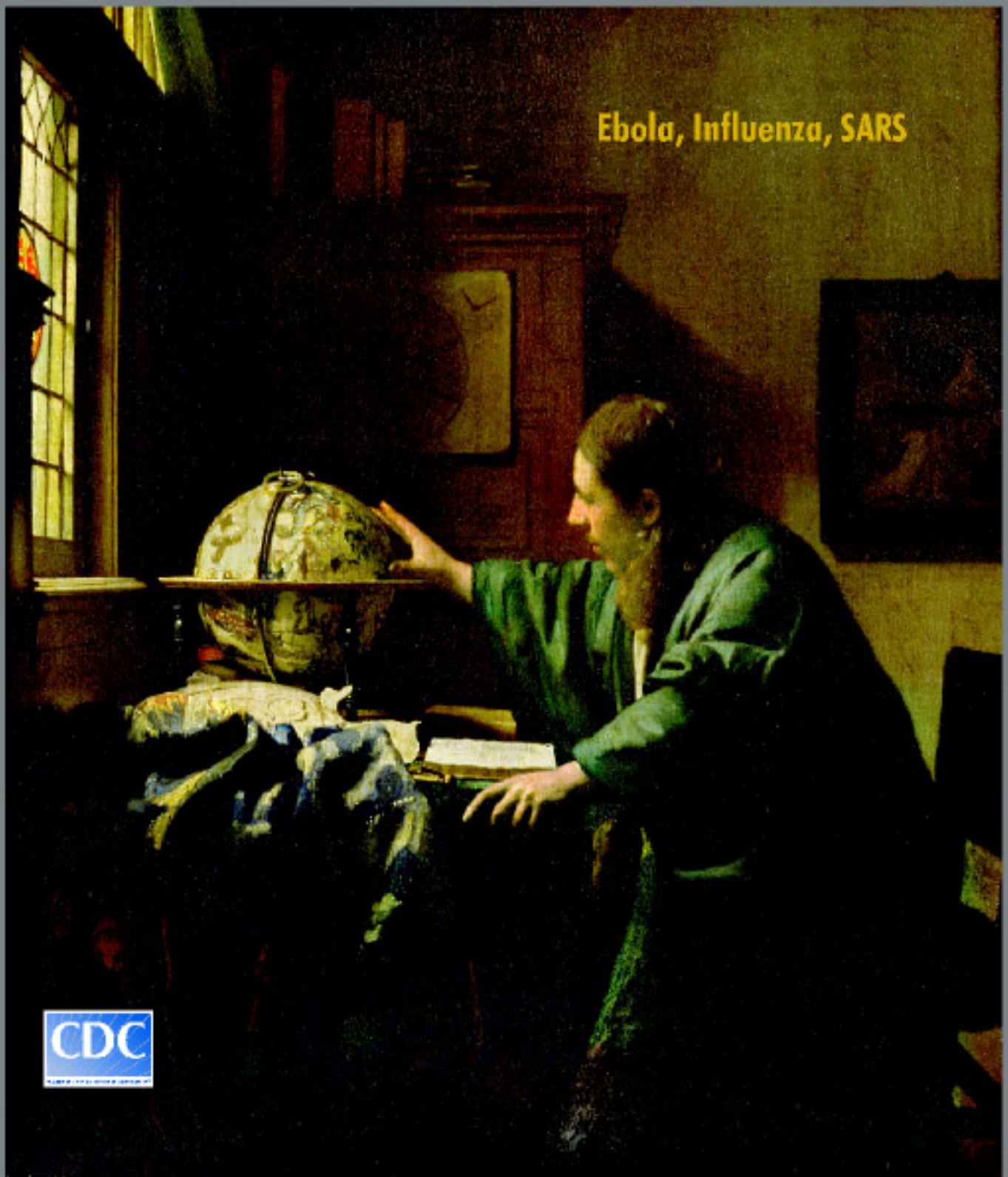

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Johannes [Jan] Vermeer (1632–1675).
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EMERGING INFECTIOUS DISEASES

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Salmonella Enteritidis Infections, United States, 1985–1999

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Salmonella enterica serotype Enteritidis emerged as an important illness during the 1980s. Investigations showed that consumption of undercooked eggs was the major risk factor for disease, and a variety of prevention and control efforts were initiated during the 1990s. We describe sporadic infections and outbreaks of *S.* Enteritidis in the United States from 1985 through 1999 and discuss prevention and control efforts. After reaching a high of 3.9 per 100,000 population in 1995, *S.* Enteritidis infections declined to 1.98 per 100,000 in 1999. While the total number of outbreaks decreased by half, those in the western states tripled. Outbreaks of *S.* Enteritidis phage type 4 infections accounted for 49% of outbreaks in 1999. Outbreak-associated deaths in health facilities decreased from 14 in 1987 to 0 in 1999. Overall, rates of sporadic *S.* Enteritidis infection, outbreaks, and deaths have declined dramatically. For further reductions, control measures should continue to be applied along the entire farm-to-table continuum.

Salmonella enterica serotype Enteritidis is one of the most common *Salmonella* serotypes worldwide, particularly in developed countries. During the 1980s, *S.* Enteritidis emerged as an important cause of human illness in the United States. In 1976, the incidence of *S.* Enteritidis was 0.55 per 100,000 population and represented only 5% of all *Salmonella* isolates. By 1985, this proportion reached 10%, and the rate increased to 2.4 per 100,000 population (1). During the same time, total *Salmonella* infection rates rose from 10.7 per 100,000 in 1976 to 24.3 in 1985. The highest rates of *S.* Enteritidis were seen in the Northeast, although rates in the western region also increased during this time.

The number of outbreaks of *S.* Enteritidis infection also increased during the 1980s (2), particularly in the northeastern United States. Laboratory subtyping of *S.* Enteritidis isolates from outbreaks indicated that phage types (PT) 8 and 13a were the most common phage types in the United States (3). Although PT4 was common in Europe, where it coincided with a large increase in *S.* Enteritidis infections (4,5), it was seen in the United States only among persons with a history of foreign travel.

Case-control studies of sporadic *S.* Enteritidis infections and outbreaks demonstrated that shell eggs were the major risk factor for disease (2,6,7). After the implicated eggs were traced back to the farm of origin, microbiologic surveys showed *S.* Enteritidis of the same phage type that caused human cases to be present in the farm environment of egg-layer poultry flocks (8–10). Studies showed that the internal contents of eggs can be contaminated with *S.* Enteritidis (11,12), and this contamination has been identified as a major risk factor in the emergence of human illness. To reduce *S.* Enteritidis in eggs, on-farm prevention and control measures and quality assurance programs were initiated in the early 1990s. Education of consumers and food workers regarding the risks of consuming raw or undercooked eggs was also begun, with special emphasis on high-risk populations such as the elderly, children, pregnant women, and others with weakened immune systems. Restaurants and health institutions were encouraged to avoid pooling eggs, to use pasteurized egg product, and to avoid raw egg recipes. Requirements for refrigeration during distribution and storage were increased.

We examined trends in *S.* Enteritidis infection in the United States from 1985 through 1999 based on surveillance data for sporadic infections and outbreaks reported to the Centers for Disease Control and Prevention (CDC). We describe prevention and control efforts and suggest a plan for further reduction of *S.* Enteritidis infections.

Methods

The CDC National Salmonella Surveillance System is a laboratory-based passive system that was developed in

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1976. In 1994, most states began reporting cases electronically to this system through the Public Health Laboratory Information System (13). Annual *S.* Enteritidis isolation rates per 100,000 population were calculated for each state and region of the United States by using 1976–1999 Census data.

Before 1985, reports of *S.* Enteritidis outbreaks were collected through the National Foodborne Disease Outbreak Surveillance System. In response to a growing number of outbreaks and the need for timely follow-up, CDC began the *S.* Enteritidis Outbreak Reporting System in 1985. This system encouraged officials from state and local health departments to report outbreaks as soon as they occurred. At the end of each year, epidemiologists from state health departments were asked to verify the information that CDC had received and to send written reports of additional outbreaks. An outbreak of *S.* Enteritidis infection was defined as ≥2 cases of laboratory-confirmed *S.* Enteritidis infection in persons who ingested a common food, or one culture-confirmed case with additional cases meeting a clinical definition of illness and *S.* Enteritidis isolated from a food specimen. Other information collected about each outbreak included the city, county, state, location where the food was prepared, and location where the food was consumed. The total number of outbreak-associated cases included all symptomatic persons with either culture-confirmed or epidemiologically linked infection.

Outbreak-associated foods were considered to be confirmed vehicles of transmission if 1) they were statistically implicated in an epidemiologic study, 2) *S.* Enteritidis was isolated from leftover foods, or 3) if the food item was the only food consumed by all ill persons (this occurred in <10 outbreaks). If eggs were implicated or were a primary ingredient in the implicated food, the outbreak was classified as egg-associated.

A subset of isolates from patients, food workers, implicated foods and farm specimens associated with outbreaks were phage typed at CDC and the U.S. Department of Agriculture (USDA) using a technique described by Ward et al. (14). Regions of the United States were defined as follows: Northeast—Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Vermont; Midwest—Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; South—Alabama, Arkansas, Delaware, the District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and West Virginia; and West—Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming.

Results

National Salmonella Surveillance Data

In the late 1980s and early 1990s, the proportion of *Salmonella* isolates that were *S.* Enteritidis continued to rise, and in 1994, it became the most common *Salmonella* serotype in the United States, representing 26% of all *Salmonella* isolates. Since then, however, the proportion has steadily decreased, reaching 16% in 1999.

The *S.* Enteritidis incidence rate increased from 2.38 per 100,000 population in 1985 to 3.9 per 100,000 in 1995. Since then, there has been a decline of 49%, to 1.98 per 100,000 in 1999 (Figure 1). This decline mirrors that of total *Salmonella* infections, which fell 51% to 12.0 per 100,000 in 1999. Rates of *S.* Enteritidis infection in the Northeast showed the greatest change, increasing from 6.39 per 100,000 in 1985 to 10.2 in 1989 and then dropping 63% to 3.8 per 100,000 in 1999 (Figure 1). Rates in the West rose from 0.87 per 100,000 in 1985 to 5.6 per 100,000 in 1994, and then fell to 2.2 per 100,000 in 1999, a decline of 61%. Rates in the Midwest rose from 1.81 per 100,000 in 1985 to 3.1 in 1997 and then decreased to 1.7 in 1999, while the rate in the South rose from 1.22 per 100,000 in 1985 to 1.85 in 1990, and then fell to 1.04 in 1999.

Outbreak Surveillance

From 1985 through 1999, a total of 841 outbreaks of *S.* Enteritidis infection were reported to CDC, affecting residents of 43 states, the District of Columbia, and Puerto Rico (Figure 2). The number of reported outbreaks increased from 26 in 1985 to 85 in 1990. Since 1990, outbreaks have declined 48%, to 44 in 1999. Reported out-

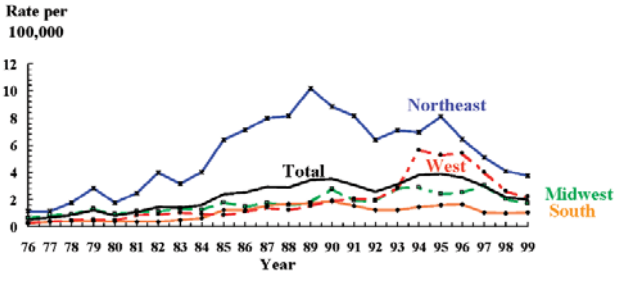

Figure 1. *Salmonella* Enteritidis isolation rates per 100,000 population, by selected regions,* United States, 1976–1999. *Northeast: Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont; Midwest: Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, Wisconsin; South: Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia; and West: Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming.

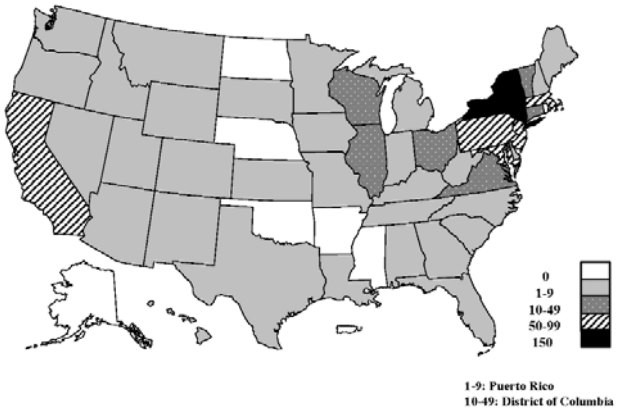

Figure 2. Reported outbreaks of *Salmonella* Enteritidis, by state, 1985–1999 (N = 841). Includes two multistate outbreaks.

breaks affected 29,762 persons; 2,904 (10%) were hospitalized (range 4%-21%), and 79 (0.3%) patients died (range 0%-0.9%) (Table 1). The median number of cases per outbreak decreased from 24 in 1985 to 10 in 1998.

Although the total number of outbreaks of *S.* Enteritidis infection declined from 1985 to 1999, regional rates have shifted dramatically. In the Northeast region, outbreaks decreased from 21 (81%) of 26 outbreaks in the United States in 1985 to 9 (20%) of 44 outbreaks in 1999 (Figure 3). Conversely, outbreaks in the Western region increased from 0 in 1985 to 22 (50%) of 44 outbreaks in 1999. Most of these outbreaks occurred in California, where the percentage of outbreaks increased from 0 in 1986 to 16 (73%) of the 22 Western region outbreaks in 1999. The percentage of outbreaks in the Midwestern region averaged 9% (range 0%-35%), while the percentages of outbreaks in the South averaged 10% (range 9%-31%).

Outbreak Settings

Five hundred twenty-two (62%) outbreaks of *S.* Enteritidis infection were associated with food prepared at commercial food establishments (restaurants, caterers, delicatessens, bakeries, cafeteria, or market), 112 (13%) were associated with food prepared in a private home, 55 (7%) with food prepared at schools or churches, and 20 (2%) with food served in prisons. Forty-three (5%) outbreaks involved foods prepared at other locations, such as camps, cruise ships, workplace, shelter, festivals, or an unknown location. Eighty-nine (11%) outbreaks involved food served to residents of hospitals or nursing homes.

Of 79 outbreak-associated deaths, 65 (82%) were among persons in healthcare facilities (55 deaths in nursing or extended-care homes and 10 deaths in hospitals) (Table 1). Deaths in healthcare facilities decreased from 14 in 1987 to 0 in 1994, 1996, 1997, and 1999 (Figure 4). Overall, the outbreak-associated case-fatality rate in healthcare institutions was 3% (range 0%-9% per year), higher than the average case-fatality rate of 0.3% for all outbreaks. The proportion of outbreaks occurring at healthcare institutions between 1992 and 1999 ranged from 2% to 14%, noticeably lower than the 11%-23% of outbreaks occurring at these facilities from 1985 through 1991.

Outbreak Vehicles

A food item was implicated in 389 (46%) outbreaks of *S.* Enteritidis infection from 1985 through1999; in 86 (22%) of these, more than one food item was implicated. Of the 371 outbreaks for which information was available, 298 (80%) were egg associated. This proportion ranged from 10 (71%) of 14 in 1985 to 19 (95%) of 20 in 1997

Table 1. Foodborne outbreaks of *Salmonella* serotype Enteritidis infection, United States, 1985–1999

	All outbreaks (N = 841)					Outbreaks in healthcare facilities[a] (N = 89)			
Y	No. outbreaks	No. ill	Median no. cases	No. hosp. (%)	No. deaths (%)	No. outbreaks	No. ill	No. hosp. (%)	No. deaths (%)
1985	26	1,159	24.0	144 (12)	1 (.08)	3 (12)	55	10 (18)	1 (2)
1986	47	1,444	12.0	107 (7)	6 (.4)	7 (15)	105	10 (10)	5 (5)
1987	58	2,616	17.5	557 (21)	15 (.6)	8 (14)	489	391 (80)	14 (3)
1988	48	1,201	12.5	155 (13)	11 (.9)	7 (15)	131	2 (2)	9 (7)
1989	81	2,518	23.0	206 (8)	15 (.6)	19 (23)	505	34 (7)	13 (3)
1990	85	2,656	18.0	318 (12)	3 (.1)	12 (14)	303	22 (7)	3 (1)
1991	74	2,461	15.0	200 (8)	5 (.2)	8 (11)	118	6 (5)	4 (3)
1992	63	2,348	13.0	233 (10)	4 (.2)	2 (3)	42	2 (5)	2 (5)
1993	66	2,215	16.5	219 (10)	6 (.3)	5 (8)	56	4 (7)	4 (7)
1994	51	5,492	14.0	214 (4)	0	0	0	0	0
1995	56	1,312	12.0	113 (9)	8 (.6)	8 (14)	156	21 (13)	7 (4)
1996	47	1,414	12.0	158 (11)	2 (.1)	3 (6)	64	9 (14)	0
1997	46	1,102	13.0	124 (11)	0	1 (2)	13	1 (8)	0
1998	49	744	10.0	93 (13)	3 (.4)	3 (6)	32	6 (19)	3 (9)
1999	44	1,080	15.0	63 (6)	0	3 (7)	12	5 (42)	0
Total	841	29,762	15.0	2,904 (10)	79 (.3)	89 (11)	2,081	523 (25)	65 (3)

[a]N = 841.

[b]Includes hospitals (hosp.) (14), nursing/extended care homes (69), assisted/independent living facilities (2), and drug/alcohol rehabilitation facilities (4).

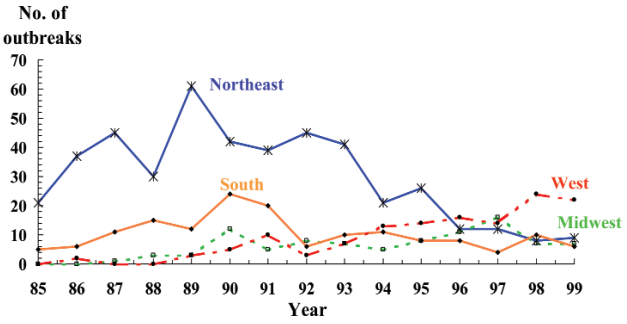

Figure 3. *Salmonella* Enteritidis outbreaks by region, United States, 1985–1999. Northeast: Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont; Midwest: Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, Wisconsin; South: Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia; and West: Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming.

(Table 2). Of outbreaks caused by a single vehicle for which information was known, 243 (83%) of 294 were egg-associated, as were 55 (71%) of 77 outbreaks in which more than one food item was implicated.

Among single foods implicated in egg-associated outbreaks, 67 (28%) of 243 were foods that contained raw eggs (e.g., homemade ice cream, Caesar salad dressing, tiramisu, egg nog). Sixty-five (27%) of the outbreaks implicated traditional egg dishes such as omelets, French toast, pancakes, and foods that use egg batter, such as crab cakes, chiles rellenos, egg rolls, and Monte Cristo sandwiches. Sixty-three (26%) outbreaks implicated dishes known to contain eggs, such as lasagna, ziti, and stuffing, which would have been expected to have been fully cooked but probably did not reach temperatures sufficient to kill *S.* Enteritidis. Thirty-six (15%) outbreaks implicated egg dishes that were "lightly cooked" (e.g., hollandaise sauce, meringue, cream pies). The food vehicles in 12 (5%) outbreaks were reported to contain eggs but could not be classified because information on how the dishes were prepared was not provided.

Seventy-three (20%) of the 371 confirmed outbreaks for which information was provided involved vehicles that did not contain eggs. Twenty (27%) of these outbreaks were associated with poultry (chicken or turkey), 8 (11%) with beef, and 6 (8%) with foods containing shrimp (3 outbreaks), bologna (1), pork (1), and pepper loaf (1). Other implicated foods included potatoes (3), beans (3), desserts (3), salad (3), macaroni and cheese (1), cheese sauce (1), goat cheese (1), chili (1), and a pureed diet (1). In 22 (30%) of the non–egg-associated outbreaks, more than one food was implicated. In four of these outbreaks, cross-contamination with raw eggs was suspected.

Phage Types

From 1988 through 1999, isolates from 455 outbreaks were submitted to CDC for phage typing. A single phage type was implicated in 436 (96%) of these outbreaks; 186 (43%) were caused by PT8, 96 (22%) by PT13a, and 64 (15%) by PT4. Other phage types included PT13 (20 outbreaks), PT34 (14 outbreaks), PT2 (13 outbreaks), and PT14b (9 outbreaks). Phage types differed by geographic region. In the Northeast and South, PT8 was the most common cause of *S.* Enteritidis outbreaks, followed closely by PT13a. Both PT8 and PT13a were common in the Midwest, while PT4 was predominant in the Western region.

The predominant phage types associated with *S.* Enteritidis outbreaks changed from 1988 through 1999. The proportion of outbreaks caused by PT8 and PT13a has decreased, while PT4 outbreaks have increased (Figure 5). In 1993, PT4 accounted for 2 (4%) of 47 *S.* Enteritidis outbreaks, while in 1999, PT4 represented 17 (49%) of 35 *S.* Enteritidis outbreaks and was the most common phage type. Most PT4 outbreaks were in the Western region; 52 (81%) of the 64 *S.* Enteritidis PT4 outbreaks reported from 1993 through 1999 occurred in California.

Hospitalization and death rates did not differ by phage type. For all years combined, hospitalization rates were 180 (9%) per 1,899 cases in PT4 outbreaks, 694 (10%) per 6,910 cases in PT8 outbreaks, and 267 (12%) per 2,281 cases in PT3a outbreaks. Death rates were not statistically different among phage types: 0.16% in PT4 outbreaks, 0.38% in PT8 outbreaks, and 0.26% in PT13a outbreaks.

Discussion

After a rapid increase in *S.* Enteritidis infection rates during the 1980s, the rate of sporadic cases and number of outbreaks declined overall. Evidence of this decline has been demonstrated through passive surveillance for sporadic cases identified through the national *Salmonella* sur-

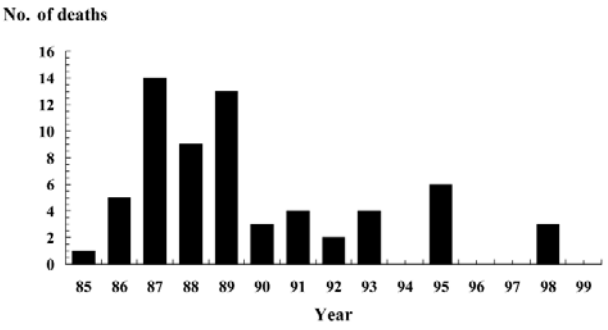

Figure 4. Deaths associated with outbreaks of *Salmonella* Enteritidis infections in healthcare facilities, 1985–1999 (N = 64).

Table 2. Reported outbreaks of *Salmonella* Enteritidis infection with confirmed vehicle that contained eggs as a principal ingredient, by year

	No. of outbreaks	Outbreaks with confirmed vehicle No. (%)	Outbreaks with a confirmed vehicle that contained eggs No. (%)
1985	26	14 (54)	10 (71)
1986	47	22 (47)	15/20 (75)
1987	58	28 (48)	21/24 (88)
1988	48	25 (52)	20/24 (83)
1989	81	30 (37)	19/28 (68)
1990	85	30 (35)	24/30 (80)
1991	74	29 (39)	20/26 (77)
1992	63	35 (56)	31/33 (94)
1993	66	40 (61)	31 (78)
1994	51	29 (57)	22 (76)
1995	56	22 (39)	15 (68)
1996	47	26 (55)	21/25 (84)
1997	46	22 (48)	19/20 (95)
1998	49	18 (37)	15/17 (88)
1999	44	19 (43)	15 (79)
Total	841	389 (46)	298/371 (80)

veillance system and the number and size of *S.* Enteritidis outbreaks that occurred throughout the United States. Success has been regional, with the greatest reductions occurring in the Northeast. One of the most notable features of this decline has been a reduction in the number of outbreaks of *S.* Enteritidis infection and outbreak-associated deaths occurring in hospitals and nursing homes during the 1990s. Although the exact reason for the overall decline in infections and outbreaks has not been proven, the many prevention and control measures implemented throughout the 1990s likely played a role. These measures include tracebacks, on-farm testing, quality assurance programs, regulations regarding refrigeration, educational messages for safe handling and cooking of eggs, and enhanced surveillance.

Tracebacks have been important in identifying farms associated with outbreaks and in tracing the spread of *S.* Enteritidis. A study of tracebacks completed in response to egg-associated outbreaks in the early 1990s showed that *S.* Enteritidis was isolated from the environment of all 14 implicated flocks tested (9). Diversion of eggs from *S.* Enteritidis–positive farms to pasteurization or hard-cooking facilities has also shown to be an effective strategy in reducing the number of human cases (10).

Quality assurance programs, first implemented in the Northeast in the early 1990s, have been essential in monitoring and controlling the spread of *S.* Enteritidis. A USDA survey found that 56% of egg-layer farms in 15 states participated in a quality assurance program and that the number of farms routinely testing for *S.* Enteritidis had increased from 16% in 1994 to 58% in 1999 (15). Other important on-farm interventions include flock-based control methods such as purchasing replacement chicks from *S.* Enteritidis–negative breeders, switching to a more *S.* Enteritidis–resistant breed of layer flock, and/or the use of vaccines in commercial pullet flocks.

Ensuring that eggs are fresh and that they are transported and stored properly are crucial steps in reducing illness. A risk assessment estimated that refrigerating eggs immediately after packing or laying could reduce *S.* Enteritidis illness by 8% and 12%, respectively (16). In August 1999, the USDA issued regulations stating that eggs packed for the consumer be stored and transported at a temperature of no higher than 45°F (7.2°C) and that containers for consumers be labeled to indicate that refrigeration is required (17). A 2000 Food and Drug Administration (FDA) rule also requires refrigeration of eggs offered for sale at retail stores (18). In addition, 17 states required an expiration or "sell-by" date on egg cartons in 1999 (19). Additional measures, such as in-shell egg heat pasteurization and irradiation, are currently available, although the effects of these on preventing human infections have not yet been measured.

Consumers and food service workers can prevent many human infections by handling and cooking eggs properly. A recent FDA rule requires that a safe handling statement be put on all cartons of shell eggs that have not been treated to destroy *Salmonella* (20). This statement explains that illness from bacteria can be prevented by keeping eggs refrigerated, by cooking eggs until the yolks are firm, and by thoroughly cooking egg-containing foods. The FDA Model Food Code advises against pooling of eggs and recommends that pasteurized eggs or egg products be substituted for raw shell eggs in the preparation of foods that are not cooked (21). The decrease in healthcare-associated deaths may be a response to the reduction in pooling of eggs, more widespread use of pasteurized eggs, and the increased education of food workers in these facilities. This decline shows that cases of *S.* Enteriditis infection were prevented in persons at highest risk for serious complications from *S.* Enteritidis infection, in particular, the elderly and persons with weakened immune systems.

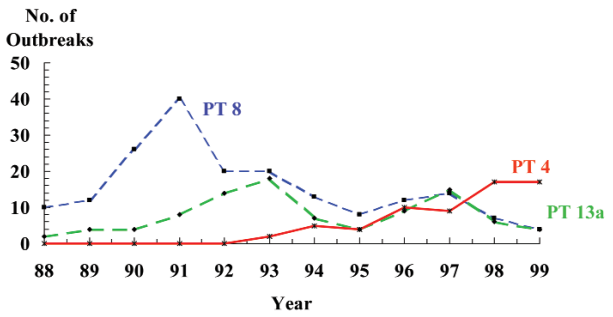

Figure 5. Frequency of outbreaks of *Salmonella* Enteritidis infection, by phage type (PT), United States, 1988–1999 (N = 346). Phage types were not collected until 1988.

Continued surveillance and outbreak investigations may help identify new vehicles of *S.* Enteritidis infection. Although a known risk factor in Europe, poultry has not previously played a large role in such infections in the United States. Our surveillance showed that in outbreaks with a known vehicle that did not contain eggs, poultry was the most common food vehicle. A study of sporadic *S.* Enteritidis infections in the United States implicated chicken as a risk factor for *S.* Enteritidis illness (22). In addition, a USDA survey of large production facilities that use Hazard Analysis and Critical Control Points (HACCP) plans found *S.* Enteritidis on 2.4% of broilers at slaughter (23). In 2000, sprouts and unpasteurized juice were identified as food vehicles in two *S.* Enteritidis outbreaks (CDC, unpub. data). These findings suggest that "new" vehicles may begin to play a larger part in future outbreaks.

Phage typing has proved to be a useful method for monitoring the spread of various strains in the United States over time. The appearance of *S.* Enteritidis PT4 in Europe led to a dramatic increase in the number of human *S.* Enteritidis infections. In the United States, PT8 and PT13a were the predominant phage types during the 1980s, and *S.* Enteritidis PT4 was not associated with domestically acquired *S.* Enteritidis infections. In 1993, the first U.S. outbreak of *S.* Enteritidis PT4 infection occurred in Texas (24), and over the next 3 years, PT4 caused human illness in California, Utah, Nevada, Arizona, and Hawaii. The introduction of PT4 in Utah caused a five-fold increase in human *S.* Enteritidis cases within 6 months (25). This paralleled the introduction of *S.* Enteritidis PT 4 into southern California in 1994, where it also caused a substantial increase in human illness (7). Since then, the number of outbreaks in the Pacific region has increased greatly. *S.* Enteritidis PT 4 has also been isolated from eggs and the farm environment of laying flocks implicated as sources for human outbreaks (25). Despite the increase in cases and outbreaks caused by PT4, severity of disease does not appear to be increasing. Hospitalization and death rates seen in PT4 outbreaks do not differ from those of other phage types. Understanding the spread of *S.* Enteritidis PT4 and other emerging phage types may give new clues to the prevention of human illness.

Despite these declines in *S.* Enteritidis infection, much more remains to be done. Cases of infection identified through outbreak investigations represent only a small fraction of reported infections. It is estimated from FoodNet[4] data that for every case of *S.* Enteritidis infection identified as many as 37 go unrecognized (D. Voetsch et al, unpub. data). So, in 1999, as many as 200,000 cases may have occurred, of which only 5,343 were reported to CDC (1). To return to the 1976 baseline rate of 0.55 *S.* Enteritidis infections per 100,000 persons, a further 72% reduction in reported infections is required. A risk assessment conducted by USDA suggests that a broad-based policy is likely to be more effective in eliminating egg-associated *S.* Enteritidis illness than a policy directed solely at one stage of the continuum from egg production to consumption (16). To meet the challenge of further reducing such infections, the President's Council on Food Safety announced an Egg Safety Action Plan on December 10, 1999, with the interim goal of reducing egg-associated *S.* Enteritidis illnesses by half by 2005 and eliminating them by 2010 (26). The plan calls for cooperation between industry, regulatory agencies, and local, state, and federal officials to implement specific controls along the entire farm to table continuum.

Conclusion

The incidence of *S.* Enteritidis illness and the number of such outbreaks in the United States have decreased by almost 50% between the mid-1990s and 1999. The most dramatic changes were the decrease in the number of outbreaks seen in the Northeast and the reduction in numbers of outbreaks and *S.* Enteritidis–associated deaths in hospitals and nursing homes. Although the exact mechanism behind these decreases has not been explained, the reductions were likely a result of intervention programs along the farm-to-table chain. Despite these accomplishments, more work needs to be done. Further success will be measured by our ability to consistently apply and successfully monitor *S.* Enteritidis prevention and control measures along the entire farm-to-table continuum.

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Emerging Issues in Virus Taxonomy

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Viruses occupy a unique position in biology. Although they possess some of the properties of living systems such as having a genome, they are actually nonliving infectious entities and should not be considered microorganisms. A clear distinction should be drawn between the terms virus, virion, and virus species. Species is the most fundamental taxonomic category used in all biological classification. In 1991, the International Committee on Taxonomy of Viruses (ICTV) decided that the category of virus species should be used in virus classification together with the categories of genus and family. More than 50 ICTV study groups were given the task of demarcating the 1,550 viral species that were recognized in the 7th ICTV report, which was published in 2000. We briefly describe the changes in virus classification that were introduced in that report. We also discuss recent proposals to introduce a nonlatinized binomial nomenclature for virus species.

In the 7th report of the International Committee on Taxonomy of Viruses (ICTV), viruses were described as elementary biosystems that possess some of the properties of living systems such as having a genome and being able to adapt to a changing environment (1). Viruses belong to biology because they possess genes, replicate, evolve, and are adapted to particular hosts, biotic habitats, and ecological niches. However, viruses cannot capture and store free energy, and they are not functionally active outside their host cells. Although they are pathogens, viruses should not be considered pathogenic microorganisms since they are not alive. The simplest system that can be said to be alive is a cell. Cells acquire the autonomy that is characteristic of living systems through a complex set of integrated, metabolic activities. However, none of the individual constituents of cells, such as organelles or macromolecules, can be said to be alive. A virus becomes part of a living system only after it has infected a host cell and its genome becomes integrated with that of the cell. Viruses are replicated only through the metabolic activities of infected cells, and they occupy a unique position in biology. They are nonliving infectious entities that can be said, at best, to lead a kind of borrowed life.

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Viruses versus Virus Particles or Virions

A virus is a general term which denotes any number of concrete objects that possess various relational properties (for instance, its host, vector, and infectivity) that arise by virtue of a relation with other objects. These relational properties, also called emergent properties, are characteristic of the viral biosystem as a whole and are not present in its constituent parts. When a virus undergoes its so-called life cycle, it takes on various forms and manifestations, for instance, as a replicating nucleic acid in the host cell or vector. One stage in this cycle is the virus particle or virion, which is characterized by intrinsic properties such as size, mass, chemical composition, nucleotide sequence of the genome, and amino acid sequence of protein subunits, among others. Virions can be fully described by their intrinsic chemical and physical properties, and that description does not entail the relational properties that belong to the virus.

Confusion sometimes arises when a virion is called the virus, as, for instance, when one refers to "the picture of the virus" or to the process of "purifying the virus." What is actually meant in such cases is a virus particle, not a virus. Confusing virus with virion is similar to confusing the entity insect, which comprises several different life stages, with a single one of these stages, such as pupa, caterpillar, or butterfly.

The Species Concept in Virology

Since viruses, like other biological entities, are concrete objects located in time and space, their classification is a purely conceptual construction, based on the use of abstract categories that have no spatiotemporal localization (1). Virus classification places the viruses in a series of classes or taxonomic categories with a hierarchical structure, the ranks being the species, genus, family, and order. These classes are abstractions, i.e., conceptual constructions produced by the mind, and they should not be confused with the real, disease-causing objects studied by

virologists. Centrifuging the family *Picornaviridae*, the genus *Enterovirus*, or the species *Poliovirus* is impossible for the simple reason that abstractions, i.e. ideas, cannot be centrifuged. For the same reason, a virus species cannot cause a disease, since abstractions do not cause diseases (with the exception of psychosomatic disease). The concrete virus entity that causes a disease can, however, be a member of an abstract virus species. Concrete objects like viruses can be members of an abstract entity, that is, an entity of a different logical type, although they cannot be a part of such an abstract concept. Class inclusion or class membership is the correct relationship between a virus and the species category to which it belongs. One often reads that the species *Mus musculus* has been inoculated with one or other virus species. The correct statement is that a mouse (a member of the species *Mus musculus*) has been inoculated with a member of viral species X.

Although species is the most fundamental taxonomic category in all biological classifications, it was only in 1991 that the ICTV agreed that the concept of virus species should be uniformly applied in virus classification. For many years, plant virologists had been arguing that the concept of species was not applicable to viruses because they are not sexually reproducing organisms (2,3). These virologists took the view that the only legitimate species concept was that of biological species, defined on the basis of gene pools and reproductive isolation. Such a concept is clearly not applicable to entities like viruses that replicate by clonal means (4). However, many other species concepts are currently used in biology, some of them applicable to asexual organisms. As many as 22 different species concepts have been applied in various fields of biology (5).

For virus species to become accepted by the virologic community, coining a definition to which virologists could subscribe was necessary. In 1991, the ICTV endorsed the following definition: "A virus species is a polythetic class of viruses that constitute a replicating lineage and occupy a particular ecological niche" (6–8). This definition was no longer based on purely phenetic criteria of similar characteristics but stressed the cohesive forces present in ancestral-descendant biological populations that share a common biotic niche. Another important feature of the definition is that a virus species is defined as a polythetic class rather than as a traditional universal class. A polythetic class consists of members which have a number of properties in common but which do not all share a single common property that could be used as a defining and discriminating property of the species because it is absent in other species. This situation is illustrated in the Figure.

The advantage of defining virus species as polythetic classes is that individual viruses that lack one or other characteristic normally considered typical of the species can be accommodated. This advantage is particularly relevant for entities like viruses that undergo continual evolutionary changes and show considerable variability. In practice, a single discriminating characteristic, such as a particular host reaction or a certain percentage of genome sequence identity, cannot be a defining property of any virus species. Rather, a combination of properties always provides the rationale for deciding whether a virus should be considered a member of a particular species. Thus, different virus species do not have sharp boundaries. Rather, they should be viewed as fuzzy sets with hazy boundaries (1,9).

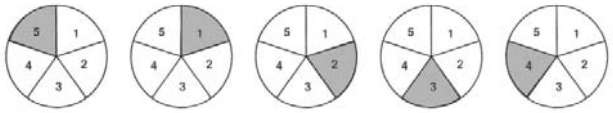

Figure. Schematic representation of five members of a polythetic class characterized by five properties, 1–5. Each member possesses several of these properties, but no single property is present in all the members of the class. This missing property in each case is represented by the gray sector.

Species are thus very different from the other taxonomic categories used in virus classification such as genera and families. A viral family, for instance, is a so-called universal class that consists of members, all of which share a number of defining properties that are both necessary and sufficient for class membership (10). Allocating a virus to a family or a genus is thus an easy task since all that is required is to consider a few morphologic or chemical features that suffice to unambiguously position the virus in the classification scheme. For instance, all members of the family *Herpesviridae* are enveloped viruses that contain an icosahedral particle and double-stranded DNA, whereas all members of the family *Adenoviridae* are nonenveloped viruses that contain an icosahedral particle and double-stranded DNA, with projecting fibers at the vertices of the protein shell. In contrast, allocating a virus to a particular species is often a matter of convenience or convention rather than of logical necessity based on an unequivocal defining property.

Demarcating Virus Species and Identifying Viruses

It is a common misperception that once the concept of virus species has been defined, deciding if a particular virus is a member of a certain virus species is easy. This expectation arises because of a failure to appreciate that definitions apply only to abstract concepts, such as the notion of species taken as a class. Individual viruses, like individual people or any other concrete entities, can be named and identified by so-called diagnostic properties, but they cannot be defined (11). The difference between definition and identification can be illustrated by the following analogy. Transportation vehicles can be classified

into categories such as buses, trucks, and cars. Cars can be defined as a type of vehicle with four wheels, capable of transporting a limited number of persons, not exceeding a certain size or weight. However, such a definition will be of no use in discriminating between a Ford and a Toyota. To ascertain whether an individual vehicle corresponds to a particular make of car, a set of distinguishing characteristics that make it possible to identify each car must be used. In a similar way, the theoretical definition of the species category that the ICTV endorsed in 1991 is not helpful for recognizing and distinguishing the viruses that are members of individual species. What is required is that virologists reach an agreement about which diagnostic properties are the most useful for identifying the individual members of a virus species. Since ICTV study groups (12) are mostly responsible for deciding which virus species should be recognized within individual genera and families, these specialty groups, with their in-depth knowledge of particular areas in virology, have been given the task of establishing which diagnostic properties are most useful for species demarcation.

To differentiate between individual species, it is necessary to rely on properties that are not present in all the members of a genus or family, since obviously such properties will not permit species demarcation. For example, characteristics such as virion morphology, genome organization, method of replication, and number and size of structural and nonstructural proteins are properties shared by all the members of a genus or family. Therefore, these characteristics cannot be used for demarcating individual species within a given genus. The following properties are useful for discriminating between virus species (13): genome sequence relatedness, natural host range, cell and tissue tropism, pathogenicity and cytopathology, mode of transmission, physicochemical properties, and antigenic properties.

All of these characteristics are not equally important for demarcating species in different viral genera and families, however. There is, in fact, no need to harmonize diagnostic criteria across all species, genera, and families. In some families, certain diagnostic criteria will be more important than in others, not the least because the practical needs for making certain distinctions are not the same in all areas of virology. The major purpose of virus classification is to partition the world of viruses into a coherent scheme of easily recognizable entities that answers to the everyday needs of practicing virologists. From a human perspective, not all hosts are equally relevant. Thus, human pathogens or pathogens that infect animals and plants of economic importance will be studied more intensively than, say, the viruses that infect the myriad species of insects. Finer distinctions based on relatively minor differences in host range, pathogenicity, or antigenicity may thus be made in the case of viruses that are of particular interest to humans. For instance, differences in the antigenic and genomic properties of individual human adenoviruses may be considered sufficient reason to allocate these viruses to separate species, whereas the same degree of antigenic dissimilarity would in other cases lead such entities to be considered serotypes of the same species. Allocating viruses to different species requires that an answer be given to the perplexing question of identity: how different must two viruses be to be considered different types of virus and therefore members of different species? Mutants or pathogenic variants that are clearly distinguishable from the wild-type virus will, however, generally be recognized as being the same type of virus, and they will, therefore, be considered, in terms of taxonomy, to be members of the same virus species.

Deciding whether individual virus isolates correspond to strains or serotypes of one species or belong to separate species remains in many cases one of the challenges that must still be addressed by many ICTV study groups. Virus identification is usually a comparative process whereby individual isolates are compared with the members of established virus species. Since virus species are polythetic, the comparison should involve a number of different characteristics rather than the presence or absence of a single key feature. However, the use of several characteristics is essential only for demarcating individual polythetic species and for constructing an acceptable classification scheme. Once a species has been established on the basis of several demarcation criteria, identifying a virus isolate as a member of that species by considering only a few properties may be possible. For instance, if a virus isolate reacts with a panel of monoclonal antibodies in the same way as an established member of a given species, the virus will be considered as a member of that species.

The 7th ICTV Report

The 7th ICTV report (14) was published in 2000, five years after the 6th report (15). Whereas the 6th report described 1 order, 50 families, and 164 genera, the 7th report contained 3 orders, 63 families, and 240 genera. In the 6th report, >3,600 viral entities were listed, in many cases without a clear indication of their status as species, strains, serotypes, or isolates. In the 7th report, the criteria used for demarcating virus species within a genus were defined for many of the genera, which resulted in a list of 1,550 officially recognized viral species. The major changes in the classification scheme introduced in the 7th report have been summarized by Fauquet and Mayo (16).

Names and Typography of Virus Species

In earlier ICTV reports, names of orders, families, subfamilies, and genera were written in italics with a capital

initial letter and had the following endings: *-virales* for orders, *-viridae* for families, *-virinae* for subfamilies and *–virus* for genera. The revised code (17) extends this typographic convention to the names of virus species in order to give a visible sign that species were recognized viral taxa, just as are genera and families. In most cases, the English common names of viruses have become the species names, and these are written in italics with the initial letter capitalized (17,18). The effect is to discriminate between virus species officially recognized by the ICTV and other viral entities such as tentative species, viral strains, serotypes, or other subspecific entities within a species. This new typography has met with some criticism (19,20) and corresponding rebuttals (21,22), but it is now generally applied in most scientific journals and books on virology (23).

The value of using italics is that it visibly reinforces the status of the corresponding species as a taxonomic entity, i.e., a formal, abstract class, distinct from the concrete viral objects that replicate and cause disease and that are written in Roman characters. Only if it is necessary to draw attention to the taxonomic position of the virus under study will it be necessary to refer to the official species name written in italics. Even then, the official name need be given only once, probably in the introduction or Materials and Methods sections (e.g., *Measles virus*, genus *Morbillivirus*, family *Paramyxoviridae*). In publications written in languages other than English, the use of italics for the English official species name would also indicate the alien nature of the term. In such publications, the common names of viruses will be those used in that language and not the English names. The use of italicized English instead of italicized Latin for the names of virus species reflects the emergence of English as the modern language of international scientific communication, and it also does away with the invidious task of having to coin new Latin names for all virus species (21).

By introducing italicized virus species names, the ICTV in no way intended to replace the existing vernacular or common names of viruses written in Roman characters (21,24). The viruses studied by virologists are concrete, disease-causing entities and not abstract classes, and they should continue to be referred to by their common, nonitalicized names. As recently reiterated by Drebot et al. (25), only the names of viral taxonomic classes are written in italics, not the names of viruses. In scientific articles, authors need to refer most of the time to the virus as a physical entity rather than as a member of a taxonomic class. Therefore, the common name written in Roman characters will most often be used; the species name, in italics, will appear only once for the purpose of taxonomic placement of the virus being discussed.

A Proposed Binomial Nomenclature for Virus Species

For many years, some plant virologists have been using an unofficial binomial system for referring to virus species (as well as to viruses). In this system, the italicized word *virus* appearing at the end of the current official species name is replaced by the genus name, which also ends in "*-virus*" (20,25). Thus *Bluetongue virus* becomes *Bluetongue orbivirus* and *Measles virus* becomes *Measles morbillivirus*. The advantage of such a system is that inclusion of the genus name in the species name indicates relationships with other viruses and therefore provides additional information about the properties of the members of the species. To nonspecialists, it would then be immediately obvious that Hepatitis A, B, and C viruses are very different entities, belonging to different genera, were their official names *Hepatitis A hepatovirus*, *Hepatitis B orthohepadnavirus*, and *Hepatitis C hepacivirus.*

Such a binomial system for species names would also have the advantage of clearly distinguishing between the species name written in italics (*Measles morbillivirus*) and the common, nonitalicized virus name, measles virus. At present, the distinction between the species name and the virus name in most cases relies only on typography (i.e., *Measles virus* versus measles virus), which can lead to confusion (24).

Whether nonlatinized binomials should become the official species names of viruses has been debated within the ICTV for many years (21,22,25–28). Although most plant virologists have favored the use of binomials for many years (29), to what extent human and animal virologists would find the system acceptable has not been known. As the ICTV strives to develop a universal system of nomenclature approved by all virologists (17), it is bound to move cautiously before changing all the current, official names of virus species. Since very few virologists express their views on matters of taxonomy (21,22), successive ICTV Executive Committees have always found it difficult to poll the representative opinion of virologists worldwide (30), and it is not clear what sort of democratic process would satisfy those who criticize ICTV decisions. During 2002, efforts were made to canvass virologists regarding their acceptance of a binomial system of species names; the results of two ballots showed that a sizeable majority (80%-85%) of the 250 virologists who expressed an opinion were in favor of a binomial system (24,31). The new ICTV Executive Committee established at the 12th International Congress of Virology, held in Paris in July 2002, will decide in the near future if binomial names of virus species should be introduced. A list of current virus species names, together with their binomial equivalents, can be found on ICTV net (available from: URL: www.danforthcenter.org/ILTAB/ICTVnet/).

Abbreviations for Virus Names

To avoid repetition, authors of virology papers use abbreviations for virus names, once the full name has been given. Since it is only the common names that are used repeatedly in a given text, abbreviating them (rather than the current official species names or their binomial counterparts if binomials were to become the official names) makes sense.

Although the ICTV does not have a constitutional responsibility for devising appropriate abbreviations, it has over the years published several lists of recommended abbreviations of virus names. Initially, these were abbreviations for the common names of viruses (32,33), but subsequently they were published as abbreviations for the names of virus species (34,35). Although the names of the viruses and of the corresponding viral species are usually the same, they are not necessarily so, and it could be argued that species names do not need to be abbreviated at all. The abbreviations recommended by ICTV should therefore apply only to the names of viruses. Although an emerging discipline, virus taxonomy is essential to the working virologist, and we need to achieve universal agreement on the principles so that we can freely communicate without misunderstanding (36,37).

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Fungal Biofilms and Drug Resistance

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Candida species, including the novel opportunistic pathogen *Candida dubliniensis*, are now emerging as major agents of nosocomial infections. Many such manifestations of infections associated with the formation of *Candida* biofilms include those occurring on devices such as indwelling intravascular catheters. Fungal biofilm-associated infections are frequently refractory to conventional therapy because of resistance to antimicrobial agents. This resistance could be in part due to the surface-induced upregulation of drug efflux pumps. Biofilm-associated *Candida* show uniform resistance to a wide spectrum of the currently available conventional antifungal agents, which implies that antimicrobial drugs that specifically target biofilm-associated infections are needed. The novel classes of antifungal agents, the lipid formulation of amphotericins, and the echinocandins have demonstrated unique antifungal activity against the resistant *Candida* biofilms, providing a breakthrough in the treatment of life-threatening invasive systemic mycoses. The use of drugs effective in combating biofilm-associated infections could lead to major developments in the treatment of fungal implant infections.

The genus *Candida* is composed of an extremely heterogeneous group of organisms that grow as yeasts. Most members of the genus also produce a filamentous type of growth (pseudohyphae) (1). In addition to pseudohyphae, *Candida albicans* and *C. dubliniensis* form true hyphae (germ tubes) and thick-walled cells referred to as chlamydospores, both of which are used by mycology diagnostic laboratories in identifying these species (1). *Candida* species are now emerging as major agents of hospital-acquired infections; they are ranked as the third or fourth most commonly isolated bloodstream pathogens, surpassing gram-negative bacilli in frequency (2–9). Although *C. albicans* is the predominant etiologic agent of candidiasis, other *Candida* species that tend to be less susceptible to the commonly used antifungal drugs such as *C. krusei*, *C. glabrata*, *C. lusitaniae*, and the newest *Candida* species, *C. dubliniensis*, have emerged as substantial opportunistic pathogens (10). *Candida dubliniensis* shares with *C. albicans* many virulence factors, such as germ tube formation, exoenzyme production, and phenotypic switching (10). This species, however, unlike *C. albicans*, has been shown to readily develop stable resistance to fluconazole in vitro and in infected patients, strongly suggesting that *C. dubliniensis* possesses a readily inducible fluconazole resistance mechanism (11–13).

Indwelling intravascular catheters represent a risk factor that is associated with nosocomial *Candida* infections. The devices become colonized by the microorganisms that form a biofilm of cells, the detachment of which can result in septicemia (2–5,8,9,14,15). Most manifestations of candidiasis are in fact associated with the formation of *Candida* biofilms on surfaces, and this phenotype is associated with infection at both the mucosal and systemic sites (8). Superficial *Candida* infections of prostheses and implanted devices are troublesome and the most frequently encountered. One of the most common is oral denture stomatitis, a *Candida* infection of the oral mucosa promoted by a close-fitting upper denture present in 65% of edentulous persons (5,8).

Microbial Biofilms

Biofilms are universal, complex, interdependent communities of surface-associated microorganisms. The organisms are enclosed in an exopolysaccharide matrix occurring on any surface, particularly aquatic and industrial water systems as well as medical devices. As such, biofilms are highly relevant for public health (4,7,15–18). Most microorganisms grow in structured biofilms rather than individually in suspensions and while in this environment may display altered phenotypes (2). Biofilms can be composed of a population that developed from a single species or a community derived from multiple microbial species (14,17). Speculations about the ecologic advantages of forming a biofilm include protection from the environment, nutrient availability, metabolic cooperation, and acquisition of new genetic traits (3,17). Biofilms are notoriously difficult to eliminate and are a source of many recalcitrant infections (15,16). A variety of microbial infections are caused by biofilms ranging from the com-

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mon, such as urinary tract infections, catheter infections, child middle-ear infections, and dental plaque, to more threatening infections, such as endocarditis and infections of heart valves (16,19). Immunocompromised patients such as those with cancer or HIV infection are often the most susceptible.

Although bacterial biofilms and their role in disease have been investigated in detail over a number of years, much less is known about fungal biofilms (2,3,8,9). Regarding oral or pharyngeal infections, to colonize and infect the oral environment, yeast cells must first adhere to host cells and tissues or prosthetic materials within the oral cavity or must coaggregate with other oral microorganisms (8,20,21). *C. albicans* biofilm formation has been shown in our laboratory and others to proceed in three distinct developmental phases: early (0–11 h), intermediate (12–30 h), and mature (38–72 h) (5) (Figure 1). The detailed structure of a mature *C. albicans* biofilm produced in vitro after 48-hour incubation has been shown to consist of a dense network of yeasts, hyphae, and pseudohypha (Figure 2). This mixture of yeasts, hyphae, and matrix material is not seen when the organism is grown in liquid culture or on an agar surface, which suggests that morphogenesis is triggered when an organism contacts a surface and that the basal cell layer may have an important role in anchoring the biofilm to the surface (2,3,5,8). In addition, bacteria are often found with *Candida* species in biofilms in vivo, indicating that extensive interspecies interactions probably occur (2,3,14,18,20).

Candida biofilms share several properties with bacterial biofilms. The two consequences of biofilm growth with profound clinical implications are the markedly enhanced resistance to antimicrobial agents and protection from host defenses, the main reasons why biofilm-associated infections are frequently refractory to conventional therapy (2,4,5,7–9,16,18,22,23). Recently, studies showed that *C. dubliniensis* has the ability to adhere to and form biofilms with structural heterogeneity and typical microcolony and water channel architecture similar to what has been described for bacterial biofilms and *C. albicans* biofilms (7,8). In addition, resistance of *C. dubliniensis* to fluconazole, as well as increased resistance to clinically applied amphotericin B (8,12,13,23,24), was demonstrated in biofilms.

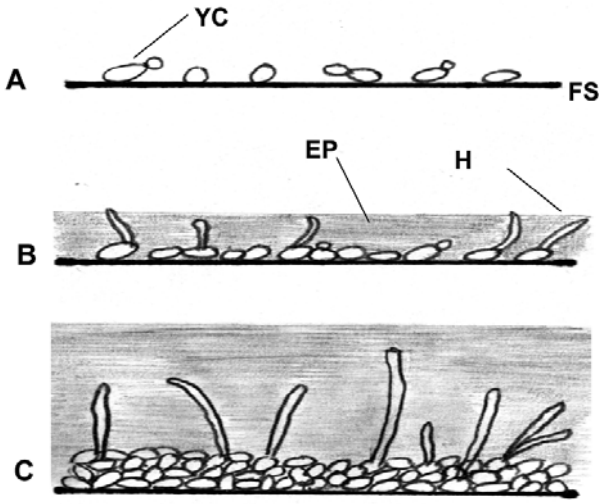

Figure 1. Illustration of biofilm development in *Candida albicans* and *C. dubliniensis*; A, early 0–11 h; B, intermediate 12–30 h; C, mature 38–72 h; FS, flat surface; YC, yeast cell; H, hyphae; EP, exopolymeric matrix.

Antifungal Drug Resistance

Antifungal drug resistance is quickly becoming a major problem in the expanding population of immunocompromised persons. It has resulted in a drastic increase in the incidence of opportunistic and systemic fungal infections. Clinical resistance is defined as persistence or progression of an infection despite appropriate antimicrobial therapy. Resistance is considered primary when an organism is resistant to the drug before exposure, whereas secondary resistance is that which develops in response to exposure to the drug (25). This latter mechanism of resistance accounts for the emergence of resistance to azoles seen over the last few years. Azole antifungal agents have become important in the treatment of mucosal candidiasis in HIV patients. Specifically, fluconazole is considered the drug of choice for the most common HIV-associated opportunistic infections in the oral cavity (26). Increased use of the azoles, coupled with the fact that they are fungistatic drugs, has likely resulted in the emergence of resistance to azoles.

Major genes that contribute to drug resistance are those coding for multidrug efflux pumps, the upregulation of which can result in a multidrug-resistant phenotype (2,5,9,26,27). *C. albicans* and *C. dubliniensis* possess two different types of efflux pumps: adenosine triphos-

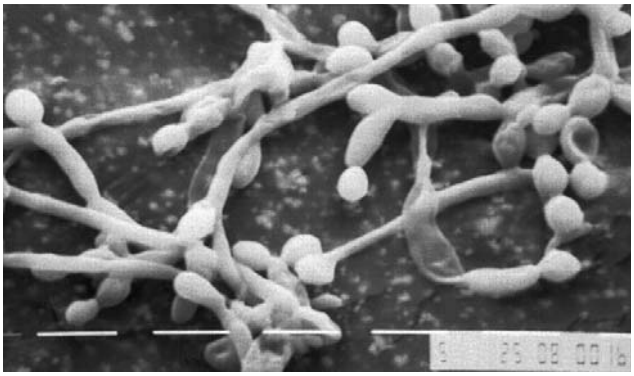

Figure 2. Typical field found in scanning electron micrograph of biofilm formed by *Candida albicans* on an intravascular disc prepared from catheter material.

phate–binding cassette (ABC) transporters encoded by the *CDR* genes (*CDR1* and *CDR2*) and major facilitators encoded by the *MDR* genes (2,12,26–28). Genes for both types of efflux pumps have been recently demonstrated to be upregulated during biofilm formation and development (2,5,9). The ABC transporters *CDR1* and *CDR2* in *C. albicans* and *C. dubliniensis* constitute a multigene family with a demonstrated role in resistance (5,9,12). The *MDR1* gene encodes a major facilitator, the overexpression of which leads exclusively to fluconazole resistance (5,9,12).

Antimicrobial Drug Resistance

Microbial biofilms not only serve as a nidus for disease but also are often associated with high-level antimicrobial resistance, a consistent phenomenon that may explain the persistence of many infections in the face of appropriate antimicrobial therapy (15,29). A study by Ramage et al. (9) analyzed the expression of *C. albicans MDR1*, *CDR1*, and *CDR2* genes during both planktonic and biofilm modes of growth. Yeast biofilms were formed in the wells of microtiter plates by pipetting standardized cell suspension of freshly grown and washed yeast cells into wells of microtiter plates and incubating at 37°C (9). After biofilm formation, the medium was aspirated and nonadherent cells were removed by thoroughly washing the biofilm. Antifungal susceptibility testing was performed by adding antifungal solution to the biofilms in serially diluted concentrations and incubating for 48 hours at 37ºC. MICs for biofilm cells were determined by using the XTT reduction assay, which semiquantitatively measures the metabolic activity of the cells within the biofilm based on a color change on the reduction of a salt that is reduced by mitochondrial dehydrogenases of metabolically active yeast cells (9).

Northern blot analysis from the study showed that mRNA levels for these genes were upregulated when the *C. albicans* cells were in a sessile mode of growth compared with planktonic cells, with mRNA levels for the *MDR1* gene transiently increased in 24-hour biofilms, which indicates that efflux pumps are upregulated in cells within a biofilm, possibly contributing to the observed azole resistance (9). However, mutant strains deficient in efflux pumps and hypersusceptible to fluconazole when grown in a planktonic mode retained a resistant phenotype during biofilm growth. This finding demonstrates that drug resistance in biofilms is complex and involves more than one mechanism (8).

The mechanisms by which *Candida* biofilms resist the functions of antifungal agents are therefore poorly understood. Factors that have been considered to be responsible for the increased resistance to antibiotics in bacterial biofilms include restricted penetration of antimicrobials caused by the exopolymeric material (EP) (14). Baillie et al. (4) analyzed the composition of *C. albicans* biofilms by isolating EP from catheter tips with adherent biofilm and, after removing the cells in suspension, concentrating and dialyzing the supernatant. The concentrated supernatant was then analyzed for total carbohydrate, phosphorous, protein, glucose, and hexosamine by chemical methods and by high-pressure liquid chromatography. Results of that study showed that the extent of matrix formation in *Candida* biofilm did not appear to affect the susceptibility of biofilms to five clinically important antifungal agents.

The potential for drug exclusion by the biofilm matrix that may act as a barrier to fluconazole penetration in biofilms of mixed species of *Candida* and oral bacteria seems to depend on a number of factors; data supporting this mechanism of resistance in bacterial biofilm are strong (2,4,7,8,17). Growth rate has been considered as an important modulator of drug activity in bacterial biofilms. Biofilms are thought to grow slowly because nutrients are limited, resulting in decreased metabolism of the microorganisms (2,7,8,16,29). A slow growth rate is frequently associated with the adoption of a different phenotype by microorganisms such as changes in the cell envelope, which in turn affect the susceptibility of the microorganism to antimicrobial agents. In addition, virtually all antimicrobial drugs are more effective in killing rapidly growing cells, and some have an absolute requirement for growth in order to kill (16).

Regarding fungal biofilms, however, a study by Chandra et al. (5), related to the increase of antifungal resistance during biofilm development, showed that the progression of drug resistance was associated with increase in metabolic activity of the developing biofilm and was not a reflection of slower growth rate, which indicates that drug resistance develops over time, coincident with biofilm maturation. This was the first report correlating the emergence of antifungal drug resistance with the development of biofilm (4).

Since the drug resistance in *C. albicans* biofilms cannot be attributed solely to matrix exclusion or slow growth rate, contact-induced gene expression for acquiring characteristic properties is probably an additional mechanism by which drug resistance is acquired (4,15). In addition, synthesis of new proteins occurs after *C. albicans* attaches to surfaces, which suggests that drug resistance might also arise as a consequence of specific surface-induced gene expression (4). Quantitative analysis of planktonic EP in comparison to *C. albicans* biofilm EP showed that glucose was more abundant in biofilm EP than planktonic EP, also suggesting that *C. albicans* might produce biofilm-specific EP by differentially regulating genes encoding enzymes involved in carbohydrate synthesis (4,5). In addition, the expression profile of *C. albicans* genes belonging to the ALS family, which encode proteins implicated in adhesion

of *C. albicans* to host surfaces, was investigated. Northern blot analysis of total RNA from planktonic and biofilm-grown cells demonstrated that *ALS* gene expression is differentially regulated between the two growth forms, with additional genes expressed in biofilms (4,5). These observations provide further evidence for contact-induced gene expression and transcriptional changes that are likely to occur during biofilm formation.

A recently proposed hypothesis on bacterial biofilm drug resistance asserts that most cells in the biofilm may not necessarily be more resistant to killing than planktonic cells. Rather, a few persisters survive and are preserved by the presence of an antimicrobial drug that slows their growth, paradoxically helping persisters to persevere and resist being killed. Thus persisters are ultimately responsible for the high level of biofilm resistance to killing (8,16,22,29). The nature of persistence and whether it even applies to fungal biofilms, however, is not clearly understood. The ability to eliminate defective cells that would otherwise drain limited resources may be a substantial adaptive value to a clonal population such as a biofilm community. Cells with serious defects undergo programmed cell death (PCD). Antimicrobial drugs that do not kill cells but cause damage trigger suicide, resulting in death from apoptosis. Persisters could represent cells with disabled PCD as a safety mechanism aimed at preventing suicide when a antimicrobial drug reaches the entire population or when nutrients are limited. Therefore, inhibition of PCD to prevent suicide allows starved cells to develop tolerance to antimicrobial drugs (16).

With fungal biofilms serving as a safe reservoir for the release of infecting cells into the oral or other environment, biofilm formation by *C. dubliniensis* and *C. albicans* likely represents a key factor in their survival, with important clinical repercussions. Treating life-threatening invasive mycoses with new antifungal agents that are active against biofilms and effective in combating biofilm-associated infections is important. Recently, studies showed some antibiofilm activity with the new lipid formulations of amphotericin B and the two echinocandins (caspofungin and micafungin), a new class of antifungals (2,24,29). These interesting findings could lead to important developments in the treatment of fungal implant infections.

Class of Antifungal Drugs

The antifungal agents currently available for the treatment of systemic fungal infections are classified by their site of action in fungal cells. The polyene antifungal agents, which include nystatin and amphotericin B, are fungicidal and have the broadest spectrum of antifungal activity of the available agents (30,31). The polyenes cause the fungal cell to die by intercalating into ergosterol-containing membranes, the major sterol in fungal membrane, to form channels and destroy the proton gradient in the cell with leakage of cytoplasmic content (30,31). Intravenous amphotericin B has been the drug of choice for invasive fungal infections (30). The most serious side effect of amphotericin B therapy is nephrotoxicity. To reduce the nephrotoxicity of conventional amphotericin B, lipid formulations are being used that have comparable antifungal activity but differ in the pharmacologic and toxicologic properties (24)

The azoles comprise the second class of antifungal agents and include the imidazoles (clotrimazole, miconazole, and ketoconazole) and the triazoles (fluconazole and itraconazole). The azoles inhibit ergosterol biosynthesis through their interactions with the enzyme lanosterol demethylase, which is responsible for the conversion of lanosterol to ergosterol in the fungal cell membrane, leading to the depletion of ergosterol in the membrane (30,31). Fluconazole is well tolerated with very low incidence of side effects and is the most effective agent for the treatment of oropharyngeal and vaginal candidiasis, as well as prophylaxis for fungal infections in neutropenic patients undergoing bone marrow transplantation and for oropharyngeal candidiasis in HIV-infected persons (30).

5-Flucytosine (5-FC) is a nucleoside analog and constitutes the third class of antifungal agents. After its uptake into the fungal cell, 5-FC ultimately leads to the disruption of DNA and protein synthesis of the fungal cell (30,31). Flucytosine is primarily used in combination with amphotericin B for the treatment of candida endophthalmitis and cryptococcal meningitis (30,31).

New Classes of Antifungal Drugs

The echinocandins and their analogs, the pneumocandins, represent the newest class of antifungal drugs (19,29,31–40). They inhibit the synthesis of 1,3-β-D-glucan, a fundamental component of the fungal cell wall by the inhibition of 1,3 β-D-glucan synthase, an enzyme complex that forms glucan polymers in the cell wall and is absent in mammalian cells. The inhibition is effective and specific, and brief exposure leads to cell death. The potent antifungal activity of the echinocandins against *Candida* species was demonstrated by Cuenca-Estrella et al. (33) and Quindos et al. (24), who evaluated the in vitro activity of LY303366, a semi-synthetic echinocandin B derivative, against 156 clinical isolates of *Candida* species and 36 *C. dubliniensis* clinical isolates, respectively. Results showed that LY303366 had potent activity against several *Candida* species including *C. albicans*, *C. tropicalis*, as well as *C. glabrata* and *C. krusei*, two species usually considered refractory to azoles. Similarly, 100% of the isolates were susceptible to the new antifungal drugs, indicating that echinocandins may provide new alternatives to fluconazole for treating *C. dubliniensis* infections (24). The excel-

lent in vitro activity of echinocandins demonstrated against fluconazole-resistant *Candida* species strains indicates that the echinocandins are very promising as novel antifungal agents with important implications for the treatment of infections by these yeasts (24,33,34). Their unique mode of action and their specificity to fungal cell walls result in minimal toxicity to mammalian cells.

Discussion

By using models of *C. albicans* biofilms, several studies have shown uniform resistance of the organisms in the biofilm to a wide spectrum of conventional antifungal agents including resistance to the new triazoles (VRC and Ravu), which have been shown to be fungicidal with extended activity against many azole-resistant organisms. Therefore, biofilm-associated infections are difficult to treat, which emphasizes the need to develop antimicrobial drugs that show activity against biofilm-associated organisms and specifically target biofilm-associated infections (5,19). The novel classes of agents, namely the lipid formulation of amphotericins and the echinocandins, have been shown to have unique activities against the resistant *Candida* biofilms (19,29). However, given their large size, that liposomal amphotericin B formulations could penetrate ECM to target the fungal cell wall is somewhat surprising. Their dispersion in phospholipids may in fact facilitate passage through the charged polysaccharide ECM, which may be the mechanism by which these compounds penetrate tissues (29). The mechanism of the echinocandins against biofilm cells is still unclear. The echinocandins probably do not exert their antibiofilm effects primarily on the fungal cell wall since only minimal cellular changes have been observed on biofilm-associated *Candida* cells. One explanation may lie in their potential effect on ECM kinetics, where the inhibition of polysaccharide production by echinocandins could lead to lysis and dissolution of the ECM (29). Further studies to determine the exact mode of action of echinocandins on *Candida* biofilms are warranted.

In conclusion, the amphotericin B lipid formulations and the echinocandins exhibit novel activity against *Candida* biofilms. The use of these drugs may represent an important step in the treatment of invasive systemic *Candida* infections by enhancing retention of affected intravascular devices and obviating the need for valve surgery in *Candida* endocarditis (2,19,29). More importantly, these antifungal drugs may be useful in management of biofilm infections by fungi and may have other clinical applications including those of oral diseases and prostheses rejection.

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Dr. Jabra-Rizk is a clinical mycologist and research associate in the Department of Diagnostic Sciences and Pathology at the University of Maryland in Baltimore. Her research focuses on the molecular characterization and immune response of fungal virulence factors with emphasis on the emerging opportunistic pathogen *Candida dubliniensis*. She is actively involved in research protocols with the Institute of Human Virology and the Greenbaum Cancer Center at the University of Maryland.

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Severe Acute Respiratory Syndrome–associated Coronavirus in Lung Tissue

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Efforts to contain severe acute respiratory syndrome (SARS) have been limited by the lack of a standardized, sensitive, and specific test for SARS-associated coronavirus (CoV). We used a standardized reverse transcription-polymerase chain reaction assay to detect SARS-CoV in lung samples obtained from well-characterized patients who died of SARS and from those who died of other reasons. SARS-CoV was detected in all 22 postmortem lung tissues (to 10^9 viral copies/g) from 11 patients with probable SARS but was not detected in any of the 23 lung control samples (sample analysis was blinded). The sensitivity and specificity (95% confidence interval) were 100% (84.6% to 100%) and 100% (85.1% to 100%), respectively. Viral loads were significantly associated with a shorter course of illness but not with the use of ribavirin or steroids. CoV was consistently identified in the lungs of all patients who died of SARS but not in control patients, supporting a primary role for CoV in deaths.

From its origins in November 2002 in Guangdong Province, China, severe acute respiratory syndrome (SARS) has become an emerging infectious disease that has spread to areas throughout the world, including Hong Kong, Vietnam, Singapore, Taiwan, and Canada (1). Although controversy remains over the etiology of SARS, the World Health Organization has declared a newly described virus known as the SARS-associated coronavirus (SARS-CoV) as the cause (2). This announcement has led to a rapid proliferation of different in-house laboratory tests aimed at detecting either SARS-CoV–specific antibodies or SARS-CoV nucleic acid in clinical specimens. The Centers for Disease Control and Prevention definition for a confirmed case of SARS includes the results of these laboratory tests (3). However, because different assays are being used, comparing results from different centers has been difficult. In addition, the inability of these nonstandardized tests to detect SARS-CoV in all cases has led to speculation that other agents may be associated with SARS. Some researchers have suggested that illnesses that progress to respiratory failure and death may not be caused by uncontrolled viral replication but rather are the result of an immunopathologic process (4). In a recent report of six fatal cases of SARS, SARS-CoV was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in postmortem lung tissue in only four patients (5).

The purpose of this study was to use a standardized, commercially available, RT-PCR assay to test for the presence of SARS-CoV RNA. Lung tissue obtained at autopsy from well-characterized patients with SARS who died during the outbreak in Canada were compared to lung samples obtained at autopsy from patients without SARS who died during the outbreak and lung samples from patients who died before the outbreak.

Methods

Patients

All patients who met the current World Health Organization case definition of probable SARS and who underwent a postmortem examination in Canada during the March–April 2003 outbreak were included in this study. Clinical details were extracted retrospectively from hospital records. Clinical descriptions of some of these cases have been published separately (6,7). As of May 14, 2003, a total of 24 patients died of SARS in Canada; all died in Toronto. Of the 24 patients, autopsies were performed on 11 patients. Results of ante- and postmortem examination for routine bacterial and viral respiratory pathogens from these 11 patients, as described elsewhere, were negative (6).

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[1]Drs. Mazzulli and Farcas contributed equally to the manuscript. All authors jointly conceived and designed the study and wrote the report. Gabriella A. Farcas performed the majority of the reverse transcription-polymerase chain reaction assays.

Lung Tissue Samples

A total of 22 discrete postmortem lung samples collected from these 11 patients were included in this analysis. An additional 13 postmortem lung samples from 7 patients who died during the SARS outbreak but whose deaths were attributed to other causes were also included. The attributed cause of death in these patients was as follows: a 46-year-old woman died of invasive group A streptococcal infection; a 93-year-old man died of congestive heart failure; a 37-year-old man died of sudden death cardiovascular disease; a 74-year-old man died of amiodarone pulmonary toxicity; a 78-year-old woman died of dementia and aspiration pneumonia; a 47-year-old woman died of diabetes and congestive heart failure; and an 81-year-old man died of bladder cancer and aspiration pneumonia. In addition, 10 lung samples collected in 1998 from 10 patients (4 women and 6 men; age range 54–75 years) with lung cancer were also included as negative controls. All samples collected at the time of autopsy were snap frozen in a mixture of absolute ethanol and dry ice and subsequently stored at –70°C until tested. The samples were coded and then processed, subjected to RT-PCR analysis, and interpreted before the identity of the samples was divulged. This study was approved by the research ethics boards at Mount Sinai Hospital and the University Health Network.

RT-PCR

Lung tissue samples were thawed and immediately homogenized in lysis buffer (QIAGEN, Mississauga, Canada) with disposable tissue grinders (Kendall Precision, Mansfield, MA). The homogenate was passed through QIAshredder columns (QIAGEN) before RNA isolation by using the RNeasy Mini Kit (QIAGEN). The sample was eluted in 30 µL of RNAse free water. The RT-PCR was carried out by using the RealArt HPA-Coronavirus LightCycler RT Reagents Assay (Artus GmbH, Hamburg, Germany) with a LightCycler real-time platform (Roche Diagnostics, Laval, Canada). The HPA-Coronavirus Master Mix contains reagents and enzymes for the specific amplification of an 80-bp region of the SARS-CoV polymerase gene from 5 µL of RNA with the primer pairs published by the Bernhard-Nocht Institute (Hamburg, Germany) as posted on the World Health Organization Web site (available from: URL: http://www.who.int/csr/sars/primers/en/).

Viral load was calculated from a standard curve based on four external positive controls (quantification standards) included in the RealArt HPA-Coronavirus LightCycler RT Reagents Assay kit (Figure A and B). The standards were treated as previously purified samples, and the same 5-mL volume was added per capillary. A standard preparation of SARS-CoV isolated from cell culture supernatants of VeroE6 cells was used as a calibrator in each

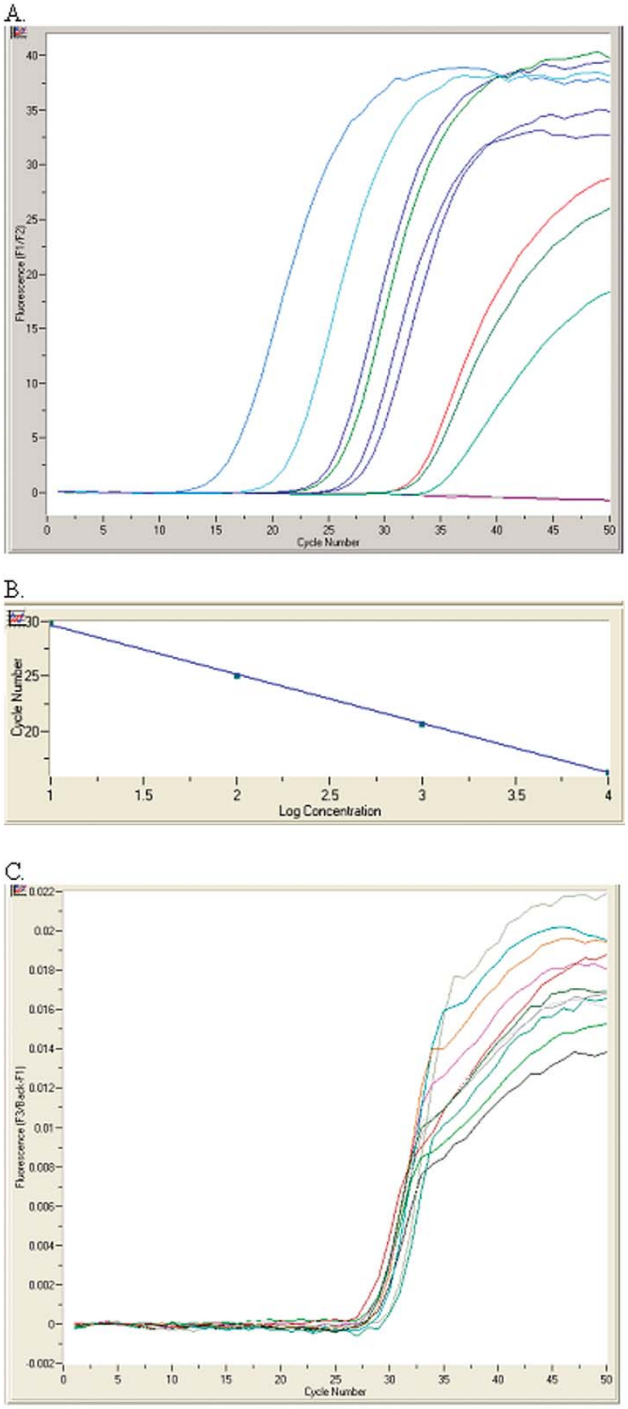

Figure. RealArt HPA-Coronavirus LightCycler reverse transcription-polymerase chain reaction (RT-PCR) assay results. PCR results from 5 µL RNA are displayed in channel F1/F2 of the LightCycler instrument (A). Four quantification standards are included in the assay to generate a standard curve (B). An internal control, added at the RNA isolation stage, is used to monitor both the quality of the RNA isolation as well as possible PCR inhibition (C).

run. In addition, the kit contains a second heterologous amplification system (i.e., an internal control) to identify either PCR inhibition exclusively, if added to the extracted RNA, or RNA isolation quality as well as PCR inhibition, if added during the RNA isolation procedure (Figure C). Although the assay insert states that the primers and probes used in the assay were checked for possible similarity to other pathogens by means of sequence comparison, 25 randomly chosen amplicons from our sample pool were independently sequenced to confirm SARS-CoV–specific amplification and detection. Univariate analysis comparing potential predictors of viral load (e.g., duration of illness, the use of ribavirin, the use of steroids) was completed by using Fisher exact test. Two-sided p values ≤ 0.05 were considered significant.

Results

The clinical description and RT-PCR results for the 11 patients with probable SARS from whom postmortem lung tissue samples were examined are summarized in Table 1. The mean age of the 11 patients was 70 years (range 43–99). Six of the 11 patients were men. All but 1 of the 11 patients had underlying coexisting conditions, the most common of which was diabetes mellitus in 6 patients. The mean duration of illness was 20 days (range 8–32). Seven patients had been intubated and mechanically ventilated before death. Three patients had requested not to be intubated (information on ventilation was not available for one patient). Ten of the 11 patients were treated with ribavirin; 6 of the 11 patients were treated with steroids.

SARS-CoV was detected in all 22 postmortem lung tissue samples collected from all 11 patients who died with a diagnosis of probable SARS. All 13 postmortem lung samples from the seven non-SARS fatalities that occurred during the SARS outbreak were negative for SARS-CoV, as were all 10 lung-tissue samples collected from patients with lung cancer 5 years before the outbreak (Table 2). The corresponding sensitivity and specificity of the RealArt HPA-Coronavirus LightCycler RT Reagent assay are both 100% (95% confidence interval [CI] for sensitivity 84.6% to 100%; 95% CI for specificity 85.1% to 100%) for the detection of SARS-CoV.

The SARS-CoV viral load in postmortem lung tissue ranged from 2.7×10^4 copies/g tissue to 3.8×10^9 copies/g

Table 1. Clinical description and SARS-CoV RT-PCR results for 11 patients who died with probable SARS[a]

		Illness and treatment duration (days)					RealArt HPA Coronavirus RT-PCR[b]	
Sex/age	Coexisting conditions	Illness	Ventilation	Ribavirin	Steroids	Postmortem lung tissue description	Results	Copies of CoV/g tissue
M/43	Type II DM, HTN	15	4	0	0	RUL	Positive	1.5×10^8
						RML (#1)	Positive	5.4×10^7
						RML (#2)	Positive	2.8×10^7
						RML (#3)	Positive	7.4×10^6
						RML (#4)	Positive	6.4×10^8
M/76	Type II DM, CAD, HTN	11	4	6 (started on day 6 of illness)	0	Lung	Positive	3.8×10^9
F/78	Type II DM, CAD, hypercholesterolemia, chronic obstructive pulmonary disease	8	5	≥5 (started on day 4 of illness)	0	RT lung	Positive	1.0×10^9
						LUL	Positive	9.4×10^7
M/62	Rectal cancer, HTN, hypercholesterolemia	8	N/A	≥5 (started on day 4 of illness)	0	LT lung	Positive	5.3×10^7
F/73	HTN, hypercholesterolemia	28	DNI	14 (started on day 5 of illness)	12 (stated on day 14 of illness)	LT lung	Positive	3.0×10^4
						RT lung	Positive	3.6×10^5
F/99	Osteoarthritis	26	DNI	13 (started on day 1 of illness)	0	Lung	Positive	5.0×10^4
M/63	Hypercholesterolemia, cerebral vascular disease	20	12	16 (started on day 4 of illness)	16 (started on day 6 of illness)	RUL lung	Positive	3.2×10^6
						LLL	Positive	2.5×10^7
F/78	Type II DM, HTN, hypercholesterolemia	24	18	10 (started on day 3 of illness)	18 (started on day 5 of illness)	LT lung	Positive	4.1×10^5
						RUL	Positive	4.9×10^5
M/44		29	18	18 (started on day 8 of illness)	17 (started on day 12 of illness)	RT lung	Positive	7.6×10^4
						LT lung	Positive	4.1×10^4
M/77	Type II DM, HTN, hypercholesterolemia	≥18	≥1	≥1 (started on day 10 of illness)	≥7 (started on day 10 of illness)	LLL	Positive	5.6×10^5
						LUL	Positive	5.7×10^5
F/79	Type II DM, HTN, hypercholesterolemia	32	DNI	11 (started on day 2 of illness)	≥4 (started on day 12 of illness)	LT lung	Positive	2.7×10^4
						Lung	Positive	2.1×10^5

[a]SARS, severe acute respiratory syndrome; CoV, coronavirus; RT-PCR, reverse transcription polymerase chain reaction; F, female; M, male; DM, diabetes mellitus; HTN, hypertension; RUL, right upper lobe; RML, right middle lobe; CAD, coronary artery disease; RT, right; LT, left; LUL, left upper lobe; LLL, left lower lobe; N/A, not available; DNI, "Do not intubate" order written.
[b]RealArt HPA Coronavirus RT-PCR (Artus GmbH, Hamburg, Germany).

Table 2. Univariate analysis of predictors of high viral loads in postmortem lung tissue

Predictor	Viral load $\geq 10^6$ copies/g lung tissue	Viral load $<10^6$ copies/g lung tissue	Fisher exact test
Short duration of illness (≤21 d)	5/5	0/6	p=0.002
Use of ribavirin	4/5	6/6	p=0.45
Use of steroids	1/5	5/6	p=0.08

tissue. Higher viral loads ($\geq 10^6$ copies/g tissue) were associated with patients who had a shorter duration of illness (≤21 days) (p=0.002, Fisher exact test). The use of ribavirin or steroids was not significantly associated with viral load levels (Table 2).

Twenty-five randomly selected amplicons from the sample pool were sequenced to assess specificity and possible cross-reactivity to other pathogens. A BLAST (available from: URL: http://www.ncbi.nlm.nih.gov/BLAST/) search performed against the SARS-CoV genomes in GenBank, European Molecular Biology Laboratory, DNA Data Bank of Japan, and Protein Data Bank on the National Center for Biotechnology Information Web site (available from: URL: http://www.ncbi.nlm.nih.gov/), indicated that all amplicon samples contained SARS-CoV polymerase gene sequence.

Discussion

By using a standardized RT-PCR assay, SARS-CoV has been unequivocally identified in the lung tissue of all patients who died with probable SARS but not in any of the controls. These observations support a primary role for this virus in patients with SARS who have fatal outcomes and provide additional, strong evidence to fulfill Koch's postulates regarding SARS-CoV as the cause of SARS (8). SARS-CoV was found in different lung samples from the same patient, suggesting that the virus is widely disseminated throughout the lung at the time of death. Previous studies suggested that progression of disease to respiratory failure may be primarily mediated by host immune response rather than viral replication (4). Although viral RNA in lung tissue does not necessarily indicate replicating virus, virus in multiple lung lobes, often in high copy number, at the time of death suggests that SARS-CoV may also be contributing to disease progression. The fact that higher viral loads were significantly associated with patients with a shorter duration from onset of illness to death supports the role of viral replication as a contributor to death. Ten of the 11 patients had received therapy with ribavirin, and 6 patients were treated with steroids. The failure to eradicate SARS-CoV despite ribavirin therapy and the lack of association between the use of ribavirin and SARS-CoV viral load are consistent with in vitro data showing that ribavirin has no activity against this agent (9).

Global efforts to contain SARS have been severely impeded by the lack of a standardized, sensitive, and specific diagnostic test for SARS-CoV. Different diagnostic strategies, including culture, serologic assays, and molecular detection methods, have been described, but each of these tests has limitations. In-house RT-PCR assays have been associated with sensitivities as low as 50% in patients with SARS (10), which raises uncertainty about the role of CoV versus co-pathogens in mediating severe or fatal SARS. By contrast, the sensitivity and specificity of the RealArt HPA-Coronavirus RT-PCR assay for detecting CoV in lung tissue samples appear to be excellent. In addition, with the real-time LightCycler system, the assay generates quantitative results within 1 hour, which is much shorter than traditional PCR reactions.

The type of specimen tested, the timing of sample collection, (i.e., acute versus convalescent phase) the method of specimen collection, as well as the method of sample preservation may have substantial impact on the results obtained from a diagnostic test. The lower sensitivity of SARS-CoV detection reported by Peiris et al. (10) may be a consequence of these confounding factors. Our study design of examining lung biopsies from clearly defined patient populations overcame confounding issues, such as sampling technique, nonspecific case definitions, and possible undocumented exposure to SARS. Given the predominance of respiratory symptoms in patients with SARS, lung samples have perhaps the highest viral titers of all specimen types; yet in nonfatal cases, obtaining routine lung biopsies is not practical. Other respiratory tract specimens may be satisfactory substitutes for biopsies, but further studies examining the prevalence of SARS-CoV in these other specimen types and in a larger population are needed. With the use of standardized commercially available assays, comparison of results from different centers may be facilitated.

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Severe Acute Respiratory Syndrome, Beijing, 2003

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The largest outbreak of severe acute respiratory syndrome (SARS) struck Beijing in spring 2003. Multiple importations of SARS to Beijing initiated transmission in several healthcare facilities. Beijing's outbreak began March 5; by late April, daily hospital admissions for SARS exceeded 100 for several days; 2,521 cases of probable SARS occurred. Attack rates were highest in those 20–39 years of age; 1% of cases occurred in children <10 years. The case-fatality rate was highest among patients >65 years (27.7% vs. 4.8% for those 20–64 years, $p < 0.001$). Healthcare workers accounted for 16% of probable cases. The proportion of case-patients without known contact to a SARS patient increased significantly in May. Implementation of early detection, isolation, contact tracing, quarantine, triage of case-patients to designated SARS hospitals, and community mobilization ended the outbreak.

By July 4, 2003, a total of 8,439 probable cases and 812 deaths from severe acute respiratory syndrome (SARS) had been identified from 30 countries (URL: http://www.who.int/csr/sars/en/). A novel coronavirus (SARS-CoV) was found to be the cause of this multicountry outbreak (1–3). Most cases of SARS occurred in China, where the virus apparently emerged first, most likely from animal sources. The largest outbreak of SARS occurred in Beijing.

In Beijing, the SARS outbreak was reported in April 2003, against a backdrop of earlier outbreaks detected in Guangdong, Hong Kong (4,5), Hanoi, Toronto (6), and Singapore (7). In contrast to Toronto, where the entire outbreak originated from a single importation (6), Beijing's outbreak involved multiple distinct imported cases, and transmission from index cases was amplified within several healthcare facilities. Widespread transmission came under control after Beijing municipal authorities aggressively implemented measures to enhance detection, isolate case-patients, and trace contacts to minimize further opportunities for transmission in community and institutional settings. This report summarizes the descriptive epidemiology of Beijing's outbreak and the emergency interventions that were implemented to control the local situation.

Methods

Setting

Beijing municipality has an estimated population of 13.8 million and includes 14 districts and four counties. Approximately 85,000 healthcare workers live there. Disease reporting and epidemic investigations of reported cases were conducted through the collaboration of the Beijing Center for Prevention and Disease Control and district centers within Beijing, using guidelines for surveillance and case investigation issued by China's Ministry of Health.

Case Definitions

China established a case definition for "infectious atypical pneumonia," also termed SARS, with minor modifications implemented during the course of the outbreak. After May 3, probable ("clinically confirmed") and suspected cases were defined according to 1) epidemiologic history (either contact with other SARS patients or exposure to a SARS-affected area); 2) symptoms and signs of fever and respiratory illness; 3) normal or decreased leukoctye count; 4) chest radiograph abnormalities; and 5) absence of substantial improvement with antibiotic treatment (Table 1). We have included cases reported as probable according to the case definition in place at the time of report, consistent with a strategy used by other investigators (8).

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[1]The Beijing Joint SARS Expert Group included public health authorities and healthcare professionals participating in the medical and public health task force constituted in April 2003 to address Beijing's SARS epidemic.

Table 1. Case definition for severe acute respiratory syndrome (infectious atypical pneumonia) in China as of May 3, 2003

Category	Criteria[a]
Probable	1.1+ 2 + 4, or 1.1+2+4+5, or 1.2+2+3+4
Suspected	1.1+2+3, or 1.2+2+4, or 2+3+4
Under medical observation	1.2+2+3

[a]1. Epidemiologic history: 1.1:Having close contact with a patient, or being a member of infected cluster, or having infected other persons; 1.2: Having visited or resided in cities or areas where SARS cases were reported with secondary transmission during the 2 weeks before onset of disease. 2. Symptoms and signs of febrile respiratory illness. 3. Normal or decreased leukocyte count. 4. Chest x-ray changes. 5. Lack of response to antibiotic treatment.

Case Reporting

When a possible case-patient is identified in a healthcare facility within Beijing, a panel of experts at the facility reviews clinical information to classify the illness as probable, suspected, or under observation. Case classification is updated on the basis of clinical progression and availability of alternative diagnoses to account for the illness, although diagnostic testing for other agents was not extensive during most of the epidemic. During May, clinical experts were dispatched to SARS hospitals to improve how consistently cases were classified according to the national case definition. For probable and suspected cases, healthcare providers complete a standard report form, which is faxed to the relevant district center for disease control. A district epidemic investigator then interviews the patient (or family member) and completes a standardized epidemic investigation form regarding demographic and clinical data, as well as the patient's contacts within the 2 weeks before symptom onset, in an attempt to identify the patient's source of infection. The district is responsible for identifying persons who had contact with the patient between the onset of symptoms and hospitalization. Those who had close contact are placed under home medical observation by community health center personnel and are quarantined to restrict their circulation in the broader community.

Laboratory Testing

Serum was collected from patients at certain hospitals for detection of anti–SARS-CoV antibodies by using one of two locally developed enzyme-linked immunosorbent assay (ELISA) kits; one was developed by the Beijing Genomics Institute in partnership with the Academy of Military Medical Sciences, and the other was developed by the China Center for Disease Control. SARS-CoV was also isolated from selected clinical specimens; substantial partial genome sequencing for four Beijing strains (AY278488, AYAY278487, AY278490, and AY279354) was submitted to GenBank April 17–April 19, 2003, by E. Qin et al. from the Academy of Military Medical Sciences and the Beijing Genomics Institute in Beijing. Details on laboratory tests are reported separately (9,10).

Data Analysis

Data were entered into either a Microsoft Excel database (case report forms) or an Oracle database (detailed epidemiologic investigation forms). Data analysis used SPSS (SPSS Inc., Chicago, IL) software. Chi-square or, when appropriate, Fisher exact test was used for comparison of proportions. Because date of onset was missing for 985 (26.8%) of the 3,665 patients with probable and suspected cases reported through May 20, we present temporal information based on date of hospitalization, which was missing in 155 (4.2%) of case-patients.

Results

Importation Phase

The earliest cases in Beijing occurred in persons who were infected with SARS in Guangdong and Hong Kong.

Index Case 1

The first apparent case of SARS in Beijing was identified on March 5 in a 27-year-old businesswoman in whom symptoms developed on February 22 while she was traveling in Guangdong (Figure 1). She sought medical attention in Shanxi Province, where SARS subsequently developed in two doctors and a nurse who cared for her. After she returned to Beijing, she was hospitalized in a military hospital, then transferred to an infectious disease hospital. SARS developed in 10 healthcare workers exposed at the two Beijing hospitals as well as 8 of the patient's family members and close colleagues or friends. Both of the patient's parents died from SARS. Healthcare workers cared for the patient before SARS was suspected and used no personal protective equipment.

Index Case 2

A 72-year-old man visited a relative in Hong Kong's Prince of Wales Hospital, and symptoms developed on March 14, 2003. On March 15, the patient flew from Hong Kong to Beijing on China Air flight 112. He was evaluated in one hospital on arrival in Beijing but was not admitted. The next day, his family brought him to a second Beijing hospital, where after a successful resuscitation in the emergency department, he was admitted to the hospital. He died there on March 20. Contact tracing and epidemic investigation suggest that at least 59 SARS cases in Beijing can be traced back to this patient, including illness in three members of his immediate family, in six of seven healthcare workers who assisted in the emergency room resuscitation, and in one other healthcare worker in the facility. The remaining cases occurred in other patients and

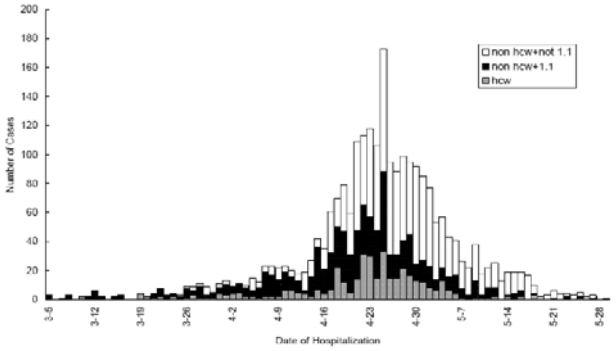

Figure 1.Epidemic curve—severe acute respiratory syndrome (SARS) probable case-patients by date of hospitalization and type of exposure, Beijing, 2003. Open bars indicate nonhealthcare workers without contact with a SARS patient; dark bars ("1.1") indicate nonhealthcare workers with contact with a SARS patient; light filled bars indicate healthcare workers.

their contacts. In addition to Beijing cases, transmission on this airplane flight has been linked to SARS cases in other areas, including Taiwan and Inner Mongolia. Besides these two index case-patients, several later SARS case-patients in Beijing had traveled to other affected areas before the onset of clinical symptoms.

Amplification in Healthcare Facilities

SARS occurred in healthcare workers in >70 hospitals throughout Beijing, and clusters of ≥20 probable SARS cases among healthcare workers occurred in four Beijing hospitals (Figure 2). Apparent transmission of SARS within fever clinics and selected hospitals prompted closure of four hospitals and numerous fever clinics. One large hospital, where 41 probable cases occurred among healthcare workers and numerous cases occurred among patients and contacts, was closed on April 23. SARS patients were transferred to designated SARS hospitals, and the remaining patients, staff, and visitors were quarantined in the hospital for 2 weeks.

Evolution of Outbreak

Through June 2003, a total of 2,521 patients with probable cases of SARS were hospitalized in Beijing. The outbreak peaked during the 3rd and 4th weeks in April, when hospitalizations for probable SARS exceeded 100 cases for several days, and an increased proportion of case-patients reported having no known contact with a SARS patient (Figure 1).

Description of Cases

Of 2,521 probable SARS cases in Beijing, 2,444 (96.7%) cases reported by May 20 had data available for review and constitute the remainder of this report. Of the 2,444 probable case-patients, 1,009 (41.3%) had a history of close contact with a patient with SARS; 395 (16.2%) of the probable cases occurred in healthcare workers. Overall, 42.9% of probable case-patients had no previous contact with a SARS case-patient or travel to affected areas outside Beijing. However, the proportion of probable case-patients with no direct contact with a SARS patient increased from 50.7% for case-patients who were hospitalized before May 1, to 75.2% for those admitted to hospitals in May ($p < 0.001$). Among probable SARS case-patients who were hospitalized in March and April, healthcare workers accounted for 18.7% (n = 329), compared with 10.7% (n = 61) for case-patients who were hospitalized on May 1 or thereafter ($p < 0.001$).

The demographic characteristics of case-patients with probable SARS are shown in Table 2. Children <10 years of age accounted for 0.9% of probable cases, and the median age of those who became ill was 33 years. Age-specific attack rates were highest in those 20–39 years of age (relative risk [RR] 1.7, 95% confidence interval (CI) 1.53 to 1.89, compared with those 40–64 years, and significantly lower in children (1–4 years of age, RR 0.12 [CI 0.05 to 0.28], 5–9 years, RR 0.17 [CI 0.09 to 0.31] and 10–19 years, RR 0.53 [CI 0.44 to 0.64], compared with those aged 40–64 years). Overall, male patients had similar rates as female patients, but the risk differed significantly in certain age groups: among those 10–19 years of age, the RR for SARS in male patients was 1.96, 95% CI 1.36 to 1.83, compared with that of females; and in those ≥75 years, RR for male patients was 1.88 (95% CI 1.08 to 3.29) (Figure 3). The attack rate for probable SARS among healthcare workers in Beijing is estimated as 465 per 100,000. Consistent with the case definitions in use in

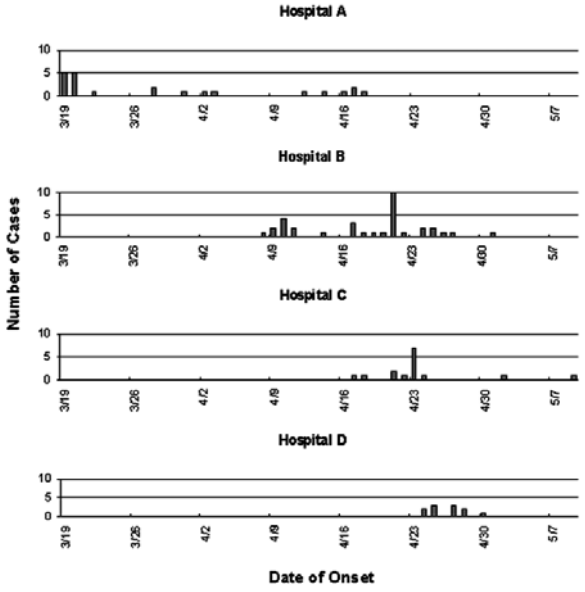

Figure 2. Clusters of severe acute respiratory syndrome (SARS) cases among healthcare workers in four hospitals, Beijing 2003.

Table 2. Characteristics of probable cases of severe acute respiratory syndrome (SARS) in Beijing, 2003

Characteristic[a]	Probable case-patients; N (%)
Demographic	
Male sex	1,217/2,406 (50.6)
Age (y)	
1–4	6/2,397 (0.2)
5–9	17/2,397 (0.7)
10–19	165/2,397 (6.9)
20–39	1,270/2,397 (53.0)
40–64	733/2,397 (30.6)
65–74	147/2,397 (6.1)
≥75	59/2,397 (2.5)
Median age (range)	33 (1–93)
Fatal outcome	156/2,444 (6.4)
Healthcare worker	395/2,444 (16.2)
Admission symptoms	
Fever	1,646/1,693 (97.2)
Cough	749/1,693 (44.2)
Difficulty breathing	166/1,693 (9.8)
Chest tightness	331/1,693 (19.6)
Diarrhea	189/1,693 (11.2)

[a]Information was not available for the sex of 38 probable case-patients and for the age of 47 probable case-patients reported through May 20.

Beijing during the outbreak, chest x-ray changes were evident in >85% of probable case-patients. As of May 20, the case-fatality rate was 6.4% for probable SARS case-patients. Case-fatality rates increased with age (0.5% in <20 year olds; 4.8% for those 20–64 years; and 27.7% for ≥65 years of age, $p < 0.001$). By June 16, 2003, a total of 190 deaths among 2,521 probable SARS case-patients were reported from Beijing, and 2,053 patients had been discharged from the hospital. The case fatality rate among probable case-patients, excluding those still hospitalized, was 8.4%.

According to clinical information available on the case investigation form, nearly all SARS case-patients had the initial symptom of fever, and many had a cough (44.2%), but only 11.0% had diarrhea. Mean leukocyte count on admission was 5.5 x 10^9/L, and 25.2% had leukocyte counts <4.0 x 10^9/L (the normal limit).

For the 1,009 probable SARS case-patients with reported contact with another SARS patient, the most recent date of such contact was collected; 595 of these patients had onset dates available, permitting approximation of the shortest possible incubation period. Among these patients, a mean of 7.8 days (median 6) occurred from most recent exposure to onset of symptoms.

Control Measures

Prompted by the rapid expansion of the epidemic from April 16 to April 19, the Beijing Municipal Government established a Joint SARS Leading Group to oversee crisis management through 10 task forces. The medical and public health task force set up an emergency command center on April 24 and organized fever clinics for triage, designated SARS areas within hospitals for isolation and specialized care, provided personal protective equipment and training for healthcare workers, and introduced community-based prevention and control through case detection, isolation, quarantine, and community mobilization. To reduce transmission within healthcare settings, Beijing authorities issued protocols for triage, isolation, case management, and administrative controls, which prohibited visitors to hospitals and separated patients who were under medical observation or suspected of having SARS from areas with other patients.

The medical emergency command center included teams for clinical diagnosis and treatment, critical care, patient transport, infection control, and information management. Local shortages of isolation rooms, intensive care facilities, and hospital beds were addressed by dispatching specially equipped ambulances to transfer SARS patients to designated facilities. An anticipated shortage of hospital beds for care and isolation of SARS patients prompted authorities to construct a new 1,000-bed hospital in 8 days.

On April 27, all patients with probable cases of SARS were moved to designated areas within hospitals. At one point, 27 municipal and 21 district hospitals were providing care to SARS patients. On May 8, 2003, the medical and public health task force finished concentrating all the probable case-patients into 16 designated municipal hospitals, with 30 district hospitals providing care for patients with suspected SARS. More than 60 fever clinics were established throughout the city to triage patients with acute febrile illness, permitting prompt isolation of patients who required further observation and referral to the appropriate level of care to rule out SARS. By June 19, 2003, a total of 30,172 people who had had close contact with probable or suspected SARS case-patients had been quarantined separately or in groups for 2 weeks after their last exposure to a SARS case-patient.

In addition to interventions directed at managing

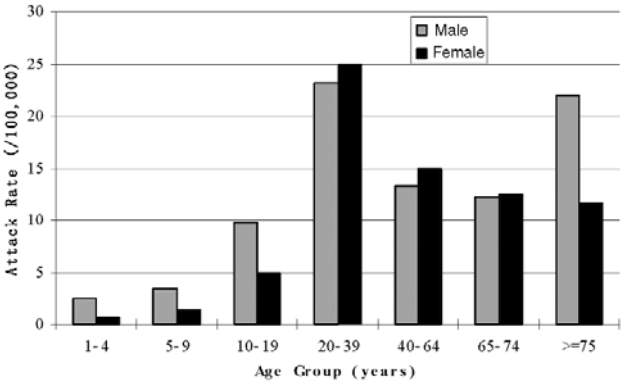

Figure 3. Attack rates (cases per 100,000 population) by age and sex of probable severe acute respiratory syndrome (SARS), Beijing 2003.

patients, their contacts, and healthcare facilities, schools were closed, travel was restricted, the community was educated about seeking care at designated sites, and temperatures were monitored at frequent check points. Service professionals were required to wear masks, and many community members donned masks as well.

Laboratory Confirmation

SARS-CoV was isolated from many patients in Beijing, and sequences from four Beijing isolates (source: GenBank) were compared with strains from other areas (11). The cause of infection was confirmed for a series of patients with severe illness who were cared for at Ditan Hospital, by using an ELISA developed by the China Center for Disease Control. Among 164 case-patients with probable SARS (who had severe illness) tested by mid-May, 98% had SARS-CoV–specific immunoglobulin (Ig) G detected from samples collected >35 days after illness onset; 55% had SARS-CoV–specific IgG detected 16–21 days after symptom onset, and 82% by 22 to 28 days after illness onset.

Discussion

Beijing experienced the largest outbreak of SARS yet recognized. The disease was transported to Beijing by multiple travelers. One case-patient originated in Guangdong and transported the virus to Shanxi along the way, and a second case-patient appears to have acquired the virus during a hospital visit in Hong Kong. He then transmitted the virus to other travelers encountered while he had symptoms on the return airline flight to Beijing and to those he came into contact with in a hospital in Beijing. The imported cases initiated cascades of illness among the family members, healthcare workers, and other hospitalized patients. Patients seeking care in multiple facilities and clinicians failing to recognize nonspecific respiratory symptoms as indications for isolation and use personal protective equipment permitted the efficient transmission of the virus to numerous healthcare workers, patients, and others throughout Beijing.

Amplification of transmission in healthcare settings was likely enhanced by resuscitation of one of the index patients in an emergency department. Since SARS patients were initially cared for on general medical wards, the virus was transmitted to other patients hospitalized for unrelated conditions; such persons may be more vulnerable to infection as well as severe outcomes (8,12,13). Designation of SARS wards and later specialized SARS hospitals facilitated control of transmission within healthcare settings in Beijing. However, the delay before these steps were taken permitted numbers of infected persons to increase in healthcare settings, which likely permitted transmission to community members through visits to hospitals, before such visits were stopped and strict isolation measures observed.

Classifying patients as having probable or suspected SARS may be difficult with the current case definitions. Although a novel coronavirus was rapidly discovered as the cause of SARS, this disease appeared to both clinicians and public health workers as a nonspecific clinical syndrome. The clinical features have substantial overlap with those caused by common respiratory viruses and bacteria. Implementation of the case definition on the basis of nonspecific clinical features was particularly challenging in areas with community transmission, since the ability to focus surveillance and isolation efforts on persons with epidemiologic links to specific travel or contact with other SARS patients was no longer appropriate. Although the case definition used by China included some components to enhance specificity (i.e., normal or low leukocyte count, lack of response to antibiotics), the definition remains nonspecific. Future surveillance and case management will benefit greatly from incorporating laboratory tests, particularly if sufficiently sensitive laboratory methods can be developed which are amenable to point-of-care use early in the clinical course. In the meantime, available assays for SARS-CoV, including ELISA and polymerase chain reaction testing, will be valuable complements to epidemiologic surveillance for understanding recent disease patterns. Elimination of SARS as a public health threat will require major commitment to laboratory testing of possible case-patients.

The age-specific attack rates of SARS in Beijing support findings from other outbreaks of SARS. High rates among those 20–39 years of age likely reflect disease among healthcare workers with occupational exposures, and high rates in the elderly may represent patients with nosocomial acquisition. The extremely low rate observed in children in Beijing is noteworthy. Pediatric cases of SARS have also been relatively rare in other countries (14), but since most other outbreaks remained concentrated at healthcare facilities, this pattern could simply reflect limited exposure to ill patients, thought to be the most efficient transmitters. Given the size of Beijing's outbreak and the spread beyond hospitals, absence of exposure is unlikely to fully account for the low incidence of SARS in children. Whether asymptomatic or mild infection is more likely to develop in children, and whether children are able to transmit the virus to others in the absence of clinical illness, are important questions which must be addressed to guide control.

The steep increase in cases of SARS in late April posed a major challenge to Beijing's healthcare and public health systems. The magnitude of ill healthcare workers, incident cases, and affected facilities necessitated a strategic response. Shortages of beds and isolation rooms, as well as

ongoing transmission in some hospitals, prompted the designation of selected hospitals for SARS patients; the preparation of special protocols for care, isolation, and healthcare worker protection; and ultimately, the construction of a large new facility to ensure that capacity could keep up with projected demand. More than 30,000 persons were placed under quarantine. Maintaining adequate case investigation challenged local public health staff, and the relatively high proportion of cases with no reported contact with a SARS patient may reflect limitations in the methods of identifying exposures during the epidemic's peak, rather than the absence of an identifiable source in all of these cases. We are reevaluating potential sources of SARS among patients initially reported to have no contact with a SARS patient (15). Given the nonspecific case definition, some of these patients may not have been infected with SARS-CoV, and we are also further evaluating this possibility. By restricting community gatherings and travel, closing schools and entertainment venues, major reductions in social contact also may have contributed to slowing transmission. The time from symptom onset to hospitalization became shorter during the course of the response efforts (before April 21, median 6 days vs. median 2 days thereafter); faster recognition of the condition and isolation of patients were likely factors in bringing the epidemic under control. Because multiple interventions were instituted simultaneously, distinguishing the effectiveness of isolation and quarantine measures from the impact of broader measures implemented for the general community will be difficult. Nevertheless, evaluation of several of these efforts is in progress.

A principal lesson learned from the Beijing experience is the importance of rapid response to SARS. Early detection of patients and prompt isolation can limit transmission, and adherence to personal and administrative infection control measures can reduce opportunities for transmission within healthcare facilities. The resources needed to respond to simultaneous outbreaks in multiple hospitals and address community transmission are much greater than those required for individual case investigation or management. Communities seeking to prepare for SARS must be alert to the speed with which one imported case can lead to dozens or hundreds of transmission chains. Thus a strong system of early detection and open communication will facilitate prompt recognition of possible problems and immediate response measures. Addressing community concerns, including fear of attending fever clinics or stigma associated with having one's neighborhood quarantined, should be an important component of planning efforts.

The Beijing epidemic has many features in common with those experienced elsewhere, including the disproportionate impact on healthcare workers and amplification of disease in hospitals. The pattern of transmission is consistent with droplet or contact spread. The apparent success of infection control, isolation, contact tracing, and quarantine in bringing the outbreak under control is encouraging, particularly because these efforts were introduced later in the epidemic in Beijing than in some other settings. Whether features particular to Beijing had a major influence on the evolution or characteristics of the outbreak is not yet clear. Careful clinical assessments of patients cared for during the outbreak will be valuable, since in addition to steroids and antiviral drugs, traditional Chinese medicine was frequently used in caring for SARS patients. Whether treatment strategies might be responsible for the lower age-specific case-fatality ratios in Beijing compared with reports from other places is not yet known, and the lower case-fatality ratio may derive in part from the nonspecific case definition with resulting misclassification of some pneumonias of other causes as probable SARS. While population density might have made the outbreak more difficult to control in Beijing, the massive and efficient mobilization of communities and health workers to respond to the outbreak was likely an asset.

In the response to SARS, opportunities exist for ensuring broad public health benefits. A stronger public health infrastructure capable of improved preparedness and response to SARS will also improve control of other diseases. Strengthening infection control practices to prevent repeated introductions of SARS epidemics is likely to reduce other healthcare-associated infections. Like other emerging infectious diseases, SARS has demonstrated the importance of enhanced communication between disparate geographic regions and diverse sectors of society.

Acknowledgments

We are grateful to the thousands of healthcare workers, epidemiologic investigators, and community members who contributed to clinical management and disease control of SARS in Beijing. We honor the memory of the healthcare workers who lost their lives battling this disease in Beijing.

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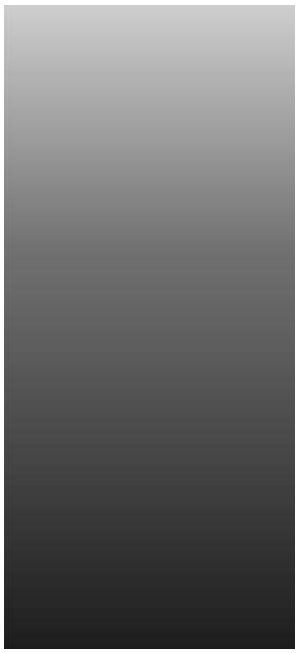

Influenza Epidemics in the United States, France, and Australia, 1972–1997[1]

Cécile Viboud,*† Pierre-Yves Boëlle,*‡ Khashayar Pakdaman,* Fabrice Carrat,*‡ Alain-Jacques Valleron,*‡ Antoine Flahault*†‡

Influenza epidemics occur once a year during the winter in temperate areas. Little is known about the similarities between epidemics at different locations. We have analyzed pneumonia and influenza deaths from 1972 to 1997 in the United States, France, and Australia to examine the correlation over space and time between the three countries. We found a high correlation in both areas between France and the United States (correlation in impact, Spearman's $\rho = 0.76$, $p < 0.001$, and test for synchrony in timing of epidemics, $p < 0.001$). We did not find a similar correlation between the United States and Australia or between France and Australia, when considering a systematic half-year lead or delay of influenza epidemics in Australia as compared with those in the United States or France. These results support a high correlation at the hemisphere level and suggest that the global interhemispheric circulation of epidemics follows an irregular pathway with recurrent changes in the leading hemisphere.

Influenza epidemics occur each year during the winter in temperate areas of the Northern and the Southern Hemispheres and result in substantial disease, death, and expense. The disease is responsible for 50 million illnesses and up to 47,200 deaths in the United States each year (1–3), with similar figures in Europe (4–6). In the last century, three global epidemics, also called pandemics, occurred in 1918-19, 1957-58, and 1968-69, and recent reports estimate that the 1918-19 influenza pandemic alone may have caused as many as 50 million deaths (7). Changing strains of the virus are responsible for these epidemics, but little is known about what triggers the epidemic in a particular location (2,8), and how epidemics observed at different locations may be linked (9–12). Travel has been thought to be a possible cause after mathematical models based on population movements successfully explained the 1968-69 pandemic (10) and represented the paths of the epidemic within a country (9,11,12). While the cause of the geographic spread of influenza is still debated, the reasons for its seasonality are even more unclear. Although markedly seasonal, the exact timing of the winter epidemics varies from year to year. Furthermore, the interannual impact of influenza epidemics varies substantially (1,3,4).

Documenting patterns in the space and time dynamics of influenza epidemics is the first step in understanding the underlying mechanisms driving epidemic fluctuations (13). Correlations over time and space have been estimated for several animal and insect populations (14–16) and diseases such as measles (17–19) but not for influenza, despite the increasing availability of time series with the recent development of surveillance networks (3,20–28). We provide data and a statistical analysis for the correlations over time and space for influenza-related deaths in the United States, France, and Australia during a 26-year period which spans 1972–1997. Our goal was to determine whether influenza epidemics are correlated in impact and synchronized in time 1) at the hemispheric level and 2) at the interhemispheric level with a systematic half-year lead of one hemisphere to the other.

Datasets and Models

Datasets

In each country, the weekly number of deaths from pneumonia and influenza from 1972 to 1997 were computed from death certificates collected by national agencies for vital statistics (United States, National Center for Health Statistics; Australia, Australian Bureau for Statistics; France, Institut National de la Santé et de la Recherche Médicale, Service Commun 8). We used codes

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470–474 and 480–486 from the International Classification of Diseases (ICD) 8th revision, before 1979, and codes 480-487 from ICD-9 from 1979 onwards to select deaths due to pneumonia and influenza. Annual population estimates were obtained from the same offices over the study period. The population sizes in 1997 in the United States, France, and Australia were 272.7 million, 58.6 million, and 18.9 million, respectively.

Data Analysis

From the pneumonia and influenza death series, we investigated two aspects of influenza epidemics: the correlation in impact as measured by seasonal excess death rates and the synchrony in timing of the epidemic peaks.

Definition of Epidemic and Excess Deaths

Influenza epidemic activity is typically observed during November–March in the United States and France and June–September in Australia (8). We conducted analyses by using the influenza year, which runs from the first week of August to the last week of July of the next year in the United States and France and contains one annual epidemic. We considered the regular calendar year as the influenza year in Australia. From the national weekly pneumonia and influenza death time series, we defined the epidemic periods by a similar procedure to that described by Serfling and used by the Centers for Disease Control and Prevention (CDC) (29,30). In summary, we excluded 25% of the weeks in which the observed death rates were the largest to exclude epidemic periods. We then fit a seasonal regression model to the truncated series to estimate the expected baseline number of deaths in the absence of epidemic activity. A nonepidemic threshold was defined by the upper limit of the 95% confidence interval derived from the seasonal regression model. Only influenza activities that remained above the threshold for $\geq$2 consecutive weeks were included in the analysis. For each influenza year, we measured the excess deaths by subtracting the expected baseline deaths, calculated from the seasonal regression model, from the observed deaths.

Estimating Correlation in Excess Deaths from Influenza Epidemics

We calculated pair-wise Spearman correlation coefficients of the excess deaths estimated for each of the 26 influenza years in the study period for the three countries. For France and the United States, epidemics were paired for contemporaneous influenza years. To test for correlation between the United States and Australia or between France and Australia, we investigated two pairing scenarios: 1) influenza epidemics in Australia were systematically 6 months in advance of those of the United States and France for the 26 years considered, and 2) influenza epidemics in Australia were systematically 6 months behind.

Estimating Synchrony in Timing of Peaks of Influenza Epidemics

For each influenza year and each country, we defined the week of the year when the peak of the epidemic occurred as the week where the maximal number of pneumonia and influenza deaths was observed. Then, we determined the distribution of the time lags between the weeks of the peak in France and in the United States in contemporaneous influenza years, with negative values of the time lag indicating that the epidemic in France preceded that in the United States. The same distribution was calculated for Australia and the United States and for Australia and France, with the two scenarios detailed in the previous section for pairing. We expected the time lags of the peaks in the United States and France to be distributed around zero if the assumption of synchronism of the peaks held true, with a small variance indicating high synchrony.

To test whether the reported distribution of the time lags was indicative of synchrony, we simulated the distribution of these lags when there was no particular synchronization mechanism between these two countries except for the seasonality of the disease. We randomly permuted the week when the pneumonia and influenza death time series peaked in the United States for the 26 influenza years and computed the distribution of the lags with the original series from France. A p value for the existence of synchrony in the peaks was derived from this randomization procedure.

With regards to Australia, because of the change of hemisphere, we expected the week of the peak to lag by 6 months on average with that of the United States or France, regardless of the pairing scenario. Here again, the small variance of distribution of the time lags would indicate synchronism. For both pairing scenarios, we performed a randomization procedure similar to that described for comparing France and the United States to derive a p value for synchrony in the peaks.

Results

Figure 1 depicts the raw time series of weekly number of deaths from pneumonia and influenza in the United States, France, and Australia, normalized by population size. The series show a typical seasonal pattern, with large interannual variability in the amplitude of epidemics in all three countries. The pneumonia and influenza death series for Australia appears less smooth than for the United States or France, probably because of noise effects caused by the smaller population size of Australia, representing 7% of the population size of the United States and 32% of that of France.

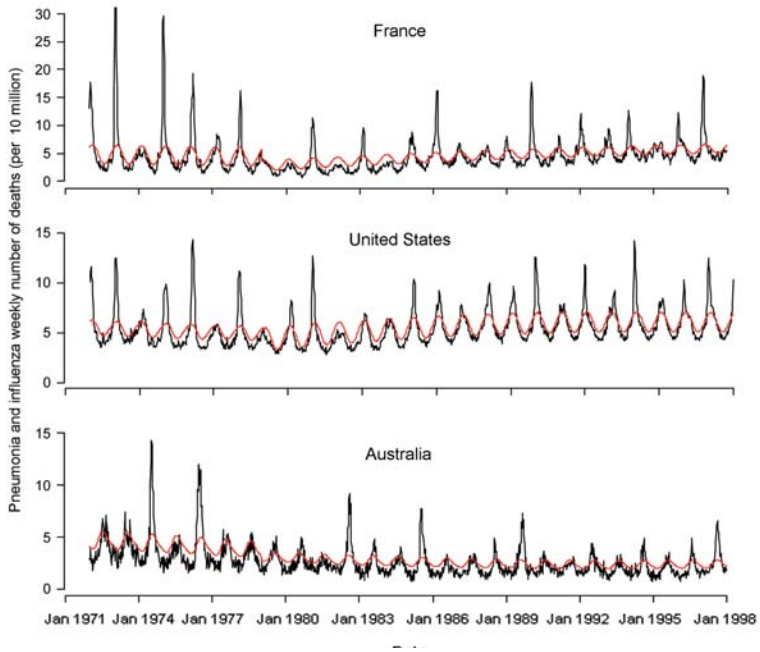

Figure 1. Weekly number of influenza and pneumonia deaths per 10 million population from January 1972 to December 1997 in the United States, France, and Australia (black line). The red line represents the epidemic threshold defined by a seasonal regression.

Excess Deaths

For the 26 influenza years (1972–1997), we estimated a median of 26 per million population pneumonia and influenza excess deaths in the United States (range 0-63), 39 per million pneumonia and influenza excess deaths in France (range 0-183), and 13 per million pneumonia and influenza excess deaths in Australia (range 0-75). The rates of excess deaths were different in the three countries (Kruskal-Wallis test, $p = 0.02$). The Table gives the detailed annual estimates of the number of excess deaths for each influenza year. Two of 26 influenza years in the United States did not result in substantial excess pneumonia and influenza deaths, 4 in France, and 3 in Australia. The duration of the epidemic periods was similar in France and in the United States, with an average duration of 11.8 weeks in the United States (standard deviation (SD) = 0.8) and 11.4 weeks in France (SD = 0.8, $p = 0.80$, Wilcoxon rank test). The epidemic periods in Australia were shorter than those in the United States and in France, with an average duration of 9.6 weeks (SD = 1.0; Kruskal-Wallis test, $p < 0.001$).

Correlation in Excess Deaths

The correlation coefficient for excess deaths in the United States and France in contemporaneous influenza years was high (Spearman's $\rho = 0.76$, $N = 26$, $p < 0.001$; Figure 2A). On the contrary, excess deaths in Australia were not correlated with excess deaths in France or the United States in the scenario in which Australia was systematically 6 months in advance (Spearman's $\rho = 0.14$ for Australia and the United States, $p = 0.50$ and $\rho = 0.37$ for Australia and France, $p = 0.06$; Figure 2B and C) or in the scenario in which Australia was systematically 6 months behind (Spearman's $\rho = 0.15$ for Australia and the United States, $p = 0.47$ and Spearman's $\rho = 0.17$ for Australia and France, $p = 0.37$).

Synchrony in Timing of Peaks

In France and in the United States, in the 26 influenza years, the median time lag between the weeks when the peak occurred was half a week ($N = 26$, range –8 through 6) reflecting a high level of synchrony (Figure 3A). The peak occurred earlier in the United States in 11 of the 26 epidemic seasons, earlier in France in 13 epidemic seasons, and on the same week for both countries in 2 epidemic seasons. Influenza epidemics did not peak earlier in France more often than in the United States, or vice versa (chi square, $p = 0.66$). Of the 100,000 randomizations we performed by permuting the week of the peak, 2 gave a variance for the time lags lower than that reported for the original data (Figure 4A; $p < 0.001$). Therefore, these results show synchronism in the times of peaks between France and the United States.

In the scenario in which the influenza season in Australia was systematically 6 months in advance of that in the Northern Hemisphere, the median time lag between the peaks in Australia and in the United States was 27 weeks (range 14-39) and 26 weeks between those in Australia and in France (range 15-41; Figure 3B and C). In the scenario in which the influenza season in Australia was systematically 6 months behind that in the Northern Hemisphere, these lags were 25.5 weeks (range 13-36) and 24.5 weeks (range 13-34), respectively. The simulated distributions obtained from the 100,000 permutations of the

Table. Number of pneumonia and influenza excess deaths and duration in weeks (wk, in parentheses) of influenza epidemics for each influenza year in the United States, France, and Australia, 1972–1997

Influenza season (Northern Hemisphere) [a]	U.S. excess deaths (wk)[b]	France excess deaths (wk)[c]	Influenza season (Southern Hemisphere) [d]	Australian excess deaths (wk)[e]
1971-72	8,100 (9)	4,200 (13)	1972	200 (11)
1972-73	11,000 (13)	9,500 (13)	1973	250 (10)
1973-74	4,100 (15)	0 (0)	1974	150 (6)
1974-75	9,000 (13)	7,000 (16)	1975	900 (14)
1975-76	13,600 (13)	4,800 (12)	1976	0 (0)
1976-77	600 (3)	1,400 (9)	1977	1,000 (16)
1977-78	10,000 (13)	2,500 (8)	1978	200 (8)
1978-79	0 (0)	300 (4)	1979	100 (7)
1979-80	4,200 (9)	0 (0)	1980	250 (13)
1980-81	8,600 (10)	3,100 (11)	1981	200 (9)
1981-82	0 (0)	0 (0)	1982	0 (0)
1982-83	1,400 (5)	1,800 (10)	1983	700 (15)
1983-84	700 (4)	0 (0)	1984	250 (12)
1984-85	7,000 (11)	2,300 (15)	1985	150 (8)
1985-86	6,000 (13)	4,700 (18)	1986	650 (15)
1986-87	2,000 (7)	900 (9)	1987	0 (0)
1987-88	8,700 (18)	700 (8)	1988	100 (7)
1988-89	7,000 (16)	1,000 (9)	1989	200 (9)
1989-90	10,400 (14)	4,300 (11)	1990	750 (17)
1990-91	3,300 (12)	1,000 (10)	1991	100 (6)
1991-92	6,800 (10)	2,900 (16)	1992	50 (3)
1992-93	6,300 (16)	2,100 (16)	1993	300 (14)
1993-94	9,800 (11)	2,600 (11)	1994	50 (3)
1994-95	5,300 (15)	900 (10)	1995	300 (10)
1995-96	6,300 (16)	2,500 (17)	1996	100 (5)
1996-97	11,400 (18)	4,500 (13)	1997	250 (10)

[a]Excess deaths reported from August of a given year to July of the next year during an influenza epidemic (e.g., for row labeled 1971-72, excess deaths occurring from August 1971 to July 1972).
[b]Population, 272.7 million.
[c]Population, 58.6 million.
[d]Excess deaths reported from January to December of a given year during an influenza epidemic (e.g., for row labeled 1972, excess deaths occurring from January 1972 to December 1972).
[e]Population, 18.9 million.

week were not different from the observed distributions (in the scenario in which the influenza season in Australia was 6 months behind, $p = 0.70$ and $p = 0.79$ for the United States and France, respectively [Figure 4B and C] and in the scenario in which it was 6 months in advance, $p = 0.13$ and $p = 0.12$). For these two pairing scenarios, no synchrony in the peaks was evident.

Discussion

The results of this study favor a high level of correlation in amplitude and synchrony in the timing of influenza epidemics in France and the United States. No correlation or synchrony was found between Australia and the United States, or between Australia and France in the two scenarios in which Australia systematically led or lagged behind the global interhemispheric circulation of epidemics by 6 months.

The level of correlation in amplitude evidenced here depends on the statistical method used for estimating seasonal excess deaths. We have used a similar procedure to that described by Serfling and used by the CDC for estimating excess deaths (3,29). In our study, periods of increased influenza activity were excluded from the baseline model by discarding upper marginal values that were above a somewhat arbitrary cutoff (25th percentile of the distribution), and we found a high correlation between excess deaths in the United States and France (Spearman's correlation coefficient $\rho = 0.76$). The correlation remained stable with a lower cutoff set at 10%: in this analysis the correlation coefficient was also 0.76. Moreover, our excess death estimates are well in line with those published for the United States (3,31,32) and for France by using a different statistical approach (4). For the United States and France, the correlation coefficients between our estimates of pneumonia and influenza excess deaths and the most recently published estimates were 0.87 and 0.94, respectively, with similar orders of magnitude (3,4). No comparable estimates were available for Australia. Pneumonia and influenza death series have been used traditionally since the work of William Farr in the late 1840s to quantify the effect of influenza epidemics in terms of death (33) because severe complications are triggered by influenza infection and result in death, in particular bacterial pneumonia (34). The use of pneumonia death series might

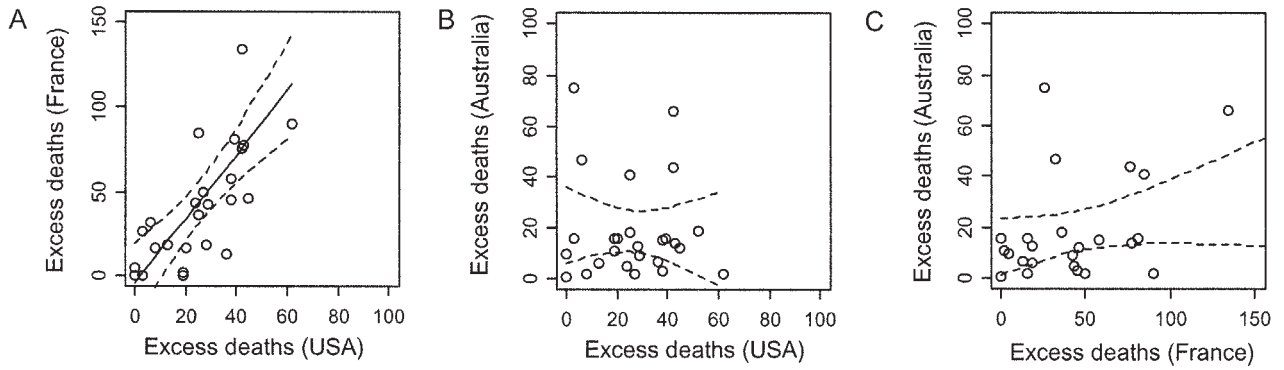

Figure 2. Correlation in the impact of influenza epidemics for 26 influenza years (1972–1997), measured by the annual number of pneumonia and influenza excess deaths. A, excess deaths per million in France (y axis) and the United States (x axis) in contemporaneous winters: Spearman correlation coefficient = 0.76 ($p < 0.001$). B, excess deaths per million in Australia (y axis) and the United States (x axis), considering the scenario in which the influenza season in Australia is systematically 6 months before that of the United States: Spearman correlation coefficient = 0.14 ($p = 0.50$). C, excess deaths per million in Australia (y axis) and France (x axis), considering the scenario where the influenza season in Australia is systematically 6 months before that of France: Spearman correlation coefficient = 0.37 ($p = 0.06$). We obtained similar results as in B and C, when we considered a reverse scenario in which the influenza season in Australia was systematically lagging 6 months behind that in the United States or France.

introduce additional background noise in estimating excess deaths caused by influenza, especially because of the high level of activity in the summer and the upward trend observed since the early 1980s (6). We performed a sensitivity analysis by restricting our calculations of excess deaths to deaths coded as influenza only and retrieved a similar correlation between the United States and France (Spearman's $\rho = 0.75$). However, we do not capture the total impact of influenza by analyzing only pneumonia and influenza death, which accounts for only part of the overall deaths from influenza (4,34). Therefore, we probably cannot rely strictly on our absolute estimates of excess deaths to study the overall impact of influenza epidemics on death. However, pneumonia and influenza deaths series provide a robust and unbiased indicator of the severity of influenza epidemics for between-country comparisons. In addition, with an indicator as simple and crude as the raw pneumonia and influenza deaths observed during the week of the peak, the correlation remains of the same order (Spearman's $\rho = 0.73$).

Demography may contribute to the lack of correlation and synchrony between Australia and the United States or between Australia and France. While the age structures of the populations in the three countries are similar, the population sizes are different. Discrepancies in the levels of vaccination might also play a role. However, the number of doses of influenza vaccine distributed was similar in France and the United States from 1980 until the early 1990s but doubled in recent years in the United States (35). The level of vaccination in Australia was about half of that of France and the United States from 1980 until 1990 and became similar to that of France in recent years

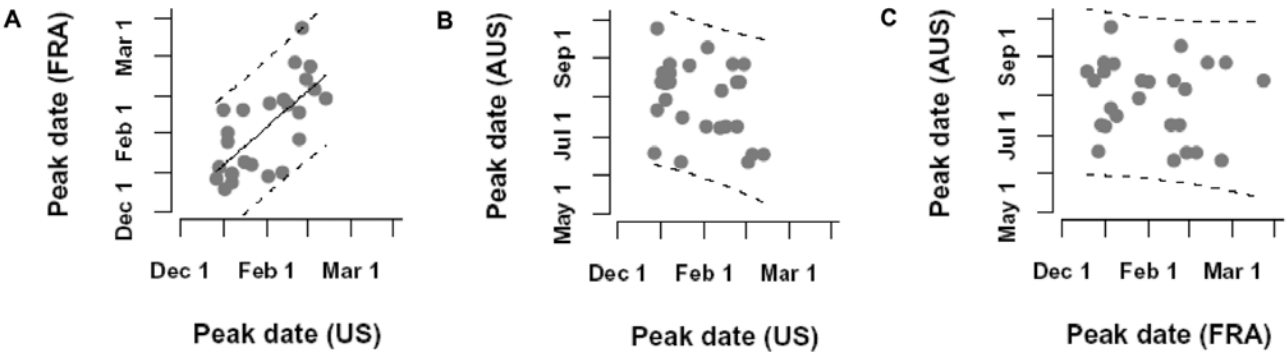

Figure 3. Synchrony in the timing of the peaks of influenza epidemics for 26 influenza years (1972–1997). Correlation between the week of year of the epidemic peak A, in the United States (x axis) and in France (y axis). B, in the United States (x axis) and in Australia (y axis). C, in France (x axis) and in Australia (y axis). Panels B) and C) illustrate the scenario in which the influenza season in Australia is systematically 6 months before that of the United States or France. Similar results are obtained for the reverse scenario.

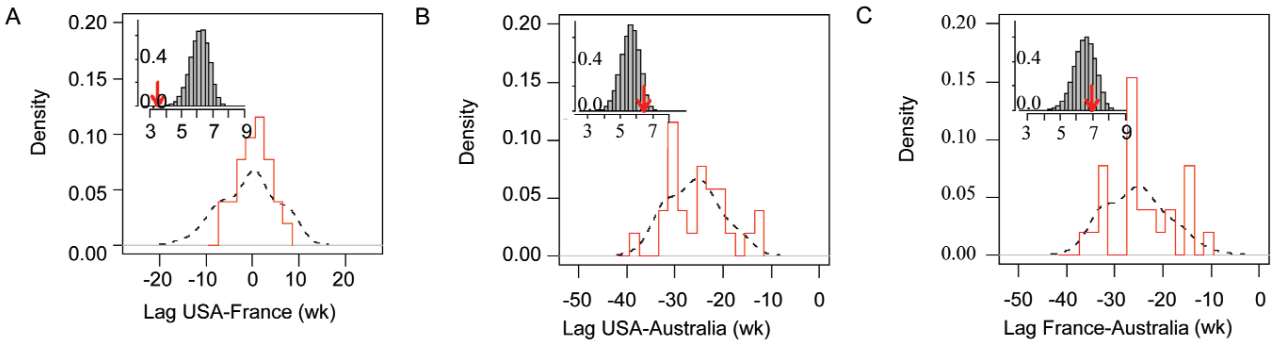

Figure 4. Synchrony in the timing of the peaks of influenza epidemics for 26 influenza years (1972–1997). Distribution of the time lags between the epidemic peaks in weeks (wk, main plot). The red bars represent the observed lags, and the dashed line represents the distribution of lags obtained by permutations. (inset plot) Distribution of the standard deviation of permuted lags under the assumption of no synchrony. Red arrow indicates the standard deviation in the observed data. A, United States and France, B) United States and Australia, C, France and Australia. Panels B and C illustrate the scenario in which the influenza season in Australia is systematically 6 months before that of the United States or France. Similar results are obtained for the reverse scenario.

(35). Therefore, no obvious link existed between trends in vaccination and our results.

An important limitation in the analysis of correlation and synchrony between countries in different hemispheres lies in the arbitrary choice for pairing epidemics. We investigated two extreme scenarios in which influenza epidemics in Australia were systematically 6 months ahead of those in France or the United States or systematically 6 months behind. We found no correlation or synchrony in either one. We cannot rule out a more complex interhemispheric pathway with recurrent changes in the leading hemisphere. We searched for an optimal theoretical pairing, allowing each epidemic in Australia in our simulations to pair with either the preceding or following epidemic in the United States (or France) to maximize the correlation in excess death. For optimal pairing, the correlation coefficients obtained were in the same order of magnitude as those observed between France and the United States (Spearman's $\rho = 0.66$ for the United States and Australia and Spearman's $\rho = 0.71$ for Australia and France).

Few reports document the circulation of influenza virus on a worldwide scale. Hope-Simpson studies of death, disease, and virologic datasets suggest that the circulation of influenza strains (and the epidemics they cause) may follow a given pathway during several years (e.g., Southern to Northern Hemisphere) and subsequently shift to the other pathway (e.g., Northern to Southern Hemisphere) (36). This finding agrees with the absence of significant time and space correlations found in this work between countries in different hemispheres when epidemics were paired 6 months ahead or behind. If this absence of correlation between the two hemispheres, observed in death records limited to an interpandemic period and a limited set of countries, held true in the case of a pandemic, information on previous routes of transmission derived from historical epidemics (10,37) could prove of little use for planning the route of future epidemics or pandemics on a worldwide scale.

Our study nevertheless strongly suggests that influenza epidemics are correlated in amplitude and synchronized in timing in the Northern Hemisphere, and collection and analysis of additional data is underway in other countries of Europe and North America. Prevaccination measles epidemics in different locales of the United States and the United Kingdom were also highly correlated in time and space, a situation that evolved to the observed absence of correlation in the last 2 decades after the level of vaccination increased from 50% to 90% (17,19). This lack of correlation is reportedly promoting persistence of the disease (19). Space and time correlations of influenza epidemics may follow the same pattern as vaccine coverage increases. How the very high rate of antigenic evolution of influenza virus, a feature not found in measles, could impact on this change requires further study, all the more as antigenic variations explain partly the impact of the disease (3,19,38–40). The current plan to control influenza advocated by the World Health Organization promotes vaccine use and, in case of emergence of a pandemic virus, extensive use of antiviral drugs and vaccine, assuming that the vaccine could be produced in a timely fashion (41). The impact of an expected loss of correlations in influenza epidemics resulting from in creased vaccine use should be further investigated, especially by mathematical modeling.

In conclusion, two factors have been reported to drive the space and time correlations of epidemics: population movements and environmental issues, such as climate or weather conditions (14–16). Population movements are assumed to play a key role, though not quantified, in the global spread of influenza epidemics (8,10), but the role of

environmental factors and virus circulation between the Southern and Northern Hemispheres (42–44) remains to be clarified. Large-scale molecular epidemiologic studies of influenza viruses, sampled from locations in different hemispheres, could assist in investigating the circulation pathways of the disease (44). Such large-scale studies seem feasible in light of the recent plan for a global sentinel laboratory that could centralize genotype and archive samples collected worldwide (45).

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Ecologic and Geographic Distribution of Filovirus Disease

A. Townsend Peterson,* John T. Bauer,* and James N. Mills†

We used ecologic niche modeling of outbreaks and sporadic cases of filovirus-associated hemorrhagic fever (HF) to provide a large-scale perspective on the geographic and ecologic distributions of Ebola and Marburg viruses. We predicted that filovirus would occur across the Afrotropics: Ebola HF in the humid rain forests of central and western Africa, and Marburg HF in the drier and more open areas of central and eastern Africa. Most of the predicted geographic extent of Ebola HF appear to have been observed; Marburg HF has the potential to occur farther south and east. Ecologic conditions appropriate for Ebola HF are also present in Southeast Asia and the Philippines, where Ebola Reston is hypothesized to be distributed. This first large-scale ecologic analysis provides a framework for a more informed search for taxa that could constitute the natural reservoir for this virus family.

The natural maintenance cycles of filoviruses (Order *Mononegavirales*, family *Filoviridae*) are unknown (1).[1] Although dynamics of filoviruses as causes of epidemic diseases among humans, great apes, and other primates have been described in detail (2–13), the natural reservoir, mode of transmission to hominids and pongids (humans, gorillas, and chimpanzees), and temporal dynamics remain unclear. Diverse taxa have been suggested as potential reservoirs, including bats, rodents, arthropods, and plants (14–18).

Two observations provide clues about the nature of the host-virus relationship. First, filovirus transmission to humans is not common, and most occurrences can be traced to a single index case (2,6,19) (exceptions occur—e.g., the Durba Marburg outbreak appears to have involved multiple independent infections of humans from a reservoir population presumably associated with a mine). We assume that introductions to nonhuman primate populations also generally begin with single index cases, but this hypothesis is more difficult to investigate. This rarity argues against a common arthropod vector for transmission: if anthrophilic arthropod vectors were to carry filoviruses, multiple index cases would be more common, as many primates in an area would have the opportunity for infection. In addition, filoviruses generally do not replicate in arthropods or arthropod cell lines, leading several authors to speculate on more incidental modes of transfer (e.g., direct contact) (20).

Second, filoviruses show clear geographically related phylogeographic structure. Viruses and subtypes from particular geographic areas cluster together phylogenetically, even when occurrences from different years are studied. This phylogeographic structure suggests a stable host-parasite relationship, in which viruses are maintained in permanent local-regional pools. This host would not experience high death rates, as primates do (7,9); evolution of avirulence in long-term host-parasite relationships is expected on theoretical grounds (21).

Searches for the natural reservoir of filoviruses have taken several paths. Epidemiologic studies designed to trace lineages of transmission in outbreaks have identified index cases, but have not succeeded in specifying the mode of "jump" to hominids (2,6,19). Testing large numbers of organisms from the vicinity of outbreaks has failed to identify even a single nonhominid infection (14–16). Finally, laboratory tests of reservoir competence of species have documented the following: 1) no, or very limited, infection of plants or arthropods; 2) a single marginally successful infection of snakes but with very low levels of virus circulation; 3) successful infection of bats and possibly rodents; and 4) frequent successful, but fatal, infection in nonhuman

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[1]Although filovirus taxonomy undergoes frequent revision, we follow nomenclature most recently established by the International Committee of Taxonomy of Viruses in 2002. Filoviruses consist of two genera. The genus *Marburgvirus* contains one species, *Lake Victoria marburgvirus*, with several recognized strains, and *Ebolavirus* contains four species: *Ivory Coast ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, and *Zaire ebolavirus*. For simplicity, we refer to the viruses using the unitalicized vernacular (e.g., Ebola Zaire). We use "Ebola viruses" to refer in general to members of *Ebolavirus* and "Marburg viruses" to refer in general to members of *Marburgvirus*. The diseases caused by filoviruses are termed Ebola hemorrhagic fever (HF; diseases caused by Ebola viruses) and Marburg HF (diseases caused by Marburg viruses).

primates (e.g., *Chlorocebus*, *Macaca*) (1,17,18). While these investigations have shed some light, they have not provided convincing evidence for a particular reservoir.

An unexplored approach to identifying the natural reservoir of filoviruses is large-scale ecologic and geographic comparisons to detect patterns of co-occurrence and codistribution of viruses with potential hosts. This approach has been applied successfully to identifying reservoir rodent species for Chagas disease (22). Our general approach is as follows: 1) to understand the large-scale ecology and geography of disease occurrences by using ecologic niche modeling (23), and 2) to compare these characteristics with ecologic and geographic patterns of potential reservoirs. Here, we address the first step and document broad-scale ecologic and geographic patterns in filovirus-associated HF occurrences.

Methods

Distributional data for filovirus-associated HF occurrences in hominids were accumulated from the literature (Table). Occurrences of unknown origin were excluded from analysis, but when reasonable guesses could be made as to point or general area of origin (e.g., 1995 outbreak of hemorrhagic fever due to Ebola Ivory Coast as originating at Plibo, Liberia), they were included. All occurrences were georeferenced (available from: URL: http://www.calle.com/world) to the nearest 0.001°. Although assigned geographic coordinates may not fix the exposure point precisely, they represent our best guess as to its position and are likely to be representative of the coarse-scale ecologic conditions. (The relatively crude spatial resolution at which analyses were conducted makes some error in georeferencing irrelevant.)

Ecologic niches and potential geographic distributions were modeled by using the Genetic Algorithm for Rule-set Prediction (GARP) (30–32) (available from: URL: http://www.lifemapper.org/desktopgarp/). In general, GARP focuses on modeling ecologic niches (the conjunction of ecologic conditions wherein a species can maintain populations without immigration) (33). Specifically, GARP relates ecologic characteristics of occurrence points to those of points sampled randomly from the rest of the study region, developing a series of decision rules that best summarize factors associated with presence (23).

Occurrence points are divided evenly into training (for model building) and test (for model evaluation) datasets. GARP works in an iterative process of rule selection, evaluation, testing, and incorporation or rejection: a method is chosen from a set of possibilities (e.g., logistic regression, bioclimatic rules) and applied to the training data to develop or evolve a rule. Predictive accuracy is evaluated on the basis of the test data. Rules may evolve in ways that mimic DNA evolution (e.g., point mutations, deletions). Change in predictive accuracy between iterations is used to evaluate whether particular rules should be incorporated into the model; the algorithm runs 1,000 iterations or until convergence. Model quality was evaluated through independent test dataset reserved prior to modeling; a chi-square test was used to compare observed success in predicting the distribution of test points with that expected under a random model (proportional area predicted present provides an estimate of occurrence points correctly predicted, were

Table. Virus, location, dates, geographic coordinates, and literature citation for filovirus-caused hemorrhagic fever occurrences

Virus	Country	Apparent origin	Dates	Latitude (°)	Longitude (°)	Reference
Ebola Ivory Coast	Côte d'Ivoire	Tai National Park	Nov 1994	5.850	-7.367	(7,24)
				5.900	-7.317	
Ebola Ivory Coast	Côte d'Ivoire or Liberia	Plibo (Liberia)	Dec 1995	4.589	-7.673	(25)
Ebola Sudan	Sudan	Nzara	June–Nov 1976	4.643	28.253	(3)
Ebola Sudan	Sudan	Nzara	July–Oct 1979	4.643	28.253	(4)
Ebola Sudan	Uganda	Gulu	Oct 2000–Feb 2001	2.783	32.300	(26)
Ebola Zaire	DRC	Yambuku	Sept–Oct 1976	2.817	22.233	(2)
Ebola Zaire	DRC	Bonduni	June 1977	2.967	19.350	(10)
Ebola Zaire	Gabon	Minkebe,	Dec 1994–Feb 1995	1.733	12.817	(8)
		Mekouka,		1.400	12.983	
		and/or Andock		1.483	12.917	
Ebola Zaire	DRC	Kikwit	Jan–Jul 1995	-5.058	18.909	(11)
Ebola Zaire	Gabon	Mayibout	Feb 1996	-1.117	-13.100	(8)
Ebola Zaire	Gabon	Booue	Jul 1996–Mar 1997	-0.100	-11.95	(8)
Ebola Zaire	Gabon and DRC	Ekata	Dec 2001–2002	0.706	14.275	(12)
Marburg	Zimbabwe	Wankie?[a]	Feb 1975	-18.367	26.483	(6)
Marburg	Kenya	Nzoia or Mt. Elgon	Jan 1980	0.450	34.617	(19)
Marburg	Kenya	Mt. Elgon?	1987	1.133	34.550	(20)
Marburg	DRC	Durba	Apr 1999–Sept 2000	3.117	29.583	(27–29)

[a]Reported location where patient received a "bite." Although some investigators felt the disease was related to the bite, the patient had traveled widely in Zimbabwe and parts of South Africa and was exposed to wildlife at several locations in Zimbabwe (6). DRC, Democratic Republic of the Congo;?,some doubt exists as to exact point of exposure.

the prediction random with respect to the distribution of the test points).

To characterize environments, we used 11 GIS coverages summarizing elevation, slope, aspect, flow direction, flow accumulation, and tendency to pool water (from the USGS Hydro-1K dataset [available from: URL: http://edcdaac.usgs.gov/gtopo30/hydro/), and climate characteristics including daily temperature range; mean annual precipitation; maximum, minimum, and mean annual temperatures; solar radiation; frost days; wet days; and vapor pressure (1960–1990; Intergovernmental Panel on Climate Change [available from: URL: http://www.ipcc.ch/]). These coverages are worldwide and provide a consistent view of ecologic variation across regions studied. GARP's predictive ability has been tested under diverse circumstances (22,23,34–47).

To optimize model performance, we developed 100 replicate models of ecologic niches based on independent random subsamples from available occurrences. We chose a "best subset" of these models on the basis of optimal error distributions for individual replicate models (34): median area predicted across all replicate modes was calculated, and the 20 models with predicted areas closest to the median were chosen for further consideration. These geographic predictions were combined to provide a summary of potential geographic distributions. Projection of the Africa-based rule-sets onto maps of Asia and the Pacific provided hypotheses of potential distributional areas in other regions (46).

To permit visualization of the ecologic dimensions of models, we combined best-subsets predictions with maps of the ecologic parameters used to build them in a GIS environment (COMBINE in ArcView 3.2). The resulting dataset represents unique combinations of environments and predictions; its attributes table provides the model prediction for all environmental combinations, to permit visualization of ecologic variation. We also compared ecologic conditions inside and outside of the modeled Ebola HF distribution within 11 regularly spaced circular windows (radius 50 km); comparisons were summarized through Mann-Whitney U-statistics, permitting a nonparametric visualization of the strength of association of each ecologic dimension (temperature, precipitation, elevation) with the range limit.

Results

The geographic distribution of filovirus disease spreads generally across the humid Afrotropics (Figure 1A). Outlier occurrences lie at the eastern extreme of the distribution, consisting of occurrences associated with Ebola Sudan and Marburg viruses. Preliminary analyses of these geographic distributions, based on random subsets of the few data points available, indicated high statistical significance to model predictions: predictions of the geographic distribution of filovirus HFs correctly included random independent subsets much better than random model expectations (all $p < 10^{-7}$). Although subsequent modeling was done without subsetting to maximize occurrence data, these preliminary results nonetheless indicated excellent predictivity of our distributional hypotheses.

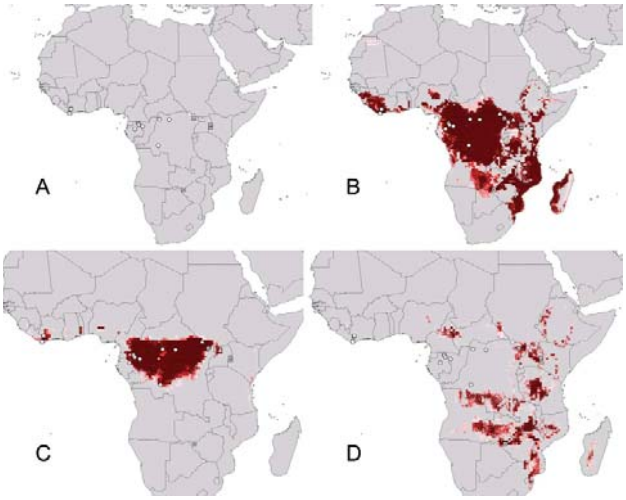

Figure 1. Summary of known and predicted geography of filoviruses in Africa. (A) Known occurrence points of filovirus hemorrhagic fevers (HFs) identified by virus species. (B) Geographic projection of ecologic niche model based on all known filovirus disease occurrences in Africa. (C) Geographic projection of ecologic niche model based on all known Ebola HF occurrences (i.e., eliminating Marburg HF occurrences). (D) Geographic projection of ecologic niche model based on all known occurrences of Marburg HF (i.e., eliminating Ebola HF occurrences). Darker shades of red represent increasing confidence in prediction of potential presence. Open squares, Ebola Ivory Coast; circles, Ebola Zaire; triangles, Ebola Sudan; dotted squares, Marburg HF occurrences.

Modeling the distribution of *Filoviridae* in general (all points in Figure 1A) produced a broad potential distribution across the Afrotropics, including areas from which filovirus HF occurrences have not been reported (Tanzania, Mozambique, Madagascar; Figure 1B). Predicted distributions of the two major *Filoviridae* clades—Ebola and Marburg viruses—showed different geographic patterns. When just the three African Ebola virus species were analyzed together, areas of overprediction in eastern Africa disappeared, and predicted distributional areas included only areas surrounding known occurrence points, except for a few small disjunct areas in West Africa (Figure 1C). The predicted distribution did not include all of the Afrotropics—coastal central Africa and most of West Africa appeared not to be included, although these models are based on very small samples of occurrences.

When we analyzed the relatively few Marburg HF occurrences for which distributional data exist (n = 4 occurrences), a complementary distributional area was

predicted (Figure 1D). Marburg HF was predicted to be absent in the humid Afrotropics, rather appearing focused in drier areas in eastern and south-central Africa. In contrast to Ebola HF, Marburg virus appears to have the potential to occur in areas from which filovirus disease has not yet been described.

Sequential omission of Ebola virus species from analyses provided a view of ecologic similarity of species (45): if omission of a particular species causes little overall change, then its ecologic characteristics are not distinct from those of the remaining species. Omission of Ebola Ivory Coast had little effect on the prediction (Figure 2A; note predicted area in Ivory Coast); similarly, predictions omitting Ebola Zaire included at least part of the distribution of Ebola Zaire (southern portion omitted; Figure 2B). Eliminating Ebola Sudan, however, yielded a prediction completely excluding the distribution of Ebola Sudan (Figure 2C), which suggests that Ebola Sudan occurs under a distinct ecologic regime.

Inspection of niche models of Ebola HF occurrences (Marburg HF excluded) in ecologic space (Figure 3) provided insight into their ecologic distribution. Predicted Ebola HF occurrences were concentrated in regions presenting high precipitation combined with moderate-to-high temperatures (Figure 3A), coinciding with the ecologic distribution of evergreen broadleaf forest, although in specific cases that forest may be highly disturbed. In fact, >50% of African evergreen broadleaf forest is predicted to be within the niche of Ebola HF; no other land-cover type exceeded 5% within the Ebola HF niche (Figure 3B). In other dimensions, Ebola HF occurrences were distributed centrically in African environments and did not include extremes (Figure 3C–D).

Distributional limits are complex results of multiple causal agents. A species is seldom limited on all sides by a single factor; rather, distributional limits are the combined result of many such factors. Inspection of the ecologic dimensions coincident with modeled geographic limits of Ebola HF occurrences (Figure 4) showed some of this complexity. At points around the distributional limit of Ebola HF distributional areas in central Africa, precipitation dominates the range limit at point 11, but temperature and elevation dominate at points 2, 3, and 6. Moreover, gradients are steeper in some areas than others (e.g., point 6 vs. 3). This preliminary analysis thus illustrates the complex relationships between ecologic dimensions and distributional limits.

Given the mysterious origin of Ebola Reston virus (Ebola HF among macaques in a breeding facility on Luzon, Philippines) (9,13), a key question regarding Ebola HF distribution and ecology is whether similar ecologic conditions exist in Southeast Asia (e.g., Philippines). Projecting ecologic niche models for Marburg HF in

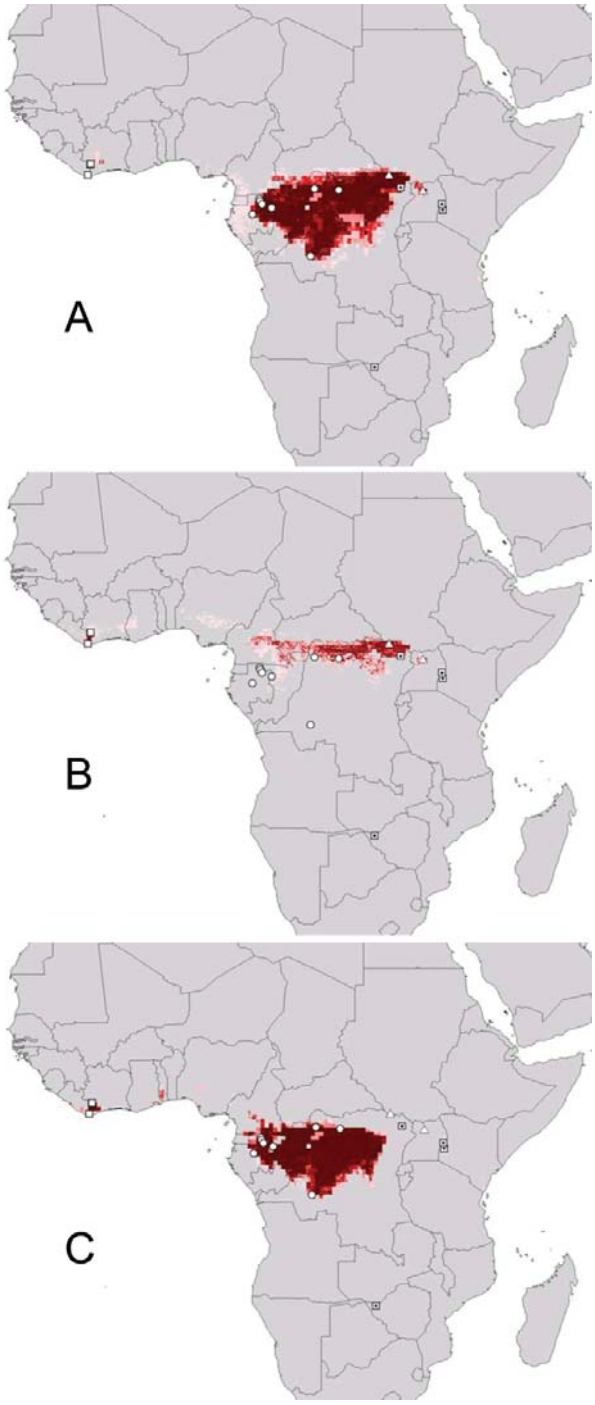

Figure 2. Geographic projection of ecologic niche models in which two Ebola virus species were modeled and used to predict the distributional area of the third. (A) Ebola Zaire and Ebola Sudan predicting (Ebola Ivory Coast omitted; note that distributional area is predicted in Ivory Coast). (B) Ebola Sudan and Ebola Ivory Coast predicting (Ebola Zaire omitted). (C) Ebola Zaire and Ebola Ivory Coast predicting (Ebola Sudan omitted). Darker shades of red represent increasing confidence in prediction of potential presence. Open squares, Ebola Ivory Coast; circles, Ebola Zaire; triangles, Ebola Sudan; dotted squares, Marburg hemorrhagic fever occurrences.

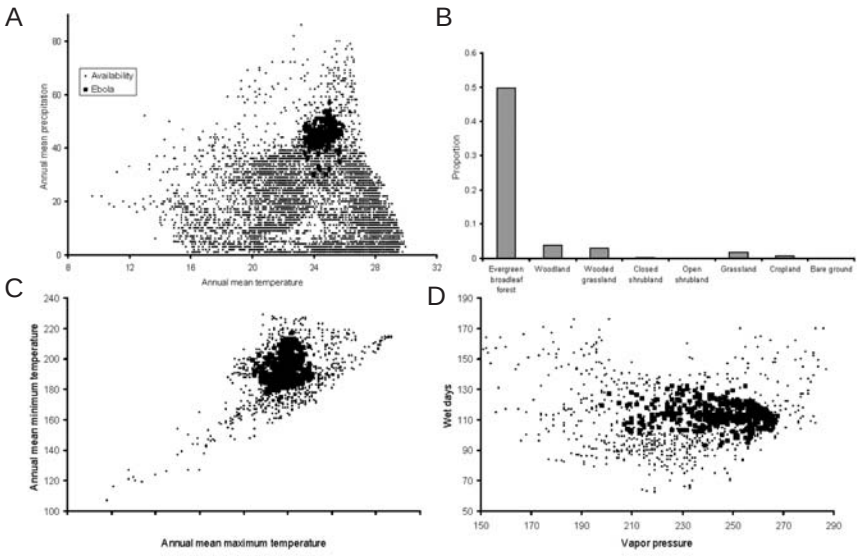

Figure 3. Ecologic distribution of predicted potential distributional areas for Ebola hemorrhagic fever (HF) occurrences, visualized in a few dimensions of climate. (A) Large-scale view (all of Africa), in which the basic concentration of Ebola HF occurrences in hot, wet climates is shown. (B) Distribution by land use/land-cover type, summarized as the proportion of overall area of land-cover types that is predicted to be present at the highest confidence level. (C,D) Regional scale (distributional area predicted by all 20 best-subsets models for Ebola HF buffered by 200 km in all directions) view of the ecology of occurrences of Ebola HF, visualized in dimensions of annual mean minimum temperature, annual mean maximum temperature, wet days, and vapor pressure.

Africa onto Asian environments identified few "appropriate" areas: only a few scattered areas in Papua New Guinea and Indonesia (Figure 5A). Projection of Ebola HF models, however, identified broader potential distributional areas in Southeast Asia (Figure 5B), including the lowlands of Mindanao (Figure 5, inset), a finding that suggests that similar ecologic conditions exist in the Philippines.

Discussion

Ecology and Geography of Filovirus Occurrences

The ecologic niche characteristics reconstructed for filovirus species disease outbreaks coincided closely with phylogenetic patterns in the group (1,48). That is, disease sites for Ebola Ivory Coast and Ebola Zaire coincided ecologically, and these viruses are phylogenetically sister taxa. Ebola Sudan is genetically and ecologically most distinct among Ebola virus species, and (with Ebola Reston) forms the sister clade to Ebola Ivory Coast + Ebola Zaire. Correspondence between phylogenetic and ecologic patterns suggests that ecologic distributions of Ebola Sudan and Ebola Reston may prove similar; hence, the ecologic characteristics of Ebola Sudan may provide clues about the origin of Ebola Reston.

Marburg HF occurrence sites are quite distinct, with minimal overlap with Ebola HF ecologic distributions, coinciding with Marburg virus' distant position in the phylogeny of the *Filoviridae*. This pattern suggests that Marburg virus and the Ebola viruses may have host species with markedly different ecologic requirements.

Ebola Reston

The geographic origin of Ebola Reston virus has been subject of controversy (9,49). Although the Ebola virus-infected monkeys initially documented in Reston, Virginia, originated in the Philippines, whether Ebola Reston occurs naturally in the Philippines has been debated. Nevertheless, the virus is distinct, and its geographic distribution is otherwise unknown. Given the phylogeny-ecology correspondence documented above, the ecology of Ebola Sudan may prove key in predicting the distribution of Ebola Reston, but the scanty occurrence data make species-specific models difficult. Our results are relevant in that ecologic conditions under which Ebola HF occurs in Africa are also found in the Philippines.

In previous analyses of animals, the conservative nature of ecologic niches has been documented to lead to prediction into regions inhabited by congener species (45). To the extent that host-parasite codistribution and cospeciation

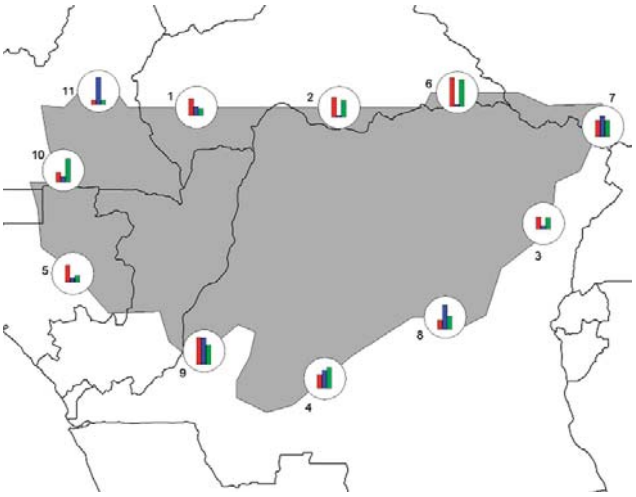

Figure 4. Preliminary exploration of patterns of ecologic variation along the modeled distributional limits (highest confidence level) for Ebola viruses in central Africa. The histograms represent relative values of Mann-Whitney U-tests for inside versus outside the prediction area for temperature (red bars), precipitation (blue bars), and elevation (green bars).

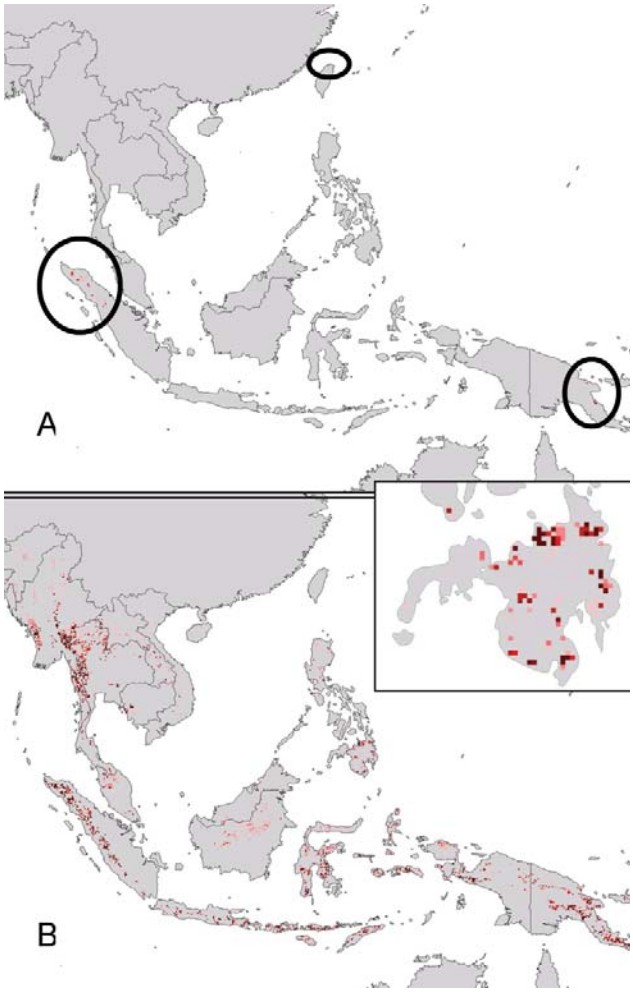

Figure 5. Projection of filovirus ecologic niche models onto southeastern Asia and the Philippines to assess the degree to which possible Philippine distributional areas are predictable on the basis of the ecologic characteristics of African filovirus hemorrhagic fever (HF) occurrences. (A) Projection of model for Marburg HF occurrences (Figure 1D) to southeastern Asia. (B) Projection of model for all filovirus disease occurrences (Figure 1B) to southeastern Asia (the projection of models for Ebola HF occurrences is identical to this map). Inset: detail of projection to the island of Mindanao, in the Philippines. Darker shades of red represent increasing confidence in prediction of potential presence.

may be involved in the virus-reservoir relationships of filoviruses, prediction of potential distributional areas in the Philippines may reflect conservative niche evolution in the host taxon. Of course, because of historical effects (e.g., limited dispersal) on species' distributions, potential distributional areas are often predicted in areas not inhabited (44), so this evidence is not definitive.

Limitations of the Approach

Limitations of our approach should be recognized. First, small sample sizes become critical. Although predictive models can be developed with relatively small samples of occurrence points (39), sample sizes for filovirus HF disease outbreaks are so minimal that single data points can change overall results. Examples of this sensitivity include the Zimbabwe Marburg HF disease outbreak and the Booue, Gabon, Ebola Zaire HF outbreak; inclusion of these points causes geographic predictions to be expanded considerably.

Other limitations center on the ecologic dimensions in which the niche is modeled. If additional dimensions exist that limit species' distributions (and they certainly do), GARP predictions will be overly large. Jackknife manipulations (systematic omission of ecologic dimensions to assess sensitivity to coverage density) can, to some degree, help in assessing sensitivity to coverage completeness (42), but dimensions more important than the set actually used may exist. Particularly relevant is climate variability—extreme events such as droughts and heavy rainfall may prove particularly relevant to filovirus transmission but are not included herein; such more complex models are under development (A.T. Peterson et al., unpub. data). Spurious associations between occurrence points and ecologic dimensions, though usually detected through independent test datasets, can limit distributional predictions overmuch.

Natural Reservoirs for Filoviruses

Detailed understanding of the geography and ecology of filovirus HF outbreaks represents an underexplored avenue of investigation regarding natural transmission cycles of filoviruses. We assembled available information regarding filovirus HF outbreaks and used various analytical tools to arrive at a detailed understanding of geography and ecology of filovirus disease occurrences. Consequently, we can now assemble criteria by which potential reservoir taxa might be judged. If one assumes a fair degree of host specificity in this host-parasite system, patterns of codistribution and cophylogeny can be expected. Hence, criteria include the following: 1) African Ebola virus reservoirs would be distributed principally in evergreen broadleaf forest; 2) the main focus of the geographic distribution of the reservoir(s) would be in the Congo Basin; 3) a disjunct (allopatric) distributional area would be present in West Africa; 4) a related taxon in eastern Africa would range in more arid habitats; 5) the reservoir would belong to a clade more broadly distributed across Africa and Southeast Asia.

Assessment of potential reservoir taxa by using these criteria has begun (A.T. Peterson et al., unpub. data), with the idea of eventually testing hypotheses of host associations through ecologic niche comparison methods (22). The goal, to be explored in future publications, is to develop reduced lists of taxa of highest priority for virus survey.

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Fatal Infectious Disease Surveillance in a Medical Examiner Database[1]

Mitchell I. Wolfe,* Kurt B. Nolte,*† and Steven S. Yoon*

Increasing infectious disease deaths, the emergence of new infections, and bioterrorism have made surveillance for infectious diseases a public health concern. Medical examiners and coroners certify approximately 20% of all deaths that occur within the United States and can be a key source of information regarding infectious disease deaths. We hypothesized that a computer-assisted search tool (algorithm) could detect infectious disease deaths from a medical examiner database, thereby reducing the time and resources required to perform such surveillance manually. We developed two algorithms, applied them to a medical examiner database, and verified the cases identified against the opinion of a panel of experts. The algorithms detected deaths with infectious components with sensitivities from 67% to 94%, and predictive value positives ranging from 8% to 49%. Algorithms can be useful for surveillance in medical examiner offices that have limited resources or for conducting surveillance across medical examiner jurisdictions.

Infectious disease deaths in the United States substantially declined during the first 8 decades of the 20th century as a result of public health interventions. However, the end of the century was marked by an increase in infectious disease deaths primarily due to AIDS and pneumonia and influenza (1,2). Increasing infectious disease deaths, the emergence of new infections, and the real or perceived threat of bioterrorist activities have made surveillance for infectious diseases a public health need (3,4).

Infectious disease mortality trends have been described by review of International Classification of Diseases (ICD)–coded death certificate data (2). Although useful in identifying trends, this process has certain limitations, including the following: causes of death are inaccurately certified, are not autopsy verified, and are erroneously coded; and ICD codes are not arranged to facilitate aggregation of infectious disease mortality data or designed to identify new infectious diseases (5). Medical examiners and coroners are also a source of surveillance data for infectious disease deaths. These investigators certify (i.e. ,enter information about the cause and manner of death on death certificates) approximately 20% of all deaths that occur within the United States (6). Medicolegal death investigation systems are often biased towards the investigation of violent or unnatural deaths. However, sudden natural deaths, unexplained deaths, and deaths of public health importance are also investigated by these agencies (5,7–11).

Natural disease deaths investigated by medical examiners and coroners are often caused by infectious processes (12). Additionally, their investigation frequently includes a complete autopsy. In recent years, medical examiners and coroners have recognized outbreaks of hantavirus pulmonary syndrome and invasive pneumococcal disease, identified cases of human plague, and participated in the investigation of West Nile encephalitis (13–16). In the 2001 outbreak of bioterrorism-related anthrax, all the deaths were investigated by medical examiners (9–11,17). Consequently, medical examiner/coroner databases can be a key source of information about infectious diseases, both in outbreak and nonoutbreak settings.

In general, medical examiners are appointed physician pathologists, usually with special training in performing forensic autopsies and medicolegal death investigations; coroners are usually elected officials, may not be physicians, and rely on other medical personnel for death investigation and autopsy services (18). Medical examiner/coroner systems are varied across the United States, ranging from states with only medical examiners, states with only coroners, and states with mixed medical examiner and coroner systems (18). Overall, medical examiner systems have larger jurisdictions and operate with more resources

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than coroner systems. Medical examiner systems are more likely to have electronic death investigation records.

Medical examiner and coroner databases contain predominantly noninfectious disease cases. Therefore, manually reviewing these databases to identify infectious disease cases is inefficient. Developing an automated system that would identify a subset of cases that are likely infectious, and then manually reviewing these cases to identify infectious disease deaths, could reduce the resources that would be necessary to perform infectious disease surveillance. We hypothesized that a computer-assisted search tool could quickly and efficiently detect infectious disease deaths from a computerized medical examiner database, thereby reducing the number of records that would need to be manually reviewed to perform infectious disease surveillance with medical examiner and coroner data.

Methods

Case Identification

The New Mexico Office of the Medical Investigator (OMI) is a statewide centralized medical examiner agency based at the University of New Mexico School of Medicine. OMI annually performs approximately 90% of the autopsies in New Mexico (5). In 1995, New Mexico had a midyear population of 1,682,417; that year, 12,545 deaths occurred in the state (Figure 1) (19,20). We obtained a database of all deaths (N_{tot} = 4,722) in New Mexico during 1995 that came under the jurisdiction of OMI. From this database, autopsied deaths were identified (n_{aut} = 1,429). A case-patient was defined as a person who died in New Mexico during 1995 who had an infectious disease identified at the time of death and who underwent autopsy by OMI. An expert review panel (Infectious Disease Death Review Team [IDDRT]) reviewed all autopsy records and identified deaths that met the case definition (n_{cd} = 125). On the basis of the findings at autopsy, we further categorized cases as an infectious cause of death (ICOD) (n_{cod} = 99) and infection incidental to death (n_{inc} = 26).

In addition to cause of death (disease or injury that initiates the fatal sequence of events), OMI cases are classified in terms of manner of death (circumstances, i.e., natural, accident, homicide, suicide, or undetermined). Deaths that were considered natural or of undetermined manner comprised 33% (471/1,429) of all OMI autopsies in 1995 and 85% (106/125) of the deaths identified as infectious disease–related by the expert panel. The manner of death was classified as an accident in 39% (561/1,429) of all autopsied persons and in 13% (16/125) of deaths that were identified as infectious disease–related by the expert panel. Homicides and suicides accounted for 28% (395/1,429) of OMI autopsies.

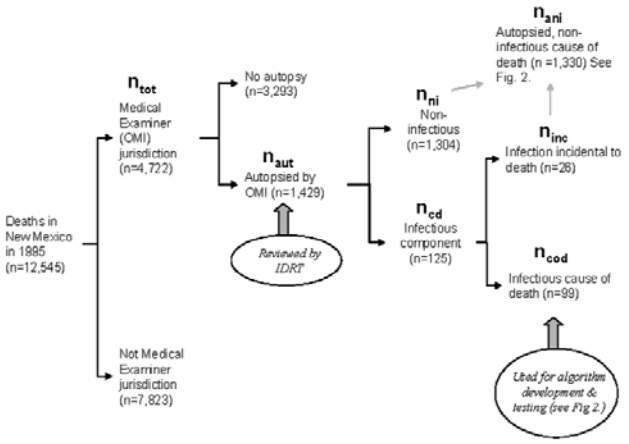

Figure 1. Flow chart for Infectious Disease Death Review Team review and determination of infectious cause of death.

Expert Review Panel

The IDDRT included specialists in infectious diseases, forensic and clinical pathology, epidemiology, and information technology and was in operation in New Mexico, under the auspices of the OMI, from late 1994 to mid-1996 (5,12). One forensic pathologist member of the IDDRT routinely reviewed all OMI autopsy records and identified those deaths that were possibly infectious disease–related for review by the expert panel.

Algorithm Development

We randomly divided ICOD cases into two groups: one group (n_{dg} = 49) was used for algorithm development (i.e., development group); the other group (n_{tg} = 50) was used for algorithm validity testing (i.e., test group). To develop the algorithm, we reviewed the autopsy record for each case in the development group. We developed two separate algorithms based on two separate, but related, datasets (Figure 2). These datasets are described below.

The first algorithm (algorithm 1) was based on data (i.e., truncated dataset) equivalent to information found on the death certificate: demographic variables (e.g., age, sex, and race); cause and manner of death; plus a brief description of the circumstances of death (i.e., a short narrative reported by the death scene investigator). This dataset is referred to as the "truncated" dataset. We used this dataset because we wanted to evaluate the usefulness of death certificate information for this method because this information may be readily available to persons performing surveillance activities. We developed an algorithm based on the development group of cases (n_{dg} = 49). After the algorithm was developed, we added the test group of cases (n_{tg} = 50) to the noninfectious cases (n_{ani} = 1,330) and applied the algorithm to this database (n_{ta} = 1,380) to test the algorithm's ability to detect infectious disease deaths from the truncated database.

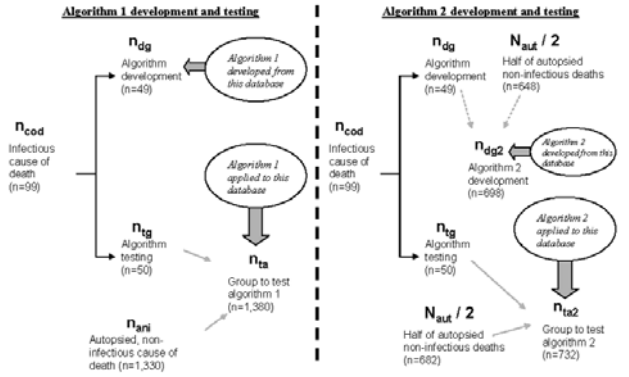

Figure 2. Flow chart for algorithm 1 and 2 development and testing.

The second algorithm (algorithm 2) was developed on the basis of the full text of the pathologists' dictated autopsy records (i.e., full-text set), which included pathologic observations, pathologic diagnoses, and causes of death. This dataset contains much more detailed information than the truncated dataset. It represents data that may be available from medical examiner offices, in addition to the death certificate. Because we found a low predictive value positive (PVP) and low specificity when algorithm 1 was applied to the full-text dataset, we chose to develop algorithm 2 in a different manner. We randomly selected approximately half (n = 649) of the autopsy-categorized deaths of noninfectious causes (n_{ani} = 1,380) to include in the development of algorithm 2 to reduce the number of false-positive cases the algorithm identified (i.e., cases identified by the algorithm as infectious disease–related but not actually infectious disease-related after expert review). Therefore, the total number of deaths from all manners used for algorithm 2 development was 698 (n_{dg2} = 698), and the total number of deaths used for testing algorithm 2 was 732 (n_{ta2} = 732). Algorithm development and text searching were performed by using a commercially available software package (AskSam 3.0 Professional, Seaside Software, Inc., Perry, FL).

For developing both algorithms, we manually searched and indexed potential keywords for identifying deaths caused by infectious diseases. From this process, a list of approximately 20 keywords and rules was compiled (i.e., algorithm; see Appendix). These keywords included entire intact words, words put in a wildcard format (e.g., *bacter**, which would flag the words bacterial and bacteremia), and words in a fuzzy search format (e.g., one letter in the word could be wrong, and the word would still be flagged in the record, thus decreasing misclassification caused by misspelling and data entry errors). Rules included searching for words in specific database fields (e.g., *undetermined* in the cause of death field) and proximity rules (e.g., *immune* within two words of *deficiency*).

Algorithm Implementation and Analysis

The algorithms were applied to the remaining set of records, which included all 1995 OMI autopsied cases except for the development group set of cases (n_{trc} = 1,380 for the truncated dataset; n_{ft} = 732 for the full-text dataset). We applied algorithm 1 to both the truncated and full-text datasets and applied algorithm 2 to the full-text dataset to determine whether an advantage existed in developing an algorithm that used the data from full-text instead of data that could be obtained from death certificates. We determined the sensitivity and PVP of the results by applying the algorithm to this database. Thus, we compared the cases identified from the database by using the algorithm with cases identified by the expert review panel.

Results

Algorithm 1: Truncated Dataset

Algorithm 1 classified 131 (10%) of 1,380 (n_{ta}) autopsied deaths from the truncated dataset as infectious dis-

Table . Sensitivity and predictive value positive (PVP) of algorithm 1 and algorithm 2 applied to the truncated and full-text datasets, compared by manner of death and infection as cause of death

	Truncated dataset				Full-text dataset			
	All causes of death		Natural and undetermined causes of death		All causes of death		Natural and undetermined causes of death	
	Sensitivity	PVP	Sensitivity	PVP	Sensitivity	PVP	Sensitivity	PVP
ICOD and incidental infections[a]								
Algorithm 1	67% (51/76) **1**	39% (51/131) **1**	73% (46/63) **2**	49% (46/94) **2**	92% (70/76) **5**	8% (70/937) **5**	87% (55/63) **6**	17% (55/315) **6**
Algorithm 2	n/a				93% (71/76) **7**	20% (71/356) **7**	94% (58/62) **8**	30% (58/196) **8**
ICOD only[a]								
Algorithm 1	92% (46/50) **3**	49% (46/94) **3**	93% (42/45) **4**	45% (42/94)**4**	88% (44/50) **9**	5% (44/937) **9**	89% (40/45)**10**	13% (40/315)**10**
Algorithm 2	n/a				90% (45/50) **11**	13% (45/356) **11**	91% (41/45) **12**	21% (41/196) **12**

[a]ICOD, infectious cause of death.
[b]Number in bold in lower right corner of each cell corresponds to results of 2 x 2 table shown in Figure 3.

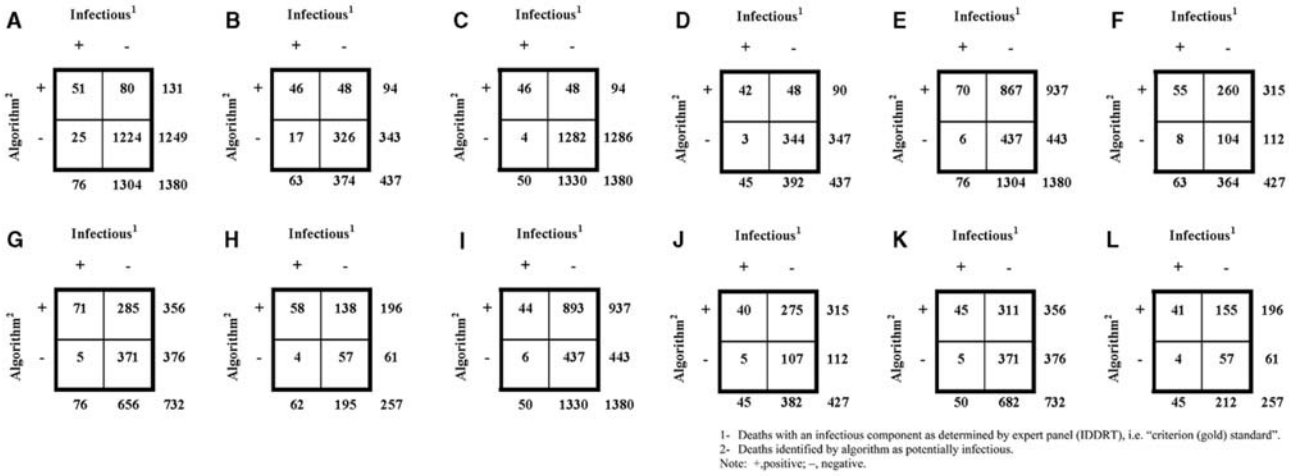

Figure 3. Two-by-two tables used to derive predictive value positive.

ease–related (Table and Figure 3). Overall sensitivity for identifying both ICOD and incidental infectious diseases was 67% (51/76), and the overall PVP was 39% (51/131). Implementation of the algorithm for surveillance for infectious disease deaths would have resulted in a 91% decrease (131 vs. 1,380) in the number of death records to review. The algorithm identified ICOD cases with a sensitivity of 92% (46/50) and a PVP of 49% (46/94).

Algorithm 1 identified deaths classified as natural or undetermined and with an ICOD and incidental infections from the truncated dataset with a sensitivity of 73% (46/63) and a PVP of 49% (46/94). Implementation of the algorithm for surveillance for infectious disease deaths would have resulted in a 78% decrease (94 vs. 437) in the number of death records to review. The algorithm identified deaths with both a natural or undetermined cause and an ICOD with a sensitivity of 93% (42/45) and a PVP of 45% (42/94).

Algorithm 1: Full-Text Dataset

When algorithm 1 was applied to the full-text dataset, it classified 937 (68%) of 1,380 deaths as infectious disease–related. Sensitivity for accurately detecting all deaths classified as natural or undetermined, for detecting deaths caused by all infections, and for detecting those with an ICOD only, ranged from 88% to 92% (Table). However, PVP ranged from 5% (for all causes of deaths, ICOD only) to 17% (for those classified as natural or undetermined, classified as ICOD, or identified as incidental infections). Implementation of the algorithm for surveillance of infectious disease deaths would have resulted in a 32% decrease (937 vs. 1,380) in the number of death records to review for all causes of death, and a 26% decrease (315 vs. 427) for only natural and undetermined causes of death.

Algorithm 2: Full-Text Dataset

Algorithm 2 (developed on the basis of the full-text dataset, which included panel-confirmed infectious disease deaths and 50% of the noninfectious disease-related deaths) was applied to the full-text dataset only. The sensitivity of the algorithm to identify infectious disease–related deaths ranged from 90% (all deaths; ICOD only) to 94% (natural or undetermined; ICOD or incidental infections). PVP ranged from 13% (all deaths; ICOD only) to 30% (natural or undetermined; ICOD or incidental infections). Implementation of the algorithm for surveillance of infectious disease deaths would have reduced by 51% (356 vs. 732) the number of death records to review for deaths from all causes and reduced by 24% the records to review (196 vs. 257) of those deaths categorized as having natural and undetermined causes.

Discussion

A simple computer text search tool (i.e., algorithm) can efficiently detect infectious disease deaths from a medical examiner's database, demonstrating that this technique can be an essential tool in the surveillance for infectious diseases of public health importance. Medical examiners are a critical public health resource for fatal infectious disease surveillance (5,12). Ideal surveillance at medical examiner offices would include active case finding, as has been implemented in a pilot program in New Mexico funded by the Centers for Disease Control and Prevention (21). Because infectious disease surveillance that uses medical examiner data does not occur in a standardized manner, a computer text search tool could be implemented by jurisdictions that otherwise might not have the resources to perform these activities. Implementation of this technique nationally would require large-scale development of electronic databases in medical examiner's offices and subse-

quent incorporation of this surveillance tool into routine activities. However, this method is also applicable to surveillance for fatal infectious diseases and other conditions at all medical facilities that collect text-based clinical data, such as emergency departments, inpatient and outpatient settings, and poison control centers.

The sensitivity of the algorithm varied depending on whether it was applied to dictated autopsy records, including all pathologic diagnoses, or to a truncated dataset containing records equivalent to that found on a death certificate (i.e., basic demographic information and causes of death). The expense of improved sensitivity is that more records must be reviewed because of false-positive results. Algorithm application on the truncated dataset achieved a sensitivity similar to that achieved with the full-text dataset, and with a higher PVP, for deaths in which an infection is a cause of death rather than incidental to the death. In addition, for the full-text dataset, sensitivity and PVP were not substantially compromised by including in the search, infections incidental to the cause of death. Clearly, incidental infections are found among persons who die from homicide, suicide, or accidents. Recognizing incidental infections could be critical for surveillance systems designed to identify chronic infections such as tuberculosis and hepatitis C. Sensitivity was not compromised by including deaths from all causes rather than deaths from natural and undetermined causes. PVP was increased somewhat by restricting the search to deaths classified as natural and deaths classified as undetermined. Still, medical examiner–based infectious disease surveillance could effectively use complete data sets rather than data subsets.

This study was possible because of the findings from the expert review of infectious disease deaths which could be compared with data generated by the algorithm. This panel reviewed records from approximately 90% of all autopsies that occurred in New Mexico in 1 year and, of these deaths, likely ascertained all OMI cases with an infectious disease component. However, the implementation of such a review process using manually retrieved cases might not be feasible in medical examiner jurisdictions with limited resources and a large case volume. A computerized algorithm could allow for surveillance in settings where it otherwise might be impossible. Minimal resources would be required to run the necessary software and review results on a daily basis. Required software is inexpensive, and running the algorithm would require minutes per day. Staff to interpret the results is the main resource that would be required. These results demonstrated a substantial decrease in the number of records that would need to be reviewed with algorithm implementation, compared with those required by manual review alone.

In the future, search algorithms could be used in settings where the records from several medicolegal jurisdictions (e.g., a region consisting of more than one city, county, or state) are combined. As outbreaks of infectious diseases, whether naturally occurring or bioterrorism-related, might span jurisdictional boundaries, computerized records could be compiled from different areas and an algorithm applied to seek patterns or clusters of deaths of one type during a given period. To carry out such cross-regional surveillance, standardized platforms of data collection that would allow for data aggregation are required. Similar algorithms could be used as permanent or temporary surveillance systems designed to detect bioterrorism-related deaths or particular outbreaks. In addition, these algorithms could be modified to evaluate notifiable disease reporting in a jurisdiction. Artificial intelligence techniques could be used to improve algorithm accuracy. Artificial intelligence technology could take algorithm development from rules derived from human testing of specific terms and conditions (as performed in this study), to algorithm development with computer intelligence techniques that develop computer-derived rules. Finally, algorithms could be developed that would identify deaths caused by noninfectious conditions of public health importance.

This study documents a first step in using computer-assisted text search tools to implement and improve infectious disease surveillance with medical examiner data. Research on computerized disease identification through medical information is in the early stages (22). Improvements in the algorithm, in algorithm development techniques (such as improving search terms), and in applying algorithms in more diverse ways could enhance the accuracy and usefulness of this method. Currently, increased national and international attention is focused on infectious disease surveillance. Novel surveillance strategies that provide timely and detailed data will likely become important adjuncts to traditional surveillance for fatal infectious diseases.

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Hospital-reported Pneumococcal Susceptibility to Penicillin[1]

Joshua P. Metlay,* Charles C. Branas,† and Neil O. Fishman†

Geographic variation in drug susceptibility among isolates of *Streptococcus pneumoniae* has influenced national treatment guidelines for community-acquired pneumonia. Whether individual hospital susceptibility data provide reliable and valid information for providers is unclear. We examined the geographic and temporal variability in hospital-reported rates of pneumococcal susceptibility. We surveyed all 52 hospitals that provided acute adult care in the five counties surrounding Philadelphia and collected data on levels of penicillin susceptibility among all pneumococcal blood isolates from 1998 to 2000. In 1998, pneumococcal nonsusceptibility to penicillin varied from 0% to 67% of all blood isolates across the 33 hospitals with ≥10 isolates in that year. Hospital location did not correlate with the level of reported pneumococcal susceptibility ($p = 0.8$). In addition, correlations were not significant in reported pneumococcal susceptibility to penicillin within individual hospitals during the 3 years.

Antimicrobial drug treatment of patients with community-acquired pneumonia is largely empirical because the pathogen is not frequently identified. Even when a bacterial pathogen is identified, antimicrobial drug susceptibility information is frequently delayed for a few days, necessitating empiric treatment decisions. Guidelines for empirical treatment have been provided by several organizations, emphasizing the selection of agents based on the probability of specific pathogens in different clinical settings (1–3). One consistent recommendation of all guidelines for all clinical settings is that antimicrobial therapy cover *Streptococcus pneumoniae* because it is the most common bacterial pathogen and among the most virulent.

A number of factors guide the selection of empirical therapy for patients with community-acquired pneumonia, including drug efficacy, side effect profile, and ease of administration. In addition, the recent emergence of antimicrobial drug resistance among clinical isolates of *S. pneumoniae* has raised considerable concern that a number of drugs historically endorsed by national treatment guidelines may no longer be effective. The recommendations to avoid β-lactams and macrolides in high-risk settings has not been preceded by clinical studies demonstrating that drug resistance in vitro translates into clinical treatment failures (4). Regardless, emerging resistance to penicillins, tetracyclines, and macrolides has prompted newer versions of treatment guidelines to recommend abandoning these therapies in favor of newer therapies, such as fluoroquinolones, in settings where the risk for a drug-resistant pneumococcal infection is high. Given that penicillin-resistant *S. pneumoniae* are increasingly multidrug resistant (5), if the risk for penicillin-resistant *S. pneumoniae* is high, the risk for resistance to macrolides and tetracyclines is correspondingly high.

Specific risk factors for infection with penicillin-resistant *S. pneumoniae* have not been clearly identified. Individual risk factors include prior exposure to antibiotics and exposure to young children in daycare (6), although no specific rules guide the interpretation of these risk factors. In addition, awareness of substantial geographic variation in the proportion of drug-resistant *S. pneumoniae* has lead some groups to recommend consideration of regional susceptibility patterns in the choice of empirical therapy for community-acquired pneumonia (2). Unfortunately, routine isolation of *S. pneumoniae* is highly dependent on the clinical setting. For example, blood and sputum samples are rarely sent from outpatient settings (7); thus, local rates of pneumococcal drug resistance are primarily determined by the phenotypes of bacteria isolated from sicker patients who require hospitalization. Whether results from local hospital microbiology laboratories provide valid information for guiding treatment is unclear.

The specific aims of this study were to compare the rates of penicillin resistance among pneumococcal blood isolates across all acute-care hospitals within a five-county region of eastern Pennsylvania. We examined the relationship between pneumococcal resistance, rates of pneu-

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mococcal isolation, and geographic location to determine the reliability of individual hospital rates for guiding treatment decisions. Our underlying hypothesis was that individual hospital rates of penicillin resistance among clinical isolates of *S. pneumoniae* would be poorly correlated across time and space, emphasizing the inherent dangers in using individual hospital rates to guide empirical treatment decisions in the community.

Methods

Design

This study was a cross-sectional study of penicillin susceptibility among clinical blood isolates reported at all acute-care hospitals in the Delaware Valley Case Control Network (DVCCN). DVCCN is a population-based network of all adult acute-care hospitals in the five Pennsylvania counties around the metropolitan Philadelphia region. These counties (Bucks, Montgomery, Chester, Delaware, and Philadelphia) contain 52 adult acute-care hospitals serving a population of 3.4 million residents.

Data Collection

Data were collected during two periods. During the first data collection, microbiology laboratory supervisors were contacted by telephone at each area hospital during January 1999 to collect data on all pneumococcal blood isolates during the preceding year. Local laboratory personnel reported cumulative numbers of pneumococcal blood isolates and the proportion of isolates that were penicillin nonsusceptible for the 12-month period. When available, laboratories separately reported susceptibility percentiles as susceptible, intermediate susceptible, and resistant; otherwise, the laboratories reported the combined percentile nonsusceptible (intermediate plus resistant). We collected information on unique isolates but did not confirm that these represented unique episodes of patient infections. However, we think hospitals generally reported only one isolate per patient because guidelines recommend that hospitals report cumulative susceptibility data on the basis of single isolates per patient per reporting period (8).

A second data collection was conducted in early 2001. The same hospital laboratories were contacted by mail with follow-up telephone calls requesting the same information on cumulative numbers of pneumococcal blood isolates and the proportion of isolates that were penicillin nonsusceptible for the 12-month period. Data were requested for 1999 and 2000 separately, if available.

Analysis

Annual number of pneumococcal blood isolates and penicillin-susceptibility rates were summarized for each hospital for each year. Standard National Committee for Clinical Laboratory Standards (NCCLS) criteria define three categories of penicillin susceptibility for *S. pneumoniae*: susceptible (MIC <0.1 μg/mL), intermediate susceptible (MIC 0.1–1.0 μg/mL) and resistant (MIC ≥2.0 μg/mL) (9). Penicillin-nonsusceptible isolates are defined as intermediate susceptible and resistant isolates combined. For all analyses, we examined the proportion of isolates that were nonsusceptible. We specifically excluded any hospital site reporting <10 isolates per year as too unreliable to provide point estimates of resistance rates. This level is consistent with NCCLS guidelines recommending hospital laboratories report cumulative susceptibility data only for organisms for which there is a minimum of 10 isolates per reporting period (8). Descriptive statistics were developed for the distributions of susceptibilities and annual pneumococcal isolation rates. Similar to prior studies of hospital-level variation (10,11), we report the proportion of hospitals with >5% deviation from the overall regional mean rate of resistance because lower levels of deviation are unlikely to influence prescribing decisions.

To analyze the reliability of individual hospital rates of pneumococcal resistance, we examined the correlation of rates across each of the 3 years of data collection. We restricted these analyses to those hospitals reporting ≥10 pneumococcal blood isolates in 1998, 1999, and 2000. Correlations between any 2 years were calculated with Spearman's correlation coefficient, testing the assumption that pneumococcal susceptibility levels at individual hospitals should be correlated year to year, regardless of whether overall susceptibility levels for the region were rising, falling, or remaining constant. In addition, we calculated an intra-class correlation coefficient across all 3 years of data at the hospital level by using a general linear model for analysis of variance (12).

Next, we tested the assumption that the proportion of nonsusceptible *S. pneumoniae* at each hospital should demonstrate an underlying geographic clustering. We tested the geographic clustering of pneumococcal resistance rates with two methods. First, we analyzed whether the location of each hospital at the county level was associated with the proportion of nonsusceptible pneumococci by using the nonparametric Kruskal-Wallis test. Second, we conducted Geographic Information System (GIS) mapping to visually judge the spatial distribution of hospital susceptibility rates. Hospitals were geographically assigned longitude and latitude coordinates on the basis of the hospital address by using GIS mapping software (Maptitude, Caliper Corporation, Newton, MA) to display the proportion of nonsusceptible pneumococci at each hospital location on the map. Using data from 1998 only, we graphical-

ly represented the proportion of nonsusceptible pneumococci as a proportional symbol map (bubble plot), where each hospital location is represented by a circle whose radius is proportional to the level of penicillin nonsusceptibility among pneumococcal blood isolates.

We tested the null hypothesis that no spatial autocorrelation of the nonsusceptibility rates occurred with Moran's I statistic, based on a proximity matrix calculated from the city-block distances between the longitude-latitude coordinate points of hospital addresses. Moran's I is a spatial cross-product coefficient that is interpreted similarly to the Pearson correlation coefficient (13,14).

Results

For the survey of 1998 susceptibility results, we received responses from 47 of 52 hospitals in the DVCCN. However, in two instances, the results from multiple hospitals were combined at a single laboratory and could not be separated for reporting purposes. Of the 45 laboratories reporting results, 33 laboratories reported ≥10 pneumococcal blood isolates during 1998. Among the 33 sites, the median number of pneumococcal blood isolates during 1998 was 19 (range 10-92).

Figure 1 displays the frequency distribution of the proportion of isolates with nonsusceptibility to penicillin as reported by each hospital in 1998. The proportion of nonsusceptible isolates ranged from 0% to 67%, with a mean proportion of 21%. Sixty-one percent of hospitals were >5% above or below the mean. If we only included hospitals with ≥20 pneumococcal isolates in 1998, a total of 16 hospitals would be included in the analysis; the proportion of nonsusceptible isolates ranged from 0% to 36%, with a mean proportion of 20%. Fifty-six percent of hospitals were >5% above or below the mean proportion of nonsusceptible isolates.

For the 1999 and 2000 survey results, we received responses from 31 hospital laboratories. Of these, 23 hospital laboratories reported ≥10 pneumococcal isolates in 1 of the 2 study years. The mean proportion of isolates with reduced susceptibility to penicillin was 19% in 1999 and 24% in 2000. Fifty-three percent of sites were >5% above or below the mean in 1999, and 65% of sites were >5% above or below the mean in 2000.

The Table summarizes the relationship between each hospital's annual proportion of nonsusceptible pneumococcal blood isolates during the 3 years of the study. For hospitals reporting ≥10 isolates in 1998, 1999, and 2000 (N = 15), the proportion of nonsusceptible isolates at each hospital was poorly correlated between any 2 years. For 1998 to 1999, the Spearman correlation coefficient was –0.07 (p = 0.82), for 1999 to 2000, the Spearman correlation coefficient was 0.28 (p = 0.32), and for 1998 to 2000, the Spearman correlation coefficient was –0.03 (p = 0.91).

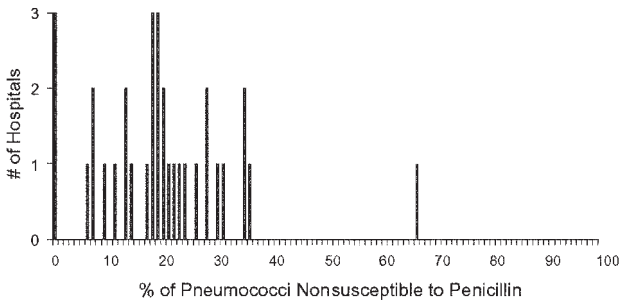

Figure 1. Proportion of penicillin-nonsusceptible pneumococcal bloodstream infections at hospitals in the Delaware Valley. The number of hospitals with each reported level of penicillin nonsusceptibility among all pneumococcal bloodstream isolates at each hospital in 1998 are shown. Penicillin nonsusceptibility was defined as any isolate with a penicillin MIC >0.1 μg/mL. Hospitals with <10 isolates in 1998 were excluded.

Across all 3 years, the overall intraclass correlation coefficient was 0.18.

Location of hospital by county did not correlate with the proportion of pneumococcal blood isolates nonsusceptible to penicillin (p = 0.45, 0.37, 0.08 in 1998, 1999, and 2000, respectively). Figure 2 is a proportional symbol map with each hospital location represented by a circle whose radius corresponds to the proportion of nonsusceptible pneumococcal blood isolates at each hospital in 1998. Overall, Figure 2 shows geographic clustering of the 33 hospitals reporting ≥10 pneumococcal blood isolates in 1998, 14 of which are gathered in Philadelphia, the most heavily populated county in our five-county study area. No geographic clustering in the proportion of nonsusceptible pneumococci isolated at each hospital was evident from our data. This finding is supported by a Moran correlation coefficient of –0.01, demonstrating a lack of statistically significant spatial autocorrelation in the proportion of nonsusceptible hospital pneumococci at hospitals across the five-county region (p = 0.80).

Discussion

Pneumococcal drug resistance has increased at a fast rate. Most resistant isolates now demonstrate reduced susceptibility to multiple antimicrobial drug classes, including β-lactams, macrolides, and tetracyclines. However, substantial geographic variability in the proportion of pneumococci resistant to different antimicrobial drugs at the international and national levels suggests that the impact of resistance on empirical treatment decisions may vary across regions. Indeed, at least one guideline recommends that the selection of empirical therapy for outpatients with community-acquired pneumonia should be influenced by regional antimicrobial drug susceptibility patterns for *S. pneumoniae* (2).

For most physicians, the obvious source of information

Table. Annual variation in proportion of penicillin-nonsusceptible isolates at hospitals in the Delaware Valley

Site	Annual no. blood isolates			% penicillin nonsusceptible		
	1998	1999	2000	1998	1999	2000
A	25	21	26	0	33	27
B	12	15	17	0	13	29
C	29	25	29	24	20	28
D	14	18	12	14	22	8
E	44	39	28	18	23	25
F	27	19	20	19	21	20
G	26	56	35	19	23	17
H	62	15	22	19	0	27
I	10	13	12	20	38	50
J	44	15	32	20	33	16
K	92	45	87	22	4	8
L	35	31	30	29	16	20
M	41	36	34	29	14	38
N	33	32	35	36	13	9
O	19	15	19	37	40	47

on community-acquired pneumonia is the microbiologic susceptibility results from their local hospital laboratory. Hospitals frequently provide this information in the form of antibiograms, specifically designed to aid in antimicrobial drug selection. The validity of this information is largely unknown. This study demonstrates that substantial variability exists in hospital-reported rates of pneumococcal drug resistance over a small geographic region and that the rates at each site are poorly correlated over time. In addition, using a variety of approaches, we were unable to demonstrate substantial geographic clustering of the data at the individual hospital level, suggesting that the individual hospital rates poorly reflect an underlying rate of resistance in the community.

Other studies have demonstrated similar heterogeneity in the proportion of drug-resistant pneumococci reported by hospitals over small geographic areas. The Centers for Disease Control and Prevention reported levels of pneumococcal resistance to penicillin among invasive isolates from all hospitals within the seven regions in the United States that comprise their Active Bacterial Core Surveillance program. The proportion of penicillin-nonsusceptible *S. pneumoniae* ranged from 15.3% to 38.3% across the seven regions. The proportion of hospitals within each region that were >5% below or above the average for the region ranged from 35% to 76%. No hospital characteristics, including proportion of isolates from children or from black patients, predicted deviation from the regional average (11). More recently, 16 hospitals in Brooklyn, New York, participated in boroughwide surveillance for *S. pneumoniae* in 1997 and 1999. Aggregating the hospitals into the western and eastern ends of the borough demonstrated a significant difference in the proportion of penicillin-susceptible isolates, 57% versus 75% (p = 0.046) (15).

Our hypothesis that individual hospital rates of drug-resistant *S. pneumoniae* should demonstrate geographic clustering is based on the assumption that rates of resistance should reflect the underlying distribution of drug resistance in the source community. Prior research has demonstrated that patients with community-acquired pneumonia are hospitalized on average <5 miles from their place of residence (16). Thus, hospitals within the same geographic region should admit patients with similar underlying rates of drug resistance. Moreover, if the pneumococcal resistance rates reported by individual hospitals should be used to guide physician antibiotic prescribing decisions for patients with community-acquired pneumococcal infections, hospitals serving similar communities should report similar rates of resistance.

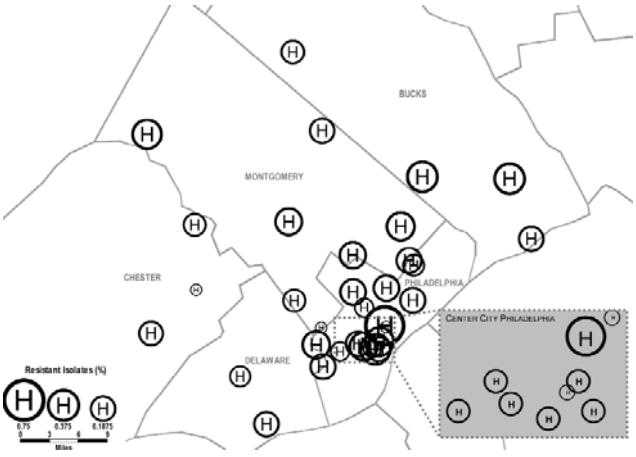

Figure 2. Geographic distribution of penicillin nonsusceptibility among pneumococcal isolates at 33 hospitals in the Delaware Valley in 1998. The figure is a proportional symbol map (bubble plot). Each hospital location is represented by a circle with an H in the center at the corresponding longitude and latitude of the hospital. The radius of the circle is directly proportional to the proportion of penicillin-nonsusceptible pneumococci at each hospital in 1998. The range is 0% to 67%. Hospitals with <10 isolates in 1998 were excluded. An insert magnifies the geographic distribution of hospitals clustered in the center of Philadelphia.

Beyond reflecting true variability in the underlying community levels of drug resistance, hospital-level variation in the proportion of penicillin-susceptible pneumococcal isolates reflects at least four phenomena. First, chance error can create significant variability in hospital-level results particularly since many hospitals have only a small number of invasive pneumococcal isolates per year. We excluded hospitals with <10 isolates in each study year from our analyses to reduce the role of chance in our findings, but the relatively small number of isolates at each site undoubtedly contributed to the significant year-to-year variability and lack of geographic clustering. However, a minimum of 10 isolates per year is the NCCLS-recommended minimum number of isolates for reporting cumulative antimicrobial susceptibility results for any species in any reporting period (8).

Second, variation in testing strategies at individual hospitals may create bias in the proportion of isolates identified as nonsusceptible if the thresholds for sending microbiologic tests vary according to characteristics that are likely to influence the probability of a drug-resistant infection. While this may be an important determinant of variability in drug-susceptibility rates among clinical isolates from respiratory and sinus samples, we believe that it is less likely to explain variability in susceptibility rates among blood isolates since thresholds for sending blood cultures are more standardized across clinical practices, particularly in the management of patients hospitalized with community-acquired pneumonia.

Third, hospitals may differ in their methodologic approach and quality assurance for pneumococcal susceptibility testing. However, a study based on a nationwide College of American Pathologists proficiency survey of hospital laboratories demonstrated that, while the use of specific susceptibility tests varies substantially, few major interpretive errors occurred in assigning the susceptibility to standardized pneumococcal isolates (17). In addition, prior studies have found that the results of susceptibility data generated at local hospital facilities provide a reasonable surrogate for susceptibility data generated at centralized facilities (18).

Finally, geographic proximity of hospitals does not ensure that the patient populations served by those hospitals come from the same communities. Indeed, substantial literature has established important variation in hospital referral patterns that creates diversity in the source of patient populations among geographically proximate hospitals. Future research will need to consider the role of referral patterns in explaining some of the hospital-level variability observed in this and other studies.

One limitation of this study is our focus on a single geographic region in eastern Pennsylvania. Patterns and determinants of hospital-level variation in pneumococcal drug susceptibility may produce different results in other regions. In addition, because we focused on hospitals providing adult care, we cannot generalize our results to pediatric hospitals.

Regardless of whether individual hospitals provide valid information on local levels of pneumococcal susceptibility, variability in these levels is only clinically meaningful if the range of susceptibility crosses some threshold for the empirical choice of specific antimicrobial drugs. Currently in the United States, empirical use of penicillins for the treatment of community-acquired pneumonia is uncommon relative to the use of fluoroquinolones and macrolides (19). However, as resistance to these classes of antimicrobial drugs continues to grow, physicians will need to determine appropriate thresholds for switching to yet newer antimicrobial agents. In these settings, valid information on local rates of pneumococcal drug susceptibility will become increasingly important. Ideally, data should be derived from large samples reflective of the region for which the empirical recommendations apply. Continued research is needed to determine whether susceptibility data provided by local hospital microbiology laboratories can ever serve this purpose.

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Ciprofloxacin-resistant *Salmonella enterica* Typhimurium and Choleraesuis from Pigs to Humans, Taiwan

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We evaluated the disk susceptibility data of 671 nontyphoid *Salmonella* isolates collected from different parts of Taiwan from March 2001 to August 2001 and 1,261 nontyphoid *Salmonella* isolates from the National Taiwan University Hospital from 1996 to 2001. Overall, ciprofloxacin resistance was found in 2.7% (18/671) of all nontyphoid *Salmonella* isolates, in 1.4% (5/347) of *Salmonella enterica* serotype Typhimurium and in 7.5% (8/107) in *S. enterica* serotype Choleraesuis nationwide. MICs of six newer fluoroquinolones were determined for the following isolates: 37 isolates of ciprofloxacin-resistant (human) *S.* Typhimurium (N = 26) and Choleraesuis (N = 11), 10 isolates of ciprofloxacin-susceptible (MIC <1 mg/mL) (human) isolates of these two serotypes, and 15 swine isolates from *S.* Choleraesuis (N = 13) and Typhmurium (N = 2) with reduced susceptibility to ciprofloxacin (MIC >0.12 μg/mL). Sequence analysis of the *gryA*, *gyrB*, *parC*, *parE*, and *acrR* genes, ciprofloxacin accumulation, and genotypes generated by pulsed-field gel electrophoresis with three restriction enzymes (*Spe*I, *Xba*I, and *Bln*I) were performed. All 26 *S.* Typhimurium isolates from humans and pigs belonged to genotype I. For *S.* Choleraesuis isolates, 91% (10/11) of human isolates and 54% (7/13) of swine isolates belonged to genotype B. These two genotypes isolates from humans all exhibited a high-level of resistance to ciprofloxacin (MIC 16–64 mg/mL). They had two-base substitutions in the *gyrA* gene at codons 83 (Ser83Phe) and 87 (Asp87Gly or Asp87Asn) and in the *parC* gene at codon 80 (Ser80Arg, Ser80Ile, or Ser84Lys). Our investigation documented that not only did these two *S. enterica* isolates have a high prevalence of ciprofloxacin resistance nationwide but also that some closely related ciprofloxacin-resistant strains are disseminated from pigs to humans.

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Infections caused by nontyphoid *Salmonella* in humans are increasingly frequent in developed and developing countries (1,2). The increasing rates of resistance to traditional anti-*Salmonella* agents (i.e., ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) and extended-spectrum cephalosporins among these isolates have made treatment of invasive salmonellosis a clinical dilemma (3–6). Of particular concern is the emergence of fluoroquinolone resistance among nontyphoid *Salmonella* and the occurrence of outbreaks caused by some resistant clones, since this class of antimicrobial agents constitutes the drug of choice for treating potentially life-threatening *Salmonella* infections caused by the multidrug-resistant strains in adult persons (7–16). Moreover, cases of treatment failure due to fluoroquinolone resistance in *Salmonella* strains have been reported (17–19).

Researchers have increasingly reported that widespread use of fluoroquinolones in food animals leads to the rapid emergence and dissemination of resistant *Salmonella* infections to humans, particularly in developing countries (4,8,20–23). In Taiwan, Chiu et al. reported that resistance to ciprofloxacin among *S. enterica* Choleraesuis isolates first appeared in 2000 and ≤60% of the isolates recovered from two hospitals in northern Taiwan in the third quarter of 2001 were resistant to ciprofloxacin (14). Molecular investigation clearly demonstrated that the primary sources of these resistant strains were herds of pigs.

To better understand the prevalence of nationwide resistance and the probable dissemination of ciprofloxacin-resistant nontyphoid *Salmonella* isolates, particularly *S.* Typhimurium and Choleraesuis, we determined the mechanisms of quinolone resistance and the genotypes of ciprofloxacin-resistant isolates from humans and pigs, collected in different parts of Taiwan. This study is part of the Surveillance from Multicenter Antimicrobial

Resistance in Taiwan (SMART) programs conducted in 2001.

Materials and Methods

Bacterial Isolates

A total of 671 nontyphoid *Salmonella* isolates were collected for the study. These isolates were recovered from various clinical specimens of patients treated at 11 major hospitals (bed capacities from 800 to 2,000) in different regions of Taiwan. These hospitals included the National Taiwan University Hospital (NTU; hospital A), Taipei; Taipei Veterans General Hospital (hospital B), Taipei; Mackay Memorial Hospital (hospital C), Taipei; Tri-service General Hospital (hospital D), Taipei; Taichung Veterans General Hospital (hospital E), Taichung; China Medical College Hospital (hospital F), Taichung; National Cheng-Kung University Hospital (hospital G), Tainan; Chi-Mei Medical Center (hospital H), Tainan; Kaohsiung Veterans General Hospital (hospital I), Kaohsiung; and Tzu-Chi General Hospital, Hualien (hospital J). Of the 671 isolates tested, 429 (64%) were recovered from stool samples, 141 (21%) from blood, and the rest from various body fluids.

Disk diffusion susceptibility results on these isolates were also provided by the hospitals and evaluated. Organisms were categorized as susceptible or resistant (including intermediate isolates) to the antimicrobial agents tested on the basis of the guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS) (24). Isolates of *Salmonella* serogroups B and C were further identified to the serotype level, according to the Kauffman and White scheme, by using somatic and flagellar antigens (Denka Seiken Co., Ltd., Tokyo, Japan) and also by conventional methods and the Phoenix System (panel type, NMIC/ID4) (Becton Dickson, Sparks, MD) at hospital A (25). Table 1 shows the number of isolates of nontyphoid *Salmonella*, *S.* Typhimurium, and *S.* Choleraesuis, and the ciprofloxacin resistance for the two serotypes of *S. enterica* isolates recovered from the 11 hospitals.

A total of 13 isolates of *S.* Choleraesuis and two isolates of *S.* Typhimurium recovered from pigs raised in southern (N = 11) and central (N = 4) Taiwan from 1997 to 2002 were also collected for study. Of the 15 isolates, 2 each were collected in 1997, 1999, and 2001 and 3 each in 1998, 2000, and 2002. These isolates were collected from various visceral organs (lungs, liver, or spleen) or from stool specimens of pigs that died of septicemia. All the isolates were stored at –70°C in trypticase soy broth (Difco Laboratories, Detroit, MI) supplemented with 15% glycerol before being further tested. *S.* Choleraesuis ATCC 13312 and *S.* Typhimurium ATCC 14028 were used as control strains.

Prevalence of Resistance at Hospital A

To determine the prevalence of antimicrobial resistance among nontyphoid *Salmonella*, we analyzed the disk diffusion susceptibility results of these organisms to ampicillin, cefotaxime/ceftriaxone, chloramphenicol, trimethoprim-sulfamethoxazole, and ciprofloxacin recovered from 1996 to 2001 at hospital A. Isolates of *Salmonella* serogroups B and C resistant to ciprofloxacin (by the disk diffusion method) were further identified to the serotype level by the methods mentioned above.

Antimicrobial Susceptibility Testing

Of the 671 isolates collected from 11 medical centers in 2001, 37 ciprofloxacin-resistant (by the disk diffusion method) *S.* Typhimurium (N = 26) and *S.* Choleraesuis (N = 11) isolates and 10 randomly selected ciprofloxacin-sus-

Table 1. Number of ciprofloxacin-resistant nontyphoid *Salmonella*, *S. enterica* Typhimurium, and *S. enterica* Choleraesuis isolates from patients treated at 11 major teaching hospitals in different regions of Taiwan, March 2001–August 2001

Hospital	% (no. ciprofloxacin-resistant isolates/no. total isolates) of ciprofloxacin-resistant isolates of total nontyphoid *Salmonella*	% (no. ciprofloxacin-resistant isolates/no. total isolates) of ciprofloxacin-resistant isolates, by serotype		
		Typhimurium	Choleraesuis	Others
Northern region	4.3 (7/231)	3.5 (3/85)	7.7 (2/26)	1.7 (2/120)
A	0.0 (0/100)	0.0 (0/40)	0.0 (0/13)	0.0 (0/47)
B	8.2 (4/49)	0.0 (0/19)	28.5 (2/7)	8.7 (2/23)
C	1.7 (1/60)	5.6 (1/18)	0.0 (0/3)	0.0 (0/39)
D	9.1 (2/22)	25.0 (2/8)	0.0 (0/3)	0.0 (0/11)
Central region	3.9 (6/154)	2.4 (2/85)	6.1 (2/33)	5.6 (2/36)
E	5.3 (3/57)	2.8 (1/36)	7.1 (1/7)	7.1 (1/14)
F	7.7 (3/97)	2.0 (1/49)	3.8 (1/26)	4.5 (1/22)
Southern region	2.0 (5/255)	0.0 (0/155)	9.5 (4/42)	1.7 (1/58)
G	0.0 (0/99)	0.0 (0/67)	0.0 (0/11)	0.0 (0/21)
H	2.3 (2/86)	0.0 (0/55)	11.8 (2/17)	0.0 (0/14)
I	4.3 (3/70)	0.0 (0/33)	14.3 (2/14)	4.3 (1/23)
Eastern region				
J	0.0 (0/31)	0.0 (0/22)	0.0 (0/6)	0.0 (0/3)
Total	2.7 (18/671)	1.4 (5/347)	7.5 (8/107)	2.3 (5/217)

ceptible (by the disk diffusion method) isolates of these two serotypes were tested for susceptibility to six fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, trovafloxacin, gatifloxacin, and garenoxacin) to determine their MICs by using the agar dilution method according to the guidelines established by NCCLS (26). The 37 ciprofloxacin-resistant isolates included 13 isolates from the 2001 SMART program (5 *S.* Typhimurium isolates and 8 *S.* Choleraesuis isolates) and 24 recovered from 1996 to 2000 at hospital A (21 *S.* Typhimurium isolates and 3 *S.* Choleraesuis isolates). These ciprofloxacin-resistant isolates were recovered from 29 patients. Six patients (patients 9, 10, 11, 15, 29, and 32) had isolates that were recovered after >7 days from various clinical specimens. The patients' ages ranged from <1 year to 84 years (mean 31 years); those <2 years of age were predominant (47%) among patients with *S.* Typhimurium isolations. None of the patients with *S.* Choleraesuis bacteremia were ≤16 years. Among the 37 human isolates of ciprofloxacin-resistant nontyphoid *Salmonella* isolates, 13 (3 of *S.* Typhimurium and 10 of *S.* Choleraesuis) were recovered from blood specimens of 12 patients with bloodstream infections. The rest of the isolates were recovered from stool or urine specimens.

Dilution susceptibilities to the aforementioned fluoroquinolones were also performed for the 15 isolates from pig herds, according to the NCCLS guidelines (26).

PCR Amplification and DNA Sequencing of *gyrA*, *gyrB*, *parC*, *parE*, and *acrR*

The sequences of the primers for the polyermase chain reaction (PCR) amplification of *gryA*, *gyrB*, *parC*, *parE*, and *acrR* have been previously described (27–30). The preparation of the template DNA and the determination of sequences of each gene followed the procedures described previously (27–29). The sequences of the quinolone resistance-determining regions (QRDRs) were determined to be between amino acids 54 and 171 of *gyrA*, 397 and 520 of *gyrB*, 12 and 130 of *parC*, and 421 and 524 of *parE*.

Ciprofloxacin Accumulation

The accumulation of ciprofloxacin, with or without 100 mM carbonyl cyanide m-chlorophenylhydrazone, was determined for two ciprofloxacin-resistant strains and one ciprofloxacin-susceptible *S.* Typhimurium (ciprofloxacin MIC = 0.06 μg/mL) as described previously (28,29). These experiments were performed twice to ensure reproducibility.

Molecular Typing

Genotyping of the human ciprofloxacin-resistant *S.* Typhimurium (N = 26) and Choleraesuis (N = 11) isolates, the 10 human ciprofloxacin-susceptible isolates of the two serotypes, and the 15 isolates from pigs was determined by the pulsotypes generated by pulsed-field gel electrophoresis (PFGE). The DNA extraction and purification were also carried out as described previously (31,32). The DNA was digested by the restriction enzymes *Spe*I, *Xba*I, and *Bln*I (9,16,23,32), and the restriction fragments were separated in a CHEF-DRIII unit (Bio-Rad, Hercules, CA). Interpretation of the PFGE profiles followed the description by Tenover et al. (33). Isolates belonging to the similar pulsotypes (within six band differences) by each of the three restriction enzymes were defined as the same genotypes (closely related clusters). Isolates with identical pulsosubtypes (no band differences) by the three restriction enzymes were defined as the same genosubtypes (clones).

Results

Nationwide Resistance in 2001

The rates of ciprofloxacin resistance among isolates of nontyphoid *Salmonella*, *S.* Typhimurium, and *S.* Choleraesuis from the 11 hospitals, stratified by region of Taiwan, is shown in Table 1. Overall, ciprofloxacin resistance was found in 2.7% (18/671) of all nontyphoid *Salmonella* isolates from humans, 1.4% in *S.* Typhimurium and 7.5% in *S.* Choleraesuis nationwide (Table 1). Among *S.* Choleraesuis isolates, the highest rate of ciprofloxacin resistance was found in hospital B (28.5%) and southern region of Taiwan (9.5%). Among *S.* Typhimurium isolates, the highest rate of ciprofloxacin resistance was found in hospital D (25.0%) and in the northern region of Taiwan (3.5%). Nontyphoid *Salmonell*a isolates recovered from patients in eastern region of Taiwan were all susceptible to ciprofloxacin. Rates of resistance to ampicillin and chloramphenicol were higher in eastern Taiwan than those from other regions of Taiwan. Resistance to cefotaxime (three hospitals tested ceftriaxone instead of cefotaxime) among all nontyphoid *Salmonella* isolates was low (<1%). However, 6% and 4% of *S.* Choleraesuis isolates recovered from central and southern Taiwan, respectively, were resistant to cefotaxime (ceftriaxone).

Prevalence of Ciprofloxacin Resistance at Hospital A

The annual number of nontyphoid *Salmonella* isolates (*Salmonella* group B and *Salmonella* group C) ranged from 294 in 1996 (182 and 46, respectively) to 90 in 2001 (76 and 8, respectively). Overall, the rate of ciprofloxacin resistance among nontyphoid *Salmonella* isolates was 2.1%. For *Salmonella* group B isolates, the rates of ciprofloxacin resistance were high (6% to 9%) during 1996 and 1997, reached a trough in 1999 (3%), and increased gradually in the following 2 years (4% in 2000 to 5% in 2001). Annual rates of resistance to ciprofloxacin among *Salmonella* group C isolates fluctuated during the same 6-

year period (data not shown). In 1996, 1998, and 2001, none of the isolates were resistant to ciprofloxacin, and the highest rate of ciprofloxacin resistance was found in 2000 (13%).

The annual rates of resistance to ampicillin, cefotaxime, chloramphenicol, and trimethoprim-sulfamethoxazole among all nontyphoid *Salmonella* isolates, *Salmonella* group B, and *Salmonella* group C at hospital A from 1996 to 2001 were evaluated. Overall, the prevalence of resistance to cefotaxime among these isolates was low (0% to 4%). Rates of resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole among all nontyphoid *Salmonella* isolates declined gradually from 1996 (64%, 64%, and 42%, respectively) to 2000 (47%, 52%, and 34%, respectively). In 2001, however, rates of resistance to ampicillin (73%) and chloramphenicol (76%) increased but that of trimethoprim-sulfamethoxazole (28%) continued to decrease. A similar scenario was found among *Salmonella* group B isolates. For *Salmonella* group C isolates, rates of resistance to these agents also fluctuated during the study period.

Antimicrobial Susceptibilities of Human Ciprofloxacin-resistant Isolates

All of the human ciprofloxacin-resistant isolates were highly resistant to ampicillin (MIC ≥128 μg/mL), chloramphenicol (MIC ≥128 μg/mL), and trimethoprim-sulfamethoxazole (≥128 μg/mL) but susceptible to cefotaxime (MIC 0.06–8 μg/mL). These ciprofloxacin-resistant isolates from humans all exhibited high-levels of resistance to ciprofloxacin (MIC 8 to 64 μg/mL), levofloxacin (MIC 32–64 μg/mL), moxifloxacin (MIC 32–28 μg/mL), gatifloxacin (MIC 16–32 μg/mL), garenoxacin (MIC 16–64 μg/mL), and trovafloxacin (MIC 8–64 μg/mL).

Fluoroquinolone Susceptibilities among Swine *S.* Choleraesuis Isolates

All 15 isolates of *S.* Typhimurium and Choleraesuis from pigs had reduced susceptibility to ciprofloxacin (MIC ≥0.125 μg/mL). Eight of the 15 isolates (53%) were susceptible or intermediate to ciprofloxacin according to the NCCLS breakpoint recommendation (MIC ≤2 μg/mL). Seven (47%) isolates had high ciprofloxacin MICs (MIC ≥64 μg/mL); these seven isolates were also highly resistant to five other newer fluoroquinolones: levofloxacin (MIC 32–64 μg/mL), moxifloxacin (MIC 32–128 μg/mL), trovafloxacin (MIC 64 μg/mL), gatifloxacin (MIC 16–32 μg/mL), and garenoxacin (MIC 32–64 μg/mL).

Nucleotide Sequence Analysis

Of human ciprofloxacin-resistant *S.* Typhimurium isolates, all were associated with two-base substitutions in the QRDR of *gyrA* at codon 83 (Ser83Phe) (TCC→TTC) and 87 (Asp87Gly) (GAC→GGC), and either Ser80Arg or Glu84Lys in the QRDR of the *parC* gene (Table 2). One base substitution in the QRDR of *gyrA* (Ser83Tyr or Ser83Phe) was found in ciprofloxacin-susceptible isolates. One isolate had a mutation in the QRDR of the *gyrB* gene, but none had mutations in the QRDR of the *parE* gene. None of the *S.* Typhimurium isolates, including ciprofloxacin-susceptible or -resistant isolates, had mutations in the acrR genes.

Of human ciprofloxacin-resistant *S.* Choleraesuis, all were associated with two-base substitutions in the QRDR of the *gyrA* gene at codon 83 (Ser83Phe) and 87 (Asp87Asn), Ser80Ile in the QRDR of the *parC* gene, and Gln78Stp in the QRDR of the *acrR* gene (Table 3). None of these isolates had mutations in the QRDR of the *gyrB* or *parE* genes. One base substitution in the QRDR of the

Table 2. Characteristics of *Salmonella enterica* serotype Typhimurium isolates from humans[a] and pigs,[b] Taiwan

		Mutation at								
		gyrA gene			*parC* gene			*acrR* gene		
Ciprofloxacin susceptibility (N)	MIC (μg/mL)	Ser83Phe Asp87Gly (N)	Ser83Phe Asp87Asn (N)	Ser83Phe (N)	Ser83Tyr (N)	Ser80Arg (N)	Glu84Lys (N)	Gln78Stp (N)	Arg107Cys (N)	Genotype: genosubtype
Humans										
Resistant (26)	16-64	24	2	0	0	24	2	0	0	I (26), Ia (4), Ic (5), Id (6), Ie (1), If (1), Ig (2), Ih (1), Ii (1) Ij (1), Ik (2), Il (1)
Susceptible (5)	0.03-0.25	0	0	1	2	0	0	0	0	IIa (1), IIb (1), III (1), IV (1), V (1)
Pigs										
Resistant (2)	128	2	0	0	0	2	0	0	0	I (2): Ic (1), Im (1)

[a]N = 31.
[b]N = 2.

gyrA (Asp87Asn) was found in the ciprofloxacin-susceptible isolates.

Of pig herd ciprofloxacin-resistant *S.* Typhimurium isolates (N = 2), both had a mutation in the QRDR of the *gyrA* and *parC* genes, respectively (Table 2). None of these isolates had mutations in the QRDR of the *gyrB*, *parE*, or *arcR* genes. Among pig herd ciprofloxacin-resistant *S.* Choleraesuis isolates, all had two mutations in the QRDR of *gyrA* (Ser83Phe plus Asp87Asn or Asp87Gly) and one mutation in *parC* (Ser80Ile) and *arcR* (Gln78Stp) (Table 3).

PFGE Analysis and Genotypes

All of the ciprofloxacin-resistant *S.* Typhimurium isolates from humans had the same pulsotype (pulsotype S) when the *Spe*I restriction enzyme was used. Figures 1 and 2 illustrate the pulsotypes and pulsosubtypes of *S.* Typhimurium (Figures 1A and 1B) and *S.* Choleraesuis (Figures 2A, 2B, and 2C) isolates by *Xba*I and *Bln*I. Using *Xba*I and *Bln*I, we observed six and eight pulsosubtypes, respectively, for *S.* Typhimurium isolates. Among *S.* Choleraesuis isolates, one pulsotype (x) and one pulsosubtype (x-1) were observed when the *Xba*I restriction enzyme was used, and two pulsotypes (a and b) with six pulsosubtypes (b-1 to b-6) were observed when the *Bln*I restriction enzyme was used. Using the three restriction enzymes, we found that all ciprofloxacin-susceptible isolates of *S.* Typhimurium and Choleraesuis had different genosubtypes (clones).

Among human ciprofloxacin-resistant *S.* Typhimurium isolates, all were closely related (genotype I) and belonged to 11 genosubtypes (genosubtypes Ia to Ik). Among the 11 genosubtypes, Ia (4 isolates), Ic (5 isolates), and Id (6 isolates) predominated. The five ciprofloxacin-susceptible isolates belonged to four genotypes (II–V) (Table 2). None of the genotypes among the *S.* Typhimurium isolates studied were identical to those of DT104.

Of human *S.* Choleraesuis isolates, 91% (10 of the 11 isolates) belonged to genotype B, which was different from those of the five ciprofloxacin-susceptible isolates from humans (genotypes C to G). None of the six genosubtypes (B1 to B6) of the genotype B isolates was predominant. Two isolates collected within 7 days of one another from patient 4 had identical genosubtypes (B2), but those from patient 7 had differing genosubtypes (B4 and B5) (Table 3).

Seven (54%) of the 13 swine *S.* Choleraesuis isolates belonged to genotype B (Table 3). Among the six genosubtypes of genotype B, two genosubtypes (B2 and B5) were also found in human isolates. Two swine isolates that showed descreased susceptibility to ciprofloxacin (MICs, 0.5 μg/mL and 2 μg/mL, respectively) also belonged to genotype B (genosubtypes B7 and B10, respectively).

Evidence for Active Efflux

Ciprofloxacin uptake appeared to be remarkably low in the two ciprofloxacin-resistant genotypes (genosubtypes Ia and B1) (Figure 3). A rapid increase in cell-associated ciprofloxacin uptake among isolates belonging to the genosubtypes was evident after addition of carbonyl cyanide m-chlorophenylhydrazone (CCCP), a proton motive force uncoupler.

Discussion

This report describes the rates of antimicrobial resistance among nontyphoid *Salmonella* isolates in a universi-

Table 3. Characteristics of *Salmonella enterica* serotype Choleraesuis isolates from humans[a] and pigs[b] in Taiwan

		Mutation at								
		gyrA gene					*parC* gene	*acrR* gene		
Ciprofloxacin susceptibility (N)	MIC (μg/mL)	Ser83Phe Asp87Asn (N)	Ser83Phe (N)	Asp87Asn (N)	Ser83Tyr (N)	Asp87Gyr (N)	Ser80Ile (N)	Gln78Stp (N)	Arg107Cys (N)	Genotype: genosubtype (N)
Humans										
Resistant (11)	16–64	11	0	0	0	0	0	0	0	A (1); B (10): B1 (2), B2 (2), B3 (2), B4 (1), B5 (2), B6 (1)
Susceptible (5)	0.03–0.25	0	0	1	0	0	0	0	0	C (1), D (1), E (1), F (1), G (1)
Pigs										
Resistant (5)	64	5	5	0	0	0	5	5	0	0 B (5): B2 (1), B5 (1), B8 (1), B9 (2)
Susceptible or intermediate (8)	0.5–2	0	1	1	2	4	0	0	0	B (2): B7 (1), B10 (1); H (1), I (1), J (3), K (1)

[a]N=16.

[b]N=13.

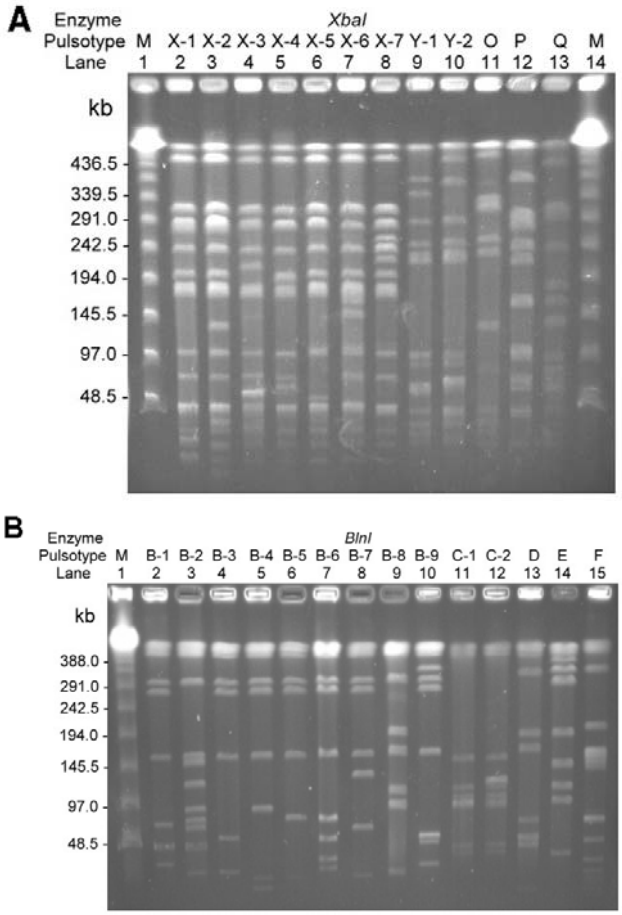

Figure 1. Pulsotypes and pulsosubtypes of *S. enterica* serotype Typhimurium from humans and pigs obtained by pulsed-field gel electrophoresis (PFGE) after digestion with *Xba*I (A) and *Bln*I. (B). Lanes M, molecular size marker. See Tables 2 and 3 for the designation of isolates for each indicated pulsotype or pulsosubtype.

ty hospital during a 6-year period. Furthermore, it characterizes the nationwide dissemination of ciprofloxacin-resistant strains of *S.* Typhimurium and Choleraesuis. Our observations in Taiwan suggest five important facets: First, ciprofloxacin resistance among our nontyphoid *Salmonella* from humans was high (2.7%), particularly among human *S.* Choleraesuis isolates. Furthermore, a previous study found a remarkably high incidence (57%, 12 of 21 isolates) of *S.* Choleraesuis at two major teaching hospitals in northern Taiwan in the second to third quarters of 2001 (14). This observation contrasts greatly with our findings (7.7%, 2 of 25 isolates, from four hospitals in northern Taiwan, or 7.5% nationwide from March 2001 to August 2001) (14).

Second, nearly all ciprofloxacin-resistant *S.* Choleraesuis isolates from humans and pigs collected in 1999–2002 were closely related to one another (genotype B) and differed from those found in pigs in 1997–1998 (these isolates had highly diverse genotypes). These find-

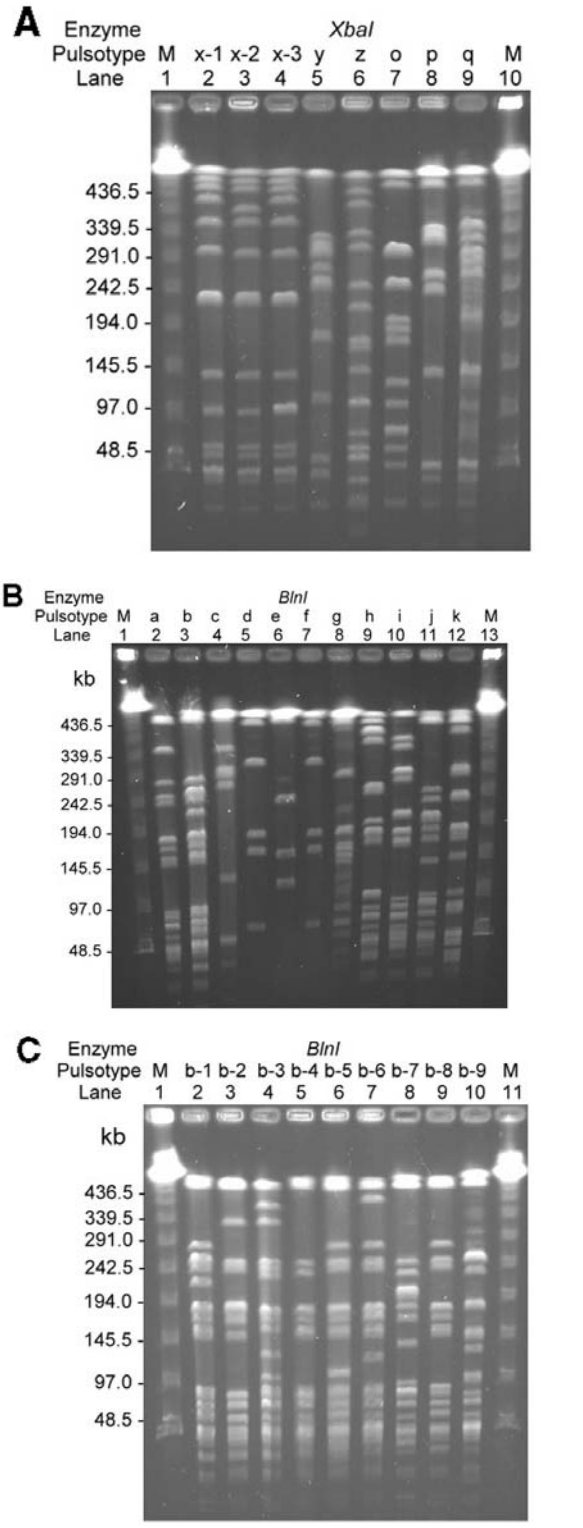

Figure 2. Pulsotypes and pulsosubtypes of *Salmonella enterica* serotype Choleraesuis from humans and pigs obtained by pulsed-field gel electrophoresis (PFGE) after digestion with *Xba*I (A) and *Bln*I (B and C). Lanes M, molecular size marker. See Tables 2 and 3 for the designation of isolates for each indicated pulsotype or pulsosubtype.

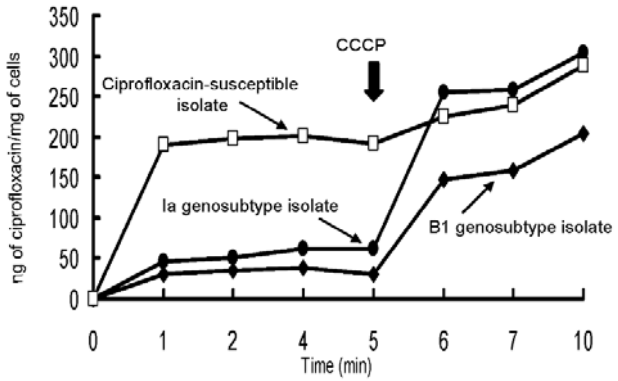

Figure 3. Accumulation of ciprofloxacin by the two ciprofloxacin-resistant isolates of genosubtype Ia of *Salmonella* Typhimurium and genosubtype B1 of *S.* Choleraesuis and one clinical isolate of *S.* Typhimurium (ciprofloxacin MIC = 0.06 μg/mL). Carbonyl cyanide m-chlorophenylhydrazone (CCCP) (100 μM) was added at the time indicated by the arrow.

ings suggest that nationwide dissemination of *S.* Choleraesuis isolates from pigs to humans occurred from 1999 to 2002. Two isolates (AC-6 and AC-10) of *S.* Choleraesuis from pigs had reduced susceptibility to ciprofloxacin (MICs, 0.5 μg/mL and 2 μg/mL, respectively). They had an identical genotype (genotype B) to that of most of the epidemic strains found in humans and pigs. These strains had high-level ciprofloxacin resistance (MIC 16 to 64 μg/mL). This indicates that the swine isolates with reduced susceptibility to ciprofloxacin might be an ancestor (a unique clone line) of the isolates that are highly resistant to ciprofloxacin and which have spread among herbs and humans nationwide (23,27).

Third, the *S.* Typhimurium strains (genotype I) with high-level fluoroquinolone resistance have been widely disseminated in humans in Taiwan since 1996. Strains belonging to genotype I and the other genotypes found in this study were domestically acquired and were not related to the clones of DT104, which were already disseminated throughout Europe and the United States (15,23). In 1998, one isolate exhibiting genotype I (genosubtype Ic) was isolated from a pig from southern Taiwan. Further studies on *S.* Typhimurium isolates from animals should be conducted to identify the primary source of the epidemic genotype strains.

Fourth, an increasing prevalence of resistance to ampicillin and chloramphenicol over time was observed in human *S.* Typhimurium isolates at hospital A. The spread of third-generation cephalosporin-resistant isolates harboring plasmid-mediated CMY-2 like cephalosporinase among *S.* Typhimurium isolates has been previously reported in Taiwan (6). Although all of the highly ciprofloxacin-resistant isolates in our study were susceptible to cefotaxime, according to NCCLS guidelines (24), five isolates from four patients with high cefotaxime MICs (MIC 4–8 μg/mL) is noteworthy. The emergence of decreased susceptibility to cefotaxime, along with the pre-existing ciprofloxacin resistance among nontyphoid *Salmonella* isolates, particularly those causing bloodstream infection, makes antimicrobial therapy more complicated.

Finally, rates of resistance varied geographically; higher rates of resistance to ampicillin and chloramphenicol were found in the eastern region of Taiwan. However, none of the nontyphoid *Salmonella* isolates collected in the eastern region of Taiwan was resistant to ciprofloxacin.

S. Typhimurium and *S.* Choleraesuis isolates with high levels of resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, cipro-floxacin, and other newer fluoroquinolones were rarely previously reported (2,19,28,30,34). In most gram-negative bacteria, including *Salmonella*, a high-level of fluoroquinolone resistance is related to the presence of multiple mutations in the QRDRs of the genes, particularly in the *gyrA* and *parC* genes (8,13,14,19,27,28,35).

Additional resistance mechanisms, such as decreased cell envelope permeability (loss of outer membrane porins or alterations of the lipopolysaccharide), decreased cellular accumulation of quinolones involving the major multidrug active efflux pump (AcrAB), or the presence of integrons, can also be responsible for fluoroquinolones resistance and resistance to a wide range of antimicrobial agents (31,35–39). Mutations in the *acrR* (regulator/repressor) gene are partly responsible for fluoroquinolone resistance in *Escherichia coli* (29). In our study, the two major genotypes (genotypes I and B) of ciprofloxacin-resistant isolates had both mutations in the *gyrA* (at least two mutations) and *parC* (at least one mutation) genes. The addition of CCCP, resulted in an increase in cell-associated ciprofloxacin uptake. This indicated that an active efflux contributed to fluoroquinolone resistance (28–31). In our study, *acrR* mutations were found in ciprofloxacin-resistant *S.* Choleraesuis, but not in Typhimurium, isolates. This finding is consistent with that of previous reports (30). Further studies are warranted to add clarification to the complexity of the mechanisms of high-level resistance among *S.* Typhimurium and Choleraesuis isolates.

PFGE analysis using restriction enzyme *Xba*I is a well-established method for epidemiologic typing of the *Salmonella* species (9,16,23,32). However, PFGE patterns by *Xba*I for most ciprofloxacin-resistant isolates investigated, including *S.* Choleraesuis isolates from human and animal origins, were indistinguishable. This scenario was also found in PFGE patterns for human *S.* Typhimurium isolates. When *Bln*I was added, the discriminatory power of pulsotyping improved among these ciprofloxacin-resistant isolates. Genotyping by using pulsotypes generated by

*Xba*I and *Bln*I clearly demonstrated that several ciprofloxacin-resistant clones (particularly, genosubtypes Ia, Ic, and Id of *S.* Typhimurium) had disseminated to humans in Taiwan. Furthermore, some genosubtypes of ciprofloxacin-resistant *S.* Choleraesuis were found only in humans, and some were found only in pigs; however, two clones (genosubtypes B2 and B5) were found in both humans and pigs.

Research studies have provided evidence that antimicrobial agents used in agriculture and closely related agents used in human medicine have been exerting selective pressure on their target bacteria, particularly *Salmonella*, *Campylobacter*, and *Escherichia coli* (1,20,40). In Taiwan, quinolones (e.g., enrofloxacin) have been used in animals and humans (from nalidixic acid to the latest fluoroquinolone moxifloxacin) for >30 years. A governmental survey among farmers and feed mill operators in 1999 indicated that 40% of farmers and 50% of feed mill operators used quinolone agents (particularly enrofloxacin) on their flocks or herds of pigs for growth promotion or therapeutic purposes (41). Previous investigations demonstrated that >90% of *Campylobacter* species and 6% of *E. coli* from chickens were resistant to ciprofloxacin (41,42). When the selective pressure of quinolones persisted, isolates, or some clones with reduced susceptibility (a single *gyrA* mutation) to quinolones, might develop full resistance (two *gyrA* mutations or multiple mutations in the QRDRs of other genes) in animals or humans and could probably jump from animals to humans (14,27). Our observations and findings from Chiu et al. indicate that outbreak-associated human *Salmonella* strains with high-level ciprofloxacin resistance might have emerged several years ago, similar to strains with antibiotypes of reduced susceptibility, but with identical genotypes, in humans or animals (14).

In conclusion, our investigation documented that *S.* Typhimurium and *S.* Choloraesuis isolates, which are highly fluoroquinolone-resistant and multidrug-resistant, have become widespread pathogens in Taiwan. The recent occurrence of ciprofloxacin resistance among *Salmonella* in animals, and its nationwide spread, is of particular concern. The remaining therapeutic options available to veterinarians and physicians for treatment of extraintestinal salmonellosis and other invasive infections include only third-generation cephalosporins. However, ciprofloxacin-resistant isolates, with reduced susceptibility to cefotaxime, have already emerged in Taiwan. Restricted use of quinolones in animal husbandry and active surveillance of quinolone resistance among *Salmonella* are crucial mitigation efforts to reduce selection and clonal spread of quinolone-resistant *Salmonella*.

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Escherichia coli Producing CTX-M-2 β-Lactamase in Cattle, Japan

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From November 2000 to June 2001, *Escherichia coli* strains producing CTX-M-2 β-lactamase were isolated from 6 (1.5%) of 396 cattle fecal samples and 2 (0.7%) of 270 surface swabs of cattle carcasses in Japan. The $bla_{CTX\text{-}M\text{-}2}$ gene responsible for CTX-M-2 production was encoded on transferable plasmids, and the gene was transferred to *E. coli* CSH2 with a very high frequency (2×10^{-4} to 6×10^{-1} per donor cells) by conjugation. Random amplified polymorphic DNA analysis of nine isolates showed at least five different patterns. These findings suggest that CTX-M-2 producers might have originated from cattle through the use of cephalosporins such as ceftiofur and that cattle could be a reservoir of CTX-M-2–producing *E. coli*. Continuous and strategic surveillance of antimicrobial-resistant bacteria in livestock is essential to suppress further dissemination of these bacteria into society at large.

Shortly after a variety of expanded-spectrum cephalosporins were introduced in the 1980s, bacterial strains producing extended-spectrum β-lactamases (ESBLs), such as TEM- or SHV-derived ESBLs, emerged in Europe (1), and since then, their variants have been proliferating around the world (2,3). More recently, CTX-M-type β-lactamases, a small but growing family of broad-spectrum class A β-lactamases, were initially discovered as MEN-1 (EMBL accession no. X92506) and also later found as Toho-1 in Japan in 1993 (4). Since the early 1990s, these β-lactamases have been identified in various bacterial species belonging to the family *Enterobacteriaceae* (5). Several questions regarding the origin and mode of proliferation of the CTX-M-type β-lactamases are unclear. Unlike TEM- and SHV-derived ESBL producers, the CTX-M-type β-lactamase producers have been incidentally and sporadically detected as single clinical isolates from patients with urinary tract infections and the like (6,7) over an extensive geographic area, including Europe, South America, and the Middle and Far East. The cause of this global distribution is not well known (5,8). Moreover, derivation of the CTX-M-type enzymes or the prototype of this enzyme with its narrow spectrum remains unknown (9–12).

In Japan, clinical isolation of the TEM- or SHV-derived ESBL producers is still rare (13,14); *Escherichia coli* strains producing CTX-M-2 β-lactamase, one of the CTX-M family, have been predominantly isolated to date (13). On the other hand, clinical isolates producing IMP-1 type metallo-β-lactamase, which show resistance to carbapenems and cephamycins as well as various expanded-spectrum cephalosporins, have been identified in Japan (15), and the proliferation of these strains has become a clinical concern (16). As for the disproportionately low isolation rate of the TEM- or SHV-derived ESBL producers in Japan, carbapenems and cephamycins, whose use has been restricted in many Western countries, have been preferentially used as first-line drugs in Japan (13,15). This practice makes it more plausible that TEM- or SHV-derived ESBL producers would be rarely isolated and that metallo-β-lactamases would be isolated often in Japan. However, it is not easy to explain the predominant isolation of *E. coli*-producing CTX-M-2 β-lactamase that is usually susceptible to carbapenems and cephamycins like TEM- or SHV-derived ESBL producers. In addition, since CTX-M-2 β-lactamase producers tend to be isolated from patients who have neither received antimicrobial drugs nor been hospitalized, the existence of healthy carriers of CTX-M-2 producers was suspected (17,18). Still, one cannot assume healthy carriers exist on the basis of the low isolation rate of strains producing broad-spectrum class A β-lactamases in Japan.

One hypothesis to address these issues is that CTX-M-2 might have emerged elsewhere than in humans and that the enzyme might have originated in livestock. Recently, a global threat developed because certain antimicrobial-resistant bacteria, such as vancomycin-resistant enterococci (19), *Salmonella enterica* Typhimurium DT104 (20) and fluoroquinolone-resistant *Campylobacter jejuni* and *C. coli* (21) emerged in food animals possibly through the use of antimicrobial drugs for growth promotion or disease treatment. However, few reports have been published

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about strains in animals producing ESBLs or CMY-type cephamycinases, which confer resistance to expanded-spectrum cephalosporins (22,23), and no CTX-M-type β-lactamase producer has been isolated from animals. Therefore, to examine this hypothesis, we conducted a study to isolate any strains producing extended-spectrum class A β-lactamases from cattle at Japanese slaughterhouses.

Materials and Methods

Sampling and Bacterial Culture

From November 2000 to June 2001, a total of 396 fecal samples of cattle and surface swabs of 270 cattle carcasses were collected at two slaughterhouses in Gifu Prefecture, Japan. ESBL screening agar plates (17), which were prepared using BTB Lactose agar (Nissui Pharmaceutical Co., Tokyo, Ltd., Japan) containing 2 μg/mL of cefotaxime (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) and 8 μg/mL of vancomycin (Shionogi & Co., Ltd., Osaka, Japan), were used to isolate gram-negative enterobacteria that produce broad-spectrum class A β-lactamases. One swab was used to sample each cattle feces, and two swabs were used for each cattle carcass. For sampling of the cattle feces, a swab was inserted into the core of a lump of feces. When several cattle were kept in the same enclosure, a direct rectal swab was sampled from each of the cattle. Shoulder and rump were swabbed separately in each cattle carcass; the size of the swabbed area was approximately 20 x 20-square centimeters for each swab. Swabs of feces were plated directly on the screening agar. Swabs of carcasses were suspended in a 10-mL Trypticase soy broth (Nissui Pharmaceutical Co.) containing 2 μg/mL of cefotaxime and 8 μg/mL of vancomycin, and then plated on the screening agar. The remaining Trypticase soy broth with bacteria was further incubated overnight. A swab of bacterial culture was then plated on the screening agar. Colonies suspected to be enterobacteria were isolated and identified by using the API 20E system (bioMérieux, Marcy l'Etoile, France). *E. coli* isolates were serotyped with a slide agglutination kit (Denka Seiken Co., Ltd., Tokyo, Japan) and were screened for genes of virulence factors, including Shiga toxins and *E. coli* attaching and effacing factor by polymerase chain reaction (PCR) (24).

Detection of β-Lactamases

The acidmetric β-lactamase test was performed by using P/Case TEST (Showa Yakuhin Kako Co., Ltd., Tokyo, Japan) to detect β-lactamase production in the isolates. According to the manufacturer's instructions, the colonies were spread on two indicator disks, containing benzylpenicillin and cephaloridine with clavulanic acid, respectively. When the strain produces class A β-lactamases, including TEM- or SHV-derived ESBLs, or CTX-M-type enzymes, the color of a disk containing benzylpenicillin turns yellow. The other disk, containing cephaloridine with clavulanic acid, remains purple because hydrolysis of cephaloridine by the class A β-lactamases is blocked in the presence of clavulanic acid. If the strain produces class C or class B β-lactamases, both disks turn yellow because these enzymes are no longer blocked by clavulanic acid. The isolates suggested to produce extended-spectrum class A β-lactamase were further investigated to determine whether they produced ESBLs by the double-disk diffusion test (25), using two Kirby-Bauer disks (Eiken Chemical Co., Ltd., Tokyo, Japan). A swab of bacterial culture (approximately 10^6 CFU/mL) to be tested was spread on a Mueller-Hinton agar plate (Eiken Chemical Co.), and one disk containing cefotaxime, ceftazidime, ceftriaxone, cefpodoxime, aztreonam, or cefepime was put on the plate. The other disk, containing amoxicillin+clavulanic acid, was also placed alongside the first disk (center-to-center distance of approximately 3 cm), and the agar plate was then incubated for 18 hours. When an expansion of the inhibitory zone between the two disks was observed, the isolates were speculated to produce ESBL.

Conjugation and Plasmid Profiles

Conjugation experiments were performed by using *E. coli* CSH2 as a recipient, as previously described (17). A mixture of donor and recipient strains was incubated in Luria-Bertani broth (Difco Laboratories, Detroit, MI) at 37°C for 18 hours. Transconjugants were selected by using BTB Lactose agar plates supplemented with 100 μg/mL of rifampicin (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) and 2 μg/mL of cefotaxime to inhibit the growth of the donor strain and the recipient strain, respectively. Frequency of transfer was calculated by dividing the number of transconjugants by the number of donors. Plasmid DNA was prepared from the isolates and their transconjugants by using Quantum Prep Plasmid Miniprep Kit (Bio-Rad Laboratories, Richmond, CA), according to the manufacturer's instructions. After agarose gel electrophoresis, the sizes of the plasmids were determined by comparing their migration distances with those of plasmids of known sizes.

Susceptibility Testing

MICs were determined by overnight broth-microdilution method using MicroScan ESBL Confirmation Panel (Dade Behring, Sacramento, CA). This panel was designed to detect ESBL producers in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) document M100-S9 (26). The MIC of ceftiofur (Pharmacia

Co., Kalamazoo, MI), an expanded-spectrum cephalosporin often used in veterinary medicine, was also determined by the broth-microdilution method in accordance with NCCLS document M7-A4 (27). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality-control strains.

PCR and DNA Sequencing

To determine the genotype of strains producing broad-spectrum class A β-lactamases, PCR was performed by using primers specific to TEM, SHV (13), CTX-M-1 (MEN-1) (28), CTX-M-2 (29), and CTX-M-9 (8) genes. The PCR products were sequenced by using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) with the same primers for PCR. The DNA sequences were analyzed in an ABI PRISM 377 XL Sequencer Analyzer (Applied Biosystems).

RAPD Analysis

Random amplified polymorphic DNA (RAPD) analysis was performed by using Ready-To-Go RAPD analysis beads (Amersham Pharmacia Biotech, Piscataway, NJ), according to the manufacturer's instructions. DNA was prepared from the isolates using InstaGene DNA Purification Matrix (Bio-Rad Laboratories), also according to the manufacturer's instructions. The reaction mixture contained 25 pmol of one of six RAPD analysis primers (Amersham Pharmacia Biotech) and 10 μL of DNA preparation in a final volume of 25 μL. Amplification was performed with initial denaturation at 95°C for 5 minutes, followed by 45 cycles of 1 minute at 95°C, 1 minute at 36°C, and 2 minutes at 72°C. The amplified products were separated by electrophoresis in 1.5% agarose gel. The fingerprints were compared visually, and patterns were considered different when they differed by at least one amplification band.

Results

Identification of β-Lactamases

Of 396 fecal samples of cattle, 104 (26.3%) samples gave colonies on the ESBL screening agar. Among the strains grown on the screening agar, 32 strains of *E. coli* and 2 strains of *Citrobacter koseri* were positive through the P/Case TEST for production of penicillinase, cephalosporinase, or both (Table 1). The double-disk diffusion test was performed on 28 strains that were speculated to produce penicillinase; 7 strains isolated from 6 (1.5%) of 396 fecal samples were positive. However, two strains, GS553 and GS554, which produced cephalosporinase and penicillinase, showed a clear expansion of the inhibitory zone only when a disk of cefepime, a better detection agent for ESBLs in the presence of an AmpC β-lactamase (30), was used. By a PCR analysis with a set of PCR primers specific for $bla_{\text{CTX-M-2}}$, a 900-bp fragment was amplified from the seven strains that were positive in the double-disk diffusion test (Table 1). However, since CTX-M-2 and Toho-1 have only one amino acid substitution, genes for $bla_{\text{CTX-M-2}}$ and $bla_{\text{Toho-1}}$ were indistinguishable by the PCR. DNA sequencing of the PCR products subsequently showed that all were 100% identical with the $bla_{\text{CTX-M-2}}$ reported (31). Similarly, two strains isolated from 2 (0.7%) of 270 surface swab samples of cattle carcasses were positive in the double-disk diffusion test and possessed $bla_{\text{CTX-M-2}}$ (Table 1).

Although all 9 isolates producing CTX-M-2 β-lactamase were *E. coli*, their serotype of O antigen could not be defined with 43 commercially available antisera that were representative serotypes of pathogenic *E. coli*. Moreover, genes of virulence factors described previously were not detected from the strains by PCR.

Antimicrobial Susceptibility Testing

The susceptibilities of two representative isolates, GS528 and GS554, and their transconjugants are shown in Table 2. All the isolates were resistant to piperacillin, cefotaxime, ceftriaxone, cefpodoxime, cefepime, and aztreonam, and more resistant to cefotaxime than to ceftazidime. Except for strains GS553 and GS554, the β-lactamase inhibitor clavulanic acid (fixed concentration of 4 μg/mL) reduced MICs of cefotaxime and ceftazidime by $>2^{10}$- and $\geq 2^{4}$-fold, respectively. These susceptibility profiles of the isolates were similar to those observed for strains that produced CTX-M-2 β-lactamase (31). Both GS553 and GS554 strains, which produced cephalosporinase as well as penicillinase, were resistant to cefotetan, cefmetazole, and cefoxitin as well as piperacillin, cefotaxime, ceftriax-

Table 1. Number of β-lactamase producers isolated from cattle[a]

Sample (no.)	Species	Acidmetric β-lactamase test				PCR typing
		Total	PC	PC and CS	CS	
Feces (396)	*Escherichia coli*	32	7	19	6	7 (CTX-M-2)
	Citrobacter koseri	2	2	0	0	0
Swab[b] (270)	*E. coli*	5	2	2	1	2 (CTX-M-2)
	C. freundii	1	0	1	0	0

[a]PC, penicillinase; CS, cephalosporinase; PCR, polymerase chain reaction.
[b]Swab, surface swab of cattle carcass.

Table 2. MICs of β-lactams for *Escherichia coli* strains isolated from cattle, transconjugants, and recipients[a]

	MIC (μg/mL) for *E. coli* strain:				
Antimicrobial drug	GS528	CSH2 trGS528	GS554	CSH2 trGS554	CSH2
Piperacillin	>64	>64	>64	>64	≤16
Cefotaxime	>128	>128	>128	>128	≤0.5
Cefotaxime + CLA[b]	≤0.12	≤0.12	32	32	≤0.12
Ceftazidime	2	4	32	32	≤0.5
Ceftazidime + CLA	≤0.12	≤0.12	16	32	≤0.12
Aztreonam	>64	64	>64	64	≤0.5
Ceftriaxone	>64	>64	>64	>64	≤0.5
Cefpodoxime	>64	>64	>64	>64	≤0.5
Cefepime	>32	>32	>32	>32	≤1
Cefotetan	≤0.5	≤0.5	>32	>32	≤0.5
Cefmetazole	1	1	>16	>16	1
Cefoxitin	≤2	≤2	>32	>32	≤2
Meropenem	≤0.5	≤0.5	8	4	≤0.5
Ceftiofur	>1,024	>1,024	>1,024	>1,024	≤0.25

[a]*E. coli* CSH2 trGS528 and trGS554 were transconjugants of *E. coli* GS528 and GS554, respectively.
[b]CLA, clavulanic acid at a fixed concentration of 4 μg/mL.

one, cefpodoxime, cefepime, and aztreonam. In addition, clavulanic acid hardly reduced the resistance levels of these two strains to cefotaxime and ceftazidime. These results, together with those obtained through the double-disk diffusion test, suggested that both GS553 and GS554 strains produced putative AmpC β-lactamase at high levels as well as the CTX-M-2 β-lactamase. All the isolates producing CTX-M-2 β-lactamase were highly resistant to ceftiofur (MIC >1,024 μg/mL).

Plasmid and RAPD Analysis

Conjugation experiments indicated that all the isolates were able to transfer their cefotaxime resistance to the recipient and that the resistance to cephamycins observed in both strains GS553 and GS554 was also transferred to the transconjugant. All transconjugants produced the same β-lactamase(s) of their donor strains, and susceptibility profiles of the transconjugants were also similar to those of donor strains (Table 2). These results demonstrated that $bla_{\text{CTX-M-2}}$ genes of the isolates might be encoded on transferable plasmids. The frequency of transfer was very high (2×10^{-4} to 6×10^{-1} per donor cells) (Table 3). Plasmid profiles of the isolates showed one to three large plasmids with five different patterns in each strain, while an approximately 33-MDa plasmid was common among all the strains. Approximately 33-MDa and 50-MDa plasmids were both transferred to recipient cells in all the strains (Table 3). RAPD analysis of a total of nine isolates gave at least five different patterns (Figure, Table 3). Although strains GS553 and GS554 were isolated from the same fecal sample, they differed in RAPD pattern and plasmid profile.

Discussion

We investigated the cause of the disproportionate emergence of CTX-M-2 β-lactamase and so-called ESBLs, including TEM- or SHV-derived enzymes, in Japan. We isolated *E. coli* strains producing CTX-M-2 β-lactamase from 6 (1.5%) of 396 fecal samples from cattle and 2 (0.7%) of 270 surface swabs of cattle carcasses. Negative results, however, do not necessarily mean the organisms are absent on the slaughterhouse carcasses because of the limited size of the overall swabbed surface area.

Table 3. Characteristics of CTX-M-2 β-lactamase–producing *Escherichia coli* isolated from cattle[a]

Strain	Source	β-lactamase[b]	Plasmid profile (MDa)	Transferred plasmid (MDa)	Frequency of transfer	RAPD pattern
GS528	Feces 1	PC	33, 50, 86	33, 50	6×10^{-4}	A
GS542	Feces 2	PC	33, 50, 86	33, 50	2×10^{-4}	A
GS547	Feces 3	PC	33, 50, 86	33, 50	3×10^{-4}	A
GS553	Feces 4	PC and CS	33, 50, 61	33, 50	3×10^{-1}	B
GS554	Feces 4	PC and CS	33, 50	33, 50	2×10^{-1}	C
GS721	Feces 5	PC	33	33	9×10^{-2}	D
GS733	Feces 6	PC	33	33	2×10^{-1}	D
GS631	Swab[c] 1	PC	33, 86	33	5×10^{-1}	E
GS671	Swab 2	PC	33, 86	33	6×10^{-1}	E

[a]PC, penicillinase; CS, cephalosporinase; RAPD, random amplified polymorphic DNA.
[b]β-lactamases were detected by acidmetric β-lactamase test.
[c]Swab, surface swab of cattle carcass.

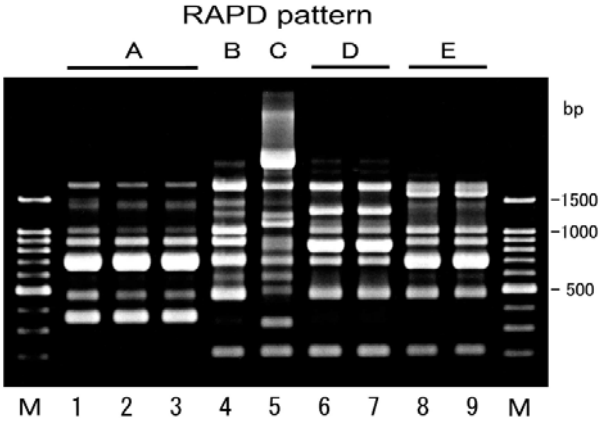

Figure. Random amplified polymorphic DNA (RAPD) patterns of CTX-M-2 β-lactamase–producing *Escherichia coli* isolated from cattle. Lanes M, 100-bp DNA ladder; lanes 1–9, strains GS528, GS542, GS547, GS553, GS554, GS721, GS733, GS631, and GS671, respectively. Five RAPD patterns, A to E, were produced with RAPD analysis primer 4 (Amersham Pharmacia Biotech, Piscataway, NJ).

Our findings raised a complex question: Did CTX-M-2 β-lactamase producers initially emerge in cattle or humans? We assume they emerged from cattle. Indeed, we found no direct evidence of transmission of *E. coli* strains producing CTX-M-2 β-lactamase from cattle to humans, but our results strongly suggested that transmission of the CTX-M-2–producing microorganism might have occurred between cattle and humans. This speculation is supported by the fact that CTX-M-2 β-lactamase-producers isolated from humans in Japan are identified predominantly as *E. coli,* as was observed in Japanese cattle. According to the survey of ESBLs in human clinical isolates in Japan, Toho-1-type β-lactamase was the most prevalent, and half of the Toho-1-type β-lactamase producers were *E. coli* (13). Moreover, the Toho-1-type β-lactamases reported in several studies in Japan were later found to be CTX-M-2 by PCR and sequencing analyses. Furthermore, according to the survey of ESBL producers in human stool specimens reported recently in Japan, Toho-1-type β-lactamase–producing enterobacteria were isolated from 2 (0.5%) of 366 specimens (17). Since the survey samples were from 231 inpatients and 135 outpatients with diarrhea, the rate of CTX-M-2 producers in healthy humans in Japan is estimated to be <0.5%. Indeed, by chi-square analysis, the isolation rate (1.5%) of CTX-M-2 producers in cattle feces obtained in our study showed no statistically significant difference from that of ESBL producers in human cases reported previously in Japan (17). However, we speculate that CTX-M-2 producers found in cattle have something to do with those from humans. Many reports substantiate that bacteria can be transmitted from food-producing animals to humans through the food chain, and we found that the surface of cattle carcasses was stained with the CTX-M-2–producing bacteria. Our speculation is also supported by the fact that TEM- or SHV-derived ESBLs have not been detected from livestock so far even in Western countries, where they have been widely detected with a high frequency in various medical institutions. In other words, if transmission of ESBL producers from human to cattle can occur with some frequency, several TEM- or SHV-derived ESBL producers would be isolated also from cattle. However, no such finding has been reported even in Western countries. Thus, prospective investigations should be conducted to understand the current status of *E. coli* strains that produce CTX-M-enzymes in livestock, especially in those countries where CTX-M-enzymes have been found in humans.

Recently, SHV-12 β-lactamase–producing *E. coli* was isolated from a dog with recurrent urinary tract infections (22). The origin of the isolate, however, was not known since the treatment with expanded-spectrum cephalosporins was not been recorded. In livestock, although penicillinases such as TEM-1 and TEM-2 have been identified from cattle (23,32–34), pigs (35), and poultry (36), isolation of ESBL producers has not been reported. On the other hand, ceftriaxone-resistant *Salmonella* isolates, which produce plasmid-mediated AmpC-type β-lactamase such as CMY-2, are proliferating globally (37). Ceftriaxone-resistant *Salmonella* and *E. coli* strains have been also isolated from cattle recently in the United States (23,32,33,35). These findings suggest that cattle can serve as an incubator or reservoir of these antimicrobial drug-resistant bacteria. The authors of the U.S. studies suggested that the emergence of the AmpC-mediated cephalosporin resistance may have been a consequence of the use of ceftiofur, the only cephalosporin approved for systemic use in food animals in the United States (23,32,35). Dunne et al. support this hypothesis, indicating that the use of ceftiofur in cattle may have contributed to the emergence of the ceftriaxone-resistant *Salmonella* because the isolate shows cross-resistance between ceftiofur and ceftriaxone (33). In our study, all the isolates producing CTX-M-2 β-lactamase were also highly resistant to ceftiofur. What antimicrobial agents had been used at Japanese cattle farms where the CTX-M-2 producers were isolated is not well known, since the samples were collected at slaughterhouses. However, ceftiofur was the only expanded-spectrum cephalosporin approved for livestock in Japan when our study was conducted. In addition, the MIC (>1,024 μg/mL) of ceftiofur for CTX-M-2 producers isolated in this study was relatively higher than those (2 to >32 μg/mL) for TEM- or SHV-derived ESBL producers (38) that have been emerging in so many humans. Thus, the emergence of CTX-M-2 β-lactamase–producing *E. coli* in Japan might also be a consequence of the use of cef-

tiofur for livestock. However, why CMY-2 type class C β-lactamase is predominantly found in livestock in the United States is not clear. The types of antimicrobial agents and their use for livestock in that country may have contributed to its high prevalence of CMY-2 producers, although no statistical data are available about the differences in usage of antimicrobial agents between the United States and Japan. Continuous and prospective investigations of veterinary usage of the antimicrobial agents as well as surveillance of antimicrobial-resistance seem necessary for preventing the emergence and further proliferation of antimicrobial-resistant bacteria in livestock.

The CTX-M-2 producers were not considered to reflect a clonal expansion of an *E. coli* strain carrying $bla_{\text{CTX-M-2}}$ because five distinct RAPD patterns and plasmid profiles were identified in the nine isolates. These findings suggest that stealthy plasmid-mediated dissemination of $bla_{\text{CTX-M-2}}$ gene among *E. coli* strains might be under way with the continuous consumption of the third-generation cephalosporin for veterinary use. Conjugal transfer of R-plasmid might occur in the intestinal tract, which is the main habitat of ESBL producers (17,39). Both strains GS553 and GS554 were isolated from the same fecal sample and produced the same β-lactamase, but they were different in terms of RAPD analysis and plasmid profile. Frequencies of transfer of the isolates were high (Table 3). These results suggested that conjugal transfer of the R-plasmids also occurred in the intestinal tract of cattle. Therefore, the possibility of further transfer of the resistance profile of *E. coli* to expanded-spectrum cephalosporins to other pathogenic bacteria such as *Salmonella* spp. and diarrheagenic *E. coli* should not be ignored.

The isolates in this study did not correspond to the serotypes of pathogenic *E. coli*, and they did not possess the virulence factors assayed. However, lack of virulence factors might contribute to subclinical increase of healthy carriers of these strains and might promote their dissemination among both cattle and human. Especially in livestock, environmental contamination and transmission among individual animals by these strains could expand rapidly because of their breeding system. Therefore, CTX-M-2 producers may well be disseminated even further in cattle farms hereafter. Although nosocomial bacteria that produce extended-spectrum class A β-lactamases have thus far been considered to emerge only among in humans, our study suggested that CTX-M-2 producers could potentially emerge in livestock and that cattle might be an original reservoir of CTX-M-2 producers. Therefore, active and continuous surveillance and strategic countermeasures are necessary for antimicrobial-resistant bacteria, including those strains producing such β-lactamases as CTX-M-type, CMY-type (37,40) and metalloenzymes (16) in livestock, especially in countries where these producers have emerged in human populations.

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Nosocomial Bloodstream Infection and Clinical Sepsis

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Primary bloodstream infection (BSI) is a leading, preventable infectious complication in critically ill patients and has a negative impact on patients' outcome. Surveillance definitions for primary BSI distinguish those that are microbiologically documented from those that are not. The latter is known as clinical sepsis, but information on its epidemiologic importance is limited. We analyzed prospective on-site surveillance data of nosocomial infections in a medical intensive care unit. Of the 113 episodes of primary BSI, 33 (29%) were microbiologically documented. The overall BSI infection rate was 19.8 episodes per 1,000 central-line days (confidence interval [CI] 95%, 16.1 to 23.6); the rate fell to 5.8 (CI 3.8 to 7.8) when only microbiologically documented episodes were considered. Exposure to vascular devices was similar in patients with clinical sepsis and patients with microbiologically documented BSI. We conclude that laboratory-based surveillance alone will underestimate the incidence of primary BSI and thus jeopardize benchmarking.

Primary bloodstream infection (BSI) is a leading, infectious complication among critically ill patients (1). It represents about 15% of all nosocomial infections (2,3) and affects approximately 1% of all hospitalized patients (4), with an incidence rate of 5 per 1,000 central-line days (5). The impact on patient outcome is tremendous; BSI increases the mortality rate (6,7), prolongs patient stay in an intensive care unit (ICU) and in the hospital (7–9), and generates substantial extra costs (7,8). For these reasons, surveillance and prevention of BSI are high priorities, and several interventions have proven to be effective (10–16).

The Centers for Disease Control and Prevention (CDC) surveillance definitions of BSI delineate two distinct entities: infections that are microbiologically documented, and those that are not, called clinical sepsis (17). Although surveillance of the former can be laboratory based, detection of clinical sepsis requires prospective on-site surveillance. The surveillance strategy determines whether clinical sepsis will be detected, thus affecting the overall BSI incidence rate.

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Because prospective on-site surveillance requires more resources than laboratory-based surveillance, the choice of the surveillance strategy should be based on knowledge of the importance of clinical sepsis. To our knowledge, clinical sepsis has never been investigated. This article describes the epidemiology of clinical sepsis in a medical ICU.

Methods

Setting

The study took place in the 18-bed medical ICU of a large teaching hospital in Geneva, Switzerland, from October 1995 to November 1997. The unit admits 1,400 patients per year; the mean length of stay is 4 days.

Surveillance and Definitions

The surveillance strategy of nosocomial infection has been described previously (12). Briefly, one infection control nurse visited the ICU daily (5 of 7 days), gathered information from medical and nursing records, microbiologic and x-ray reports, and interviews with nurses and physicians in charge. All patients staying $\geq$48 hours were included and followed up for 5 days after ICU discharge (18). Nosocomial infections were defined according to CDC criteria (17), except that asymptomatic bacteriuria was not considered an infection (19). Collected variables included all nosocomial infections, demographic characteristics, admission and discharge diagnoses, exposure to invasive devices and antibiotics, and ICU and hospital survival status.

Microbiologically documented BSI required one of the following: 1) recognized pathogen in the blood and pathogen not related to an infection at another site; or 2) fever, chills, or hypotension; and any of the following: a) a common skin contaminant is isolated from at least two blood cultures drawn on separate occasions, and the organism is not related to infection at another site; b) a common skin contaminant is isolated from blood culture in a patient with an intravascular device, and the physician institutes appropriate antimicrobial therapy; c) a positive antigen test

on blood and the organism is not related to infection at another site (17).

Clinical sepsis was diagnosed when the patient had either fever, hypotension, or oliguria, and all of the following: 1) blood not cultured or no microorganism isolated; 2) no apparent infection at another site; and 3) physician institutes appropriate antimicrobial therapy for sepsis (17).

The surveillance strategy, definitions, and the discharge policy did not change over the study period. Patients were discharged from the ICU, according to specific guidelines designed for this unit, and compliance with these guidelines was checked daily by a senior staff member. An ongoing intervention aiming to reduce catheter-related infection was begun in March 1997. Reports on the intervention and its effect have been published previously (12).

Statistical Analysis

All primary BSI were considered in the first part of the analysis. Episodes of BSI that were not associated with a central line were identified. Infection rates were expressed as the total number of episodes per 1,000 ICU patient days, or the number of episodes associated with a central line per 1,000 central-line days. Their corresponding 95% confidence intervals (CI) were computed, according to the normal approximation of the Poisson distribution.

The study population was then divided into three groups to describe the epidemiology of clinical sepsis. The first group included all patients who remained free of any ICU-acquired BSI; the second group comprised all patients whose first episode was a microbiologically documented BSI, and the third group included those whose first episode was clinical sepsis. Only the first episode of BSI was considered. We then performed a subgroup analysis comparing patients with and without BSI but with at least a 5-day stay in the ICU. This analysis was conducted to exclude patients who died or were discharged quickly after ICU admission to ensure that patients without BSI were sufficiently exposed to the risk of acquiring nosocomial BSI.

Exposure to invasive devices was estimated by the proportion of patients exposed to the device and the duration of the exposure. We separately investigated peripheral, arterial, and central vascular lines. Among patients with BSI, the duration of the exposure to the vascular line was censored at onset of the first episode of BSI.

Continuous variables were summarized by means or medians and compared with the Student t-test or a nonparametric test, when appropriate. Categorical variables were compared by using chi-square or the Fisher exact test. All tests were two-tailed, and p values <0.05 were considered statistically significant. All statistical analyses were conducted with Stata 7.0 (Stata Corporation, College Station, TX).

Results

We surveyed 1,068 patients who stayed in the ICU $\geq$48 hours, for a median length of stay of 5 days (range 2–134), totaling 7,840 ICU patient days. Median age was 62.9 (range 16.2–92.0), and male-to-female ratio 622/446. The main admission diagnoses were infectious (38.7%), cardiovascular (24.2%), and pulmonary (17.7%) conditions. We detected 554 ICU-acquired infections, yielding an infection rate of 71 episodes per 1,000 patient-days (95% CI 64.8 to 76.5). The leading sites were the lungs (pneumonia, 28.7%), bloodstream (20.4%), skin and soft tissue (15.3%), catheter exit site (13.5%), and urinary tract (11.2%). We detected nine episodes of secondary BSI, six secondary to a urinary tract infection, two to a lower respiratory tract infection, and one to a skin and soft tissue infection.

Of 113 episodes of BSI, 33 (29.2%) were microbiologically confirmed, and 80 (70.8%) were clinical sepsis. Four episodes (three of clinical sepsis and one of microbiologically confirmed BSI) were not associated with a central line. Blood cultures were drawn in most of the clinical sepsis episodes (66/80, 82.5%). Exposure to systemic antimicrobial drugs before blood culture was 39.4% (13/33) among patients with microbiologically documented BSI and 77.3% (51/66) among patients with clinical sepsis ($p < 0.001$). Among the 20 patients with microbiologically documented BSI who had not received antimicrobial drugs during the 48 hours before the blood culture, 6 were in a therapeutic window (antibiotherapy was suspended before drawing blood cultures to increase the culture's sensitivity).

Among the 33 episodes of microbiologically confirmed BSI, 4 were polymicrobial. The most frequently isolated microorganisms were coagulase-negative staphylococci (n = 21). Other gram-positive cocci were *Staphylococcus aureus* (n = 1) and *Enterococcus faecalis* (n = 2). Gram-negative rods included *Enterobacter aerogenes* (n = 2), *Serratia marcescens* (n = 2), *Escherichia coli* (n = 1), *Proteus mirabilis* (n = 1), and *Pseudomonas* non-*aeruginosa* (n = 1). Other microorganisms found were *Candida albicans* (n = 1) and *Propionibacterium acnes* (n = 2).

Table 1 displays BSI infection rates per 1,000 patient days and central-line days. The overall rate of BSI was 19.8 per 1,000 central-line days (CI 95%, 16.1 to 23.6) and markedly differed when only microbiologically documented BSI were considered. These 113 BSIs occurred in 91 patients; 73 patients had a single episode, 14 had two, and 4 had three episodes. The first episode was microbiologically documented for 28 patients and diagnosed as clinical sepsis for 63.

Selected characteristics of patients with and without BSI are displayed in Table 2. Patients without BSI tended to be older; the distribution of admission diagnosis was similar in both groups, but intoxication was more prevalent

Table 1. Primary bloodstream infection rates

	N	Incidence rate/1,000 patient days (CI 95%)[a]	N	Incidence rate/1,000 central-line days (CI 95%)
All primary bloodstream infections	113	14.4 (11.8 to 17.1)	109	19.8 (16.1 to 23.6)
Microbiologically documented	33	4.2 (2.8 to 5.6)	32	5.8 (3.8 to 7.8)
Clinical sepsis	80	10.2 (8.0 to 12.4)	77	14.0 (10.9 to 17.1)

[a]CI; confidence interval.

in patients without BSI, although the difference was not statistically significant. Illness appeared more severe in patients with BSI, as estimated by a higher number of discharge diagnoses, a longer ICU length of stay, and a higher mortality rate. After patients who stayed <5 days were excluded, 558 patients remained in this analysis. The picture remained the same. In particular, both groups were of similar age ($p = 0.054$); the proportion of patients admitted for intoxication was 1.9% in those without BSI and 2.3% in patients with BSI ($p = 0.82$).

The occurrence of pneumonia, urinary tract infection, and other infections was similar in patients with microbiologically documented BSI and clinical sepsis, but less frequent in patients without BSI. However, catheter exit-site infection was more frequent in patients with clinical sepsis (Figure).

The results of exposure to invasive devices are shown in Table 3. Exposure to vascular lines was censored at the time of the first episode of BSI. Exposure to central lines and arterial lines was similar in patients with a microbiologically documented episode of BSI and in those with clinical sepsis but much lower in patients without BSI. Three episodes of primary BSI occurred in patients without a central line in place before onset of infection. Similarly, exposure to urinary catheter and mechanical ventilation was lower in patients without BSI. After patients who stayed <5 days in the ICU were excluded, exposure to central vascular lines remained more important in patients with BSI (96.6% of exposed patients vs. 76.4%, $p < 0.001$), and duration of the exposure was also longer in this group (median [range], 9 days [1-39], vs. 7 days [1-117], $p = 0.002$).

Median ICU length of stay was longer among patients with microbiologically documented BSI (15.5 days; range 4–67) and clinical sepsis (14.0 days; range 3–48) than among patients with no BSI (4 days; range 2–134), (both $p < 0.001$). The hospital mortality rates among patients without BSI, with a microbiologically confirmed BSI, and with clinical sepsis were 22.7%, 32.1%, and 39.7%, respectively; the difference was statistically significant between the first and last group ($p = 0.01$).

Discussion

This study shows the importance of primary BSI; the bloodstream was the second most frequent infection site, representing 20% of all infections. We also found that a minority of BSI were microbiologically documented and that ignoring clinical sepsis has a large impact on the BSI infection rate. To our knowledge, this is the first report that provides a detailed epidemiologic description of clinical sepsis.

Whether clinical sepsis represents a primary BSI or whether it is a systemic reaction accompanying an unrecognized infection at another site or a noninfectious systemic inflammatory response are valid concerns (1,20–23). The definition is not specific because it requires, among other criteria, only one of three clinical signs (fever, hypotension, or oliguria). Also, this condition mandates antimicrobial therapy prescribed by the physician for suspected sepsis. Thus, we decided to use unmodified definitions, elaborated by CDC and widely used because they are still considered the standard operational definitions for surveillance of nosocomial infections. An epidemiologic description of patients without BSI, with microbiological-

Table 2. Selected characteristics of the study population[a]

Characteristic	Patients without BSI, n = 977	Patients with BSI, n = 91	p value
Sex			0.28
Male (%)	562 (57.5)	60 (65.9)	
Female (%)	415 (42.5)	31 (34.1)	
Median age (range)	63.0 (16.2–92.0)	59.2 (18.7–86.8)	0.05
Admission diagnosis			
Infectious (%)	377 (38.6)	36 (39.6)	0.86
Cardiovascular (%)	241 (24.7)	17 (18.7)	0.2
Pulmonary (%)	171 (17.5)	18 (19.8)	0.59
Neurologic (%)	68 (7.0)	10 (11.0)	0.16
Intoxication (%)	50 (5.1)	2 (2.2)	0.22
Others (%)	70 (7.2)	8 (8.8)	0.57
No. of discharge diagnoses (range)	5 (1–30)	6 (1–19)	<0.001
ICU length of stay (range)	4 (2–134)	14 (3–67)	<0.001
ICU mortality rate	154 (15.8)	25 (27.5)	0.004

[a]BSI, bloodstream infection; ICU, intensive care unit.

Table 3. Exposure to invasive devices among patients with and without primary bloodstream infection

	No BSI, n = 977	Microbiologically confirmed BSI, n = 28	Clinical sepsis, n = 63
Peripheral catheter			
Exposed patients (%)	858 (87.8)	24 (85.7)	58 (92.1)
Catheter-days [days, median (range)]	3 (1-30)	4 (1-10)[b]	5.5 (1-20)[c]
Central line			
Exposed patients (%)	627 (64.2)[d]	27 (96.4)	61 (96.8)
Catheter-days [days, median (range)]	4 (1-117)[d]	8 (2-39)	8 (1-33)
Arterial line			
Exposed patients (%)	791 (81.0)[d]	28 (100)	62 (98.4)
Catheter-days [days, median (range)]	3 (1-47)[d]	7 (2-23)	8 (1–21)
Mechanical ventilation			
Exposed patients (%)	380 (38.9)[d]	19 (67.9)	53 (84.1)[e]
MV-days [days, median (range)]	3 (1–123)[d]	12 (2–61)	11 (1–35)
Urinary catheter			
Exposed patients (%)	665 (68.1)[d]	27 (96.4)	58 (92.1)
Catheter-days [days, median (range)]	3 (1–77)[d]	12 (1–63)	14 (1–45)

[a]BSI, bloodstream infection; MV, mechanical ventilation.
[b]p = 0.059 when compared to no BSI.
[c]p < 0.001 when compared to no BSI, and p = 0.053 when compared to microbiologically confirmed BSI.
[d]p < 0.005 when compared to microbiologically confirmed BSI and clinical sepsis.
[e]p = 0.097 when compared to microbiologically confirmed BSI.

ly documented BSI, and with clinical sepsis provides valuable information. First, approximately 90% of primary BSIs occur in patients with intravascular devices, especially central lines, and these represent the most powerful risk factors for BSI (24). In our study population, exposure to central and arterial lines was similar in both groups of patients with BSIs, but the frequency and duration of the exposure were of greater importance than they were in the group of patients without BSIs. The longer exposure to vascular devices does not reflect the impact of BSI because exposure was censored at time of BSI. Consequently, the most powerful risk factor for clinical sepsis is the same as that for microbiologically documented BSI.

Second, during the same study period we implemented an intervention targeted at vascular-access care to reduce the incidence of catheter-related BSIs (12). We observed a dramatic decrease in the incidence of all catheter-related infections: catheter exit-site infection dropped from 9.2 to 3.3 episodes per 1,000 ICU-patient days (64% reduction), and microbiologically documented BSI dropped from 3.1 to 1.2 episodes per 1,000 ICU-patient-days (61% reduction). A parallel sharp decrease occurred in the rate of clinical sepsis, which went from 8.2 to 2.6 episodes per 1,000 ICU-patient days (68% reduction). Rates of ventilator-associated pneumonia and urinary tract infection did not change over time. These two sets of results, same exposure and same response to a prevention program, strongly suggest that clinical sepsis is indeed primary BSI.

Blood cultures were performed in most (82.5%) cases of clinical sepsis and were negative. The absence of microorganisms can be explained in several ways. First, bacteremia is not constant, and sensitivity of the blood culture increases with the number of cultures drawn and the volume of the sample (25–27). Second, most of our patients (77%) with clinical sepsis were receiving broad-spectrum antimicrobial drugs for other conditions, thus decreasing the sensitivity of the test. This pattern of antimicrobial prescription is usual in critical care, as reported in large studies which showed that >60% of the patients were receiving antimicrobial drugs on the day of the study (2,28,29). In further studies to delineate the epidemiology and pathophysiology of clinical sepsis, the sensitivity of blood cultures should be maximized and should include genomic approaches to identify pathogens, especially if antimicrobial therapy has been initiated.

The question arises regarding whether to include clinical sepsis in surveillance of BSI, considering the amount of work generated by on-site prospective surveillance, compared to laboratory-based surveillance. In response, the following elements should be considered. Benchmarking is increasingly performed and is part of the quality improvement process. However, the sensitivity of

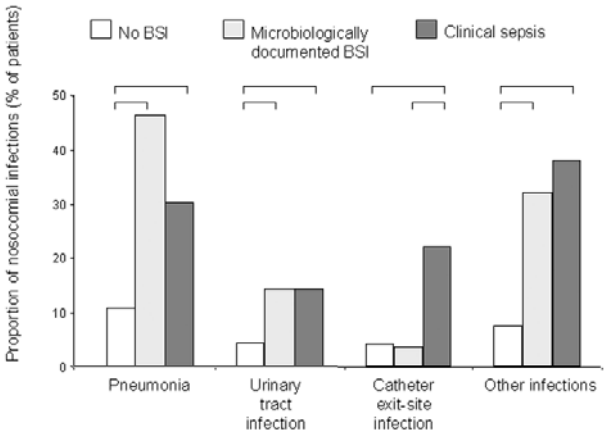

Figure. Frequency of nosocomial infections among patients with and without primary bloodstream infection (BSI). Columns represent the proportion of patients with each type of infection. Brackets indicate a significant (p < 0.05) difference between groups.

the surveillance method to detect clinical sepsis will greatly impact the infection rate and make benchmarking difficult. Our overall BSI rate was high (19.8 episodes per 1,000 central-line days), well above that reported by the National Nosocomial Infection Surveillance (NNIS) system (3,5,30). This difference is due to the proportion of clinical sepsis, 80% in our study and 8% in NNIS (3). When microbiologically documented BSI alone is considered, our BSI rate is comparable to that reported in the literature, including the rate reported by NNIS hospitals (5,14,16,31). Surveillance estimates the incidence of a disease. Underdetection of clinical sepsis will grossly underestimate its incidence, and data generated by the system will be misinterpreted, affecting the allocation of resources. Finally, demonstrating the effectiveness of a prevention program that aims to reduce BSI will require a much greater sample size than if cases of clinical sepsis were considered in the surveillance system. Conversely, the cost-effectiveness of prevention programs will be underestimated if only microbiologically documented BSI is considered.

This study has some limitations. Whether our results can be extrapolated to other ICUs needs to be tested. Indeed, the surveillance criteria for clinical sepsis might be sensitive to local case management policies, for instance, regarding antimicrobial drug prescription. In addition, the situation in surgical ICUs might be quite different, as systemic inflammatory reactions after surgery that mimic clinical sepsis are frequent (22,23). Neither can we rule out some degree of misclassification of clinical sepsis that is actually catheter infection. This possibility is suggested by the fact that catheter exit-site infections were more prevalent in the group of patients with clinical sepsis than in the group of patients with microbiologically documented BSI. This misclassification would be very important if we were investigating the impact of clinical sepsis. However, this is not relevant in terms of surveillance and infection control and prevention because both clinical sepsis and catheter infection have the same risk factors, are sensitive to the same prevention strategies, and are equal markers of poor quality of care.

In conclusion, clinical sepsis is an epidemiologically important syndrome. We believe that surveillance strategies that can detect this syndrome should be favored because prevention, benchmarking, program evaluation, and ultimately, quality of patient care depend on the accuracy of surveillance data.

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Experimental Infection of Cats and Dogs with West Nile Virus

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Domestic dogs and cats were infected by mosquito bite and evaluated as hosts for West Nile virus (WNV). Viremia of low magnitude and short duration developed in four dogs but they did not display signs of disease. Four cats became viremic, with peak titers ranging from $10^{3.0}$ to $10^{4.0}$ PFU/mL. Three of the cats showed mild, non-neurologic signs of disease. WNV was not isolated from saliva of either dogs or cats during the period of viremia. An additional group of four cats were exposed to WNV orally, through ingestion of infected mice. Two cats consumed an infected mouse on three consecutive days, and two cats ate a single infected mouse. Viremia developed in all of these cats with a magnitude and duration similar to that seen in cats infected by mosquito bite, but none of the four showed clinical signs. These results suggest that dogs and cats are readily infected by WNV. The high efficiency of oral transmission observed with cats suggests that infected prey animals may serve as an important source of infection to carnivores. Neither species is likely to function as an epidemiologically important amplifying host, although the peak viremia observed in cats may be high enough to infect mosquitoes at low efficiency.

The appearance of the West Nile virus (WNV) in New York in 1999 and the subsequent establishment and spread of the virus have aroused public anxiety about the potential of companion animals to become infected, show illness and die, and transmit WNV to other species, including humans. Little information is available concerning the susceptibility of dogs and cats to WNV infection. Approximately one third of a large sample of dogs from a WNV-endemic region of South Africa had neutralizing antibody to WNV, and viremia was detected in one of three dogs inoculated with a large dose of WNV (1). Recently, WNV was isolated from the brain of a cat with "neurologic disease" (2), but nothing is known about the natural history of WNV infection in this species. Most dogs and cats spend at least some time out of doors and thus risk exposure from hematophagous insect vectors. Also, as carnivores, they may be exposed to WNV through ingestion of infected small mammals and birds, which are known to have large quantities of WNV in blood and tissue during the course of infection (2,3). Since there are an estimated 68 million companion dogs and 73 million pet cats in the United States alone (4), evaluating their response to WNV infection, assessing their potential to serve as amplifying hosts for this virus, and obtaining some estimate of the clinical consequences of infection are important concerns.

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Materials and Methods

Animals and Examinations

Four young adult female, crossbred hounds (Harlan, Indianapolis, IN) and eight adult, female domestic, crossbred cats (Liberty Laboratories, Waverly, NY) were used. All were purchased as specific pathogen-free animals. Each cat was negative for antibodies to feline immunodeficiency virus and negative for feline leukemia virus antigen in serum. Animals were gang housed by species under animal biosafety level three conditions with ad libitum access to food and water. They were examined clinically by a veterinarian twice daily for the duration of the study but did not receive formal neurologic evaluations. Body temperature and general appearance were recorded twice daily from days –0.5 to 7 or 9 (day 0 being the day of infection). With few exceptions, blood was collected twice daily from days –1 (dogs) or 0 (cats) to 9, at 2 and 3 weeks postinfection, and at the time of euthanasia. For the animals exposed to WNV through mosquito bites, blood was collected into EDTA tubes for hematology once daily from days –1 (dogs) or 0 (cats) to 10, and on days 14 and 21. Hematologic parameters (erythrocyte and leukocyte counts, platelet counts, plus relative and absolute mononuclear and granulocyte counts) were evaluated by using a QBC-V analyzer (Clay-Adams, Parsippany, NJ). Saliva was collected from mosquito-inoculated animals once daily from days 0 to 7 by swabbing the oral cavity with a cotton swab premoistened with BA-1 medium (M-199

salts, 1% bovine serum albumin, 250 mg/L sodium bicarbonate, 100 U penicillin G/mL, 100 µg/mL streptomycin, 1 µg/mL amphotericin B in 50 mM Tris, pH 7.6). All animals were euthanized between days 22 and 50 after infection by pentobarbital overdose and necropsied; their carcasses were incinerated within the containment facility.

Hematologic and body temperature data were analyzed by repeated measures analysis of variance and compared to a baseline by using the differences of least squares means statistical test (Proc Mixed, SAS Statistical Software, SAS Institute, Cary, NC). Baseline values were obtained on day 0 (body temperature) or represent the mean of values obtained on days 0 and 21 (hematologic data).

Infection by Mosquito Feeding

Four dogs and four cats were exposed to WNV through the bites of infective *Aedes albopictus* mosquitoes. The mosquitoes were from a colony strain from Lake Charles, Louisiana, which had been maintained in the insectary for several years with periodic additions of field-collected stock. Mosquitoes were reared in an insectary maintained at 26.7°C, approximately 80% relative humidity and a light:dark cycle of 16:8 hours. Larvae were fed liver powder and rabbit chow ad libitum. Cohorts of adult female *Ae. albopictus* 3–5 days of age were inoculated intrathoracically with approximately 170 Vero PFU of the NY99 strain of WNV, isolated originally from an infected crow. Inoculated mosquitoes were placed in 3.8-L cages, given 5% sucrose for maintenance, and incubated for 12 days under the same insectary conditions described as before virus inoculation. On the afternoon before feeding on dogs and cats, mosquitoes were transferred to cylindrical cartons with netting on both ends, denied sucrose solution, and held overnight with a pad moistened lightly with water on their cages.

Cats were lightly anesthetized with ketamine, and dogs were sedated with xylazine during mosquito feeding. Before feeding, a 10- to 15-cm diameter area of fur over the thorax was clipped using a #40 blade. Feeding was accomplished by holding the mosquito cage against the clipped area of skin for approximately 5 minutes. Individual lots of 14 mosquitoes were given the opportunity to feed on each animal, then transported to a secure laboratory. They were anesthetized with CO_2 and sorted on wet ice. Three engorged mosquitoes per animal were ground individually in BA-1 using a mixer mill. Samples were then clarified in a refrigerated microcentrifuge (5,000 x *g* for 5 min) and tested for virus by plaque assay.

Oral Exposure to WNV

Four cats were exposed to WNV by ingestion of mice previously infected by intraperitoneal inoculation of approximately 100 PFU of WNV (NY99), incubated for 5 to 8 days, and euthanized by CO_2 inhalation immediately before being given to the cats. To estimate the minimal virus load of the mice, cohorts of the mice fed to cats were euthanized at the same time and frozen at –70°C; later, their brains were assayed for virus by plaque assay. The cats were housed individually for feeding, by leaving the infected mouse on a plate overnight. In most but not all cases, the mouse was observed to have been eaten within 1 hour. If a cat had not eaten the mouse by the following morning, it was not considered an exposure, the mouse was discarded, and another mouse was presented the following evening. Two cats were fed one infected mouse each on three successive days, and two other cats were each fed a single infected mouse.

Plaque and Neutralization Assays

Serum, saliva, mosquitoes, and mouse brain homogenates were tested for virus concentration by plaque assay. Briefly, serial 10-fold dilutions of the samples were made in BA-1 medium and 0.1-mL volumes were inoculated onto monolayers of Vero cells in 6-well tissue culture plates. The plates were incubated at 37°C in an atmosphere containing 5% CO_2, then overlaid with 3 mL of 0.5% agarose in M-199 medium supplemented with 5% fetal bovine serum and antibiotics. After 48 hours, a second 3-mL overlay containing 0.004% neutral red was added. Plaques were scored on days 3 and 4 of incubation. Selected serum samples were tested for antibodies to WNV with a 90% plaque-reduction neutralization test, as described previously (5).

Results

The number of mosquitoes that fed on dogs 1 to 4 and cats 1 to 4 ranged from 8 to 11. The quantity of WNV present in the three mosquitoes assayed from each animal after feeding ranged from $10^{7.4}$ to $10^{7.9}$ PFU per mosquito.

Viremia was detected in the four dogs from 12 to 60 hours after exposure to WNV (Table). The quantity of virus in serum was low and fluctuated, and viremia was not detected past day 4.5. The peak viremia detected in the dogs ranged from $10^{1.6}$ to $10^{2.2}$ PFU/mL. Virus was not isolated from any of the oral swab samples. Clinical signs of disease were not observed in any of the infected dogs. They ate readily and remained energetic and afebrile, with the exception of dog 4, which had a slightly elevated body temperature 12 hours after mosquito feeding (Figure). Dog 4 was in proestrus and estrus during the challenge period. Statistical analysis of canine hematologic changes indicated a decrease in leukocyte count from days 2 through 7 and in hematocrit from days 4 through 9. However, the least squares means for those parameters were within the normal clinical reference range throughout the sampling period, suggesting that hematologic analysis is not clinically

Table. West Nile virus titers in serum of dogs and cats following bites of virus-infected mosquitoes or ingestion of virus-infected mice

Route of exposure	Animal	WNV virus titer (log_{10} PFU/mL serum) at days postexposure																		
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0
Mosquito	Dog 1	<1	1.6	<1	<1	<1	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	-[a]	-	-	-
Mosquito	Dog 2	<1	<1	2	2	<1	2.2	2	<1	<1	<1	<1	<1	<1	<1	<1	-	-	-	-
Mosquito	Dog 3	<1	<1	<1	<1	<1	1	1.6	1.6	1	1	<1	<1	<1	<1	<1	-	-	-	-
Mosquito	Dog 4	<1	<1	1.6	1.6	<1	1.6	1.6	1.6	1	1	<1	<1	<1	<1	<1	-	-	-	-
Mosquito	Cat 1	<1	1.6	2.3	2.9	2.4	2.8	2.7	3.2	2	2	<1	<1	<1	<1	<1	-	-	-	-
Mosquito	Cat 2	<1	<1	2.1	2.3	2.4	2.7	2.8	3.0	2.6	1	<1	<1	<1	<1	<1	-	-	-	-
Mosquito	Cat 3	<1	<1	<1	1	1.6	1.9	2.0	2.8	3.4	3.0	<1	<1	<1	<1	<1	-	-	-	-
Mosquito	Cat 4	<1	<1	1	2.5	3.2	3.2	4.0	3.2	3.0	1	<1	<1	<1	<1	<1	-	-	-	-
Oral	Cat 14	<1	-	1	3.2	2.9	3.2	3.4	3.7	3.6	3.2	<1	<1	<1	<1	<1	-	-	-	-
Oral	Cat 16	<1	-	2	2.7	2.8	3.4	3.6	3.9	3.6	2.7	<1	1	<1	<1	<1	-	-	-	-
Oral	Cat 17	-	<1	<1	<1	<1	<1	<1	<1	1.8	1.0	1.6	2.1	2.5	2.3	1.8	1.0	1.3	<1	<1
Oral	Cat 18	<1	<1	<1	<1	<1	1.8	1.3	1.5	1.0	1.6	2.2	1.5	1.3	<1	<1	<1	<1	<1	<1

[a]-, Not done.

predictive. At necropsy on day 50, gross abnormalities were not observed in any dog.

Viremia developed in the four cats exposed to WNV by feeding of infected mosquitoes 12 to 36 hours after exposure (Table). The peak titers of virus in serum ranged from $10^{3.0}$ to $10^{4.0}$ PFU/mL, and virus was not isolated from any samples collected >4.5 days after mosquito feeding. Three of the four cats (numbers 1, 2, and 4) displayed both lethargy and a fluctuant febrile response (Figure), but specific neurologic signs were not observed in any cat. Significant ($p < 0.05$) elevations of rectal temperature occurred from days 1 through 6. A number of isolated significant deviations from baseline in hematologic parameters were identified, but meaningful trends were not observed. Cat 4 was anemic at the time of challenge (hematocrit 21%), became more severely anemic during the first week after challenge (hematocrit as low as 13%), then rebounded by days 9 to 14 (hematocrit 18%-22%). WNV was not isolated from any of the oral swab samples. At necropsy on day 50, gross abnormalities were seen in cat 2 (pale, reticulated liver) and cat 4 (hydrocephalus and a nodular, fibrous spleen). These findings were considered incidental and unrelated to WNV infection; hydrocephalus in the absence of overt neurologic signs is not uncommon in cats (D. Baker, pers. commun.).

Clinical signs, including pyrexia and neurologic abnormalities, were absent throughout the study period in cats infected with WNV by ingesting three (n = 2) or one (n = 2) infected mice. Viremia developed in cats that ingested three mice (cats 14 and 16) within 24 hours of ingesting the first mouse (these cats were not bled at 12 hours), and viremia resolved by day 6 (Table). The peak viremia in these two animals was $10^{3.7}$ and $10^{3.9}$ PFU/mL, respectively. Cohort mice of those fed at the first of the three feedings yielded $10^{9.5}$ and $10^{9.7}$ PFU/g of brain. Cats 17 and 18 ingested a single mouse each; these two mice were euthanized approximately 2 days earlier in the course of their infection than those fed to cats 14 and 16, and virus titration of their cohorts showed $10^{5.3}$ and $10^{6.5}$ PFU/g of brain. Cats fed a single mouse had viremia of similar duration but delayed onset (day 4 and 2.5 for cats 17 and 18, respectively), and lower peak titer ($10^{2.5}$ and $10^{2.2}$ PFU/mL serum) than occurred in the cats ingesting mice with higher virus content (Table). Neutralizing antibody titers to WNV of at least 1:10 developed in three of the four cats fed infected

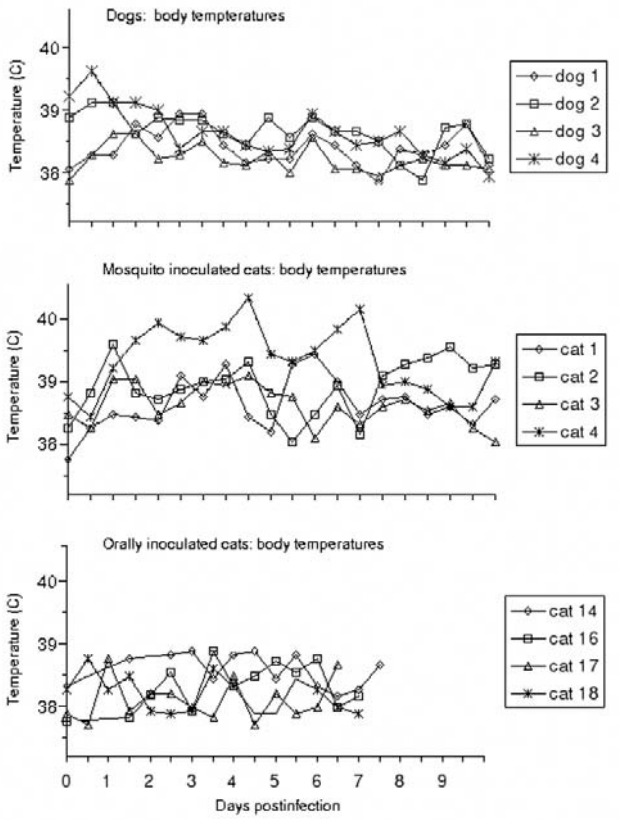

Figure. Body temperatures during the course of experimental West Nile virus infection. Reference ranges for clinically normal dogs and cats were considered to be 38.3°C–39.5°C and 38.0°C–39.2°C, respectively.

mice by day 14 postexposure (range 1:10-1:40, 90% neutralization). Cat 18, which had a neutralizing titer <1:10 on day 14, had a titer of 1:40 on day 21.

Discussion

Little attention has been paid to dogs and cats as hosts for WNV, and neither species is a recognized natural host for this virus. However, considering the large populations of these animals in North America and their close association with humans, understanding their clinical response to infection and the likelihood that they might serve as amplifying hosts are important. Dog and cat owners have also expressed concern about the potential effects of WNV infection on their pets. Roughly one in three dogs from a WNV-endemic region of South Africa were found to have neutralizing antibody to WNV (1). More recently, 10 of 139 dogs and none of 10 cats sampled from New York City during the fall of 1999 were reported to have WNV-neutralizing antibody (6); the number of these animals, particularly the cats, that spent a considerable amount of time out of doors was not known. WNV was isolated from the brain of a cat from New York that displayed neurologic disease (2).

Ae. albopictus mosquitoes were used to challenge the animals in these experiments because they are known to be capable of transmitting WNV by bite (7,8) and to reproduce a natural route of infection. In addition, WNV has been isolated from *Ae. albopictus* in New York (9).

None of the four dogs infected by mosquito bite showed clinical signs of disease, and although each became viremic, the quantity of virus in blood was low and fluctuated considerably. These results are similar to those described by Blackburn and co-workers, who found no clinical signs and viremia in one of three dogs inoculated by subcutaneous and intravenous inoculation with a South African strain of WNV (1). Collectively, these observations and the paucity of clinical reports of WNV disease in dogs suggest that WNV infection in dogs is typically subclinical in nature.

Three of the four cats infected by mosquito bite showed mild, nonspecific signs of disease at times during the first week after challenge. Signs included lethargy and modest decreases in appetite, none to the extent that would typically alarm pet owners. Two of the four cats (numbers 2 and 4) did show periods of distinctly elevated body temperature. Cat 4 had a preexisting anemia, which may have contributed to the course of disease. The duration of viremia in these four animals ranged from 3.5 to 4.5 days, with peak titers between $10^{3.2}$ and $10^{4.0}$ PFU/mL.

The oral transmission experiments conducted with cats were designed to determine whether carnivores are likely to become infected with WNV through eating infected birds or small mammals, which often contain high quantities of virus. Transmission of a vaccine strain of Japanese encephalitis virus to mice by instillation of virus into the oral cavity has previously been reported (10). Efficient transmission to adult mice by oral instillation of WNV has been reported (3), but similar attempts failed to transmit the virus (11). Further, isolation of WNV from a Red-tailed Hawk found dead in New York during midwinter was suggested to be due to predation, although the source of an infected prey animal at that time of year was not clear (12). Finally, milk-borne transmission from sheep to humans of the tick-borne encephalitis flavivirus has been reported (13). The current report provides the first experimental demonstration of oral transmission of a flavivirus using methods that approximate a natural type of exposure in mammals. The four cats that ate one or three infected mice became infected, indicating that this route of transmission is quite efficient. Whether such transmission results simply from oral exposure to virus, depends upon inoculation through the oral mucosa by small splinters of bone, or proceeds through some other pathway is not known. Nonetheless, transmission of WNV by predation or scavenging seems another likely important route of WNV transmission in mammals and birds.

The magnitude of WNV viremia necessary to efficiently infect feeding mosquitoes varies with mosquito species. Based on experiments using viremic chicks as a blood source, Jupp estimated 10% infection thresholds for South African strains of *Culex univittatus*, *Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. theileri* of $<10^{2.7}$, $10^{2.7}$, $10^{2.7}$ and $<10^{4.1}$ adult-mouse lethal dose $(LD)_{50}$/mL, respectively (14). More recently, Turell and co-workers fed a variety of mosquito species from the eastern United States on viremic chicks (15). Infection rates in these experiments ranged from 0% to 17% for mosquitoes ingesting blood with a WNV titer of $10^{5.2}$ Vero cell PFU/mL, and from 0% to 92% for mosquitoes that fed on chicks circulating $10^{7.0}$ ($\pm$ 0.3) PFU/mL, respectively. We did not test the ability of any species of mosquito to become infected by feeding on viremic dogs or cats. The low serum virus titers reported here and by Blackburn and colleagues (1) suggest that dogs are very unlikely to serve as an amplifying host for WNV. In contrast, the peak viremias observed in all of the mosquito-fed and orally exposed cats suggest that they may support infection of mosquitoes, albeit with low efficiency relative to many avian hosts.

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Capture-Recapture Analysis and Pneumococcal Meningitis Estimates in England

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To improve estimates of disease incidence and deaths from pneumococcal meningitis among adults in England, we performed a capture-recapture analysis for 1996 through1999. We compared data from Hospital Episode Statistics (HES) and the Public Health Laboratory Services (PHLS) for incidence estimates and from HES and the Office for National Statistics (ONS) for estimates of deaths. Estimated sensitivities for the examined systems were 46% (95% confidence interval [CI] 42% to 50%) for HES and 40% [95% CI 37% to 44%] for PHLS. Sensitivities for mortality rates were found to be similar, 48% [95% CI 41% to 55%] for HES and 49% [95% CI 42% to 56%] for ONS. Stratification analysis showed that the sensitivity in those >85 years of age was significantly lower than the sensitivity for other ages. The estimated case-fatality rate was 24% [95% CI 21% to 26%]. These estimates indicate that a cost-benefit analysis of adult pneumococcal vaccination programs is required.

Streptococcus pneumoniae is a leading cause of pneumonia, bacteremia, meningitis, and otitis media in children and adults. In the United Kingdom, respiratory infections account for an estimated 55% of all antimicrobial drug prescriptions (1). The emergence of pneumococci that are resistant to single or multiple antimicrobial drugs (2,3) and their association with outbreaks in child care centers and nursing homes underscore the need for new preventive strategies (4).

Pneumococcal meningitis represents a small but important component of the total illness and deaths from pneumococccal disease, resulting in a mortality rate of 25% and sequelae in excess of 50% of affected cases (5,6). Public health officials have emphasized the prevention of pneumococcal meningitis and invasive pneumococcal disease (IPD) (7–10). Compelling data support administration of pneumococcal conjugate vaccine to children (11) and considerable, although arguably less robust, evidence supports the view that the elderly should receive pneumococcal vaccination with a polysaccharide vaccine (12,13). If pneumococcal vaccination is introduced into the general adult population, however, accurate estimates of the extent and impact of invasive pneumococcal disease are needed.

Geographic differences in the distribution of IPD and the underreporting of infectious diseases are widely acknowledged (14). As an alternative to population-based surveys and active surveillance systems, which are resource intensive, methods such as capture-recapture analysis (15) have been used effectively for both chronic disease and infectious disease epidemiology (16,17).

We performed a capture-recapture analysis to provide better estimates of illness and deaths caused by invasive adult pneumococcal disease among adults in England. We have focused on meningitis, which is the most reliably identifiable manifestation of invasive pneumococcal disease in the available U.K. data sources (18).

Materials and Methods

Data Sources

Hospital Episode Statistics (HES) and Public Health Laboratory Services (PHLS)-reconciled laboratory reports (RLR) on pneumococcal disease incidence for England were compared, and HES and Office for National Statistics (ONS) reports of deaths in England were compared. Private hospitals and private laboratories rarely manage bacterial meningitis in England. Each of the three sources—RLR, HES, and ONS—covers the entire population of England, 39.4 million adults ages ≥16 years in 1999. HES data were available only for hospitals in England, preventing an analysis of a larger U.K. dataset.

HES includes medical information on all patients treated in or admitted to National Health Service (NHS) hospi-

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tals in England. Diagnoses are recorded using the 10th International Classification of Diseases (ICD X) coding system, based on the clinical diagnosis, which may be supported by laboratory diagnostic data. RLR comprise data from laboratory reports of invasive disease from PHLS and NHS microbiology laboratories to PHLS Communicable Disease Surveillance Centre, and the PHLS Respiratory and Systemic Infection Laboratory, which actively collects pneumococcal isolates from invasive disease cases since 1996, in England. ONS contains reports of all certified deaths in England and Wales. Information on the cause of death is recorded by using the ICD IX coding system on the basis of the diagnosis made by the coroner or medical officer in charge of patient.

Extraction of Data

All adults in England >16 years of age in whom pneumococcal meningitis was diagnosed were identified from the three data sources. Mortality data were available for 17% of the RLR cases and were therefore unreliable. RLR data for the year 2000 were not available at the time of the study, and the HES data were recorded as encompassing financial years 1996–1999, that is, from April of the respective year to March of the consecutive year. Therefore, the incidence analysis was restricted to April 1996 to December 1999. The analysis of mortality rates was conducted by using records from April 1996 to March 2000. For case-fatality estimates, mortality rate data were restricted to December 1999 to be comparable with incidence data.

Cases were extracted from the RLR data when *S. pneumoniae* was isolated from cerebrospinal fluid (CSF) or when the clinical diagnosis recorded was pneumococcal meningitis; from HES when ICD X: G001 was recorded in the primary diagnostic field; and from the ONS data for England when pneumococcal meningitis (ICD IX: 3021) was the primary cause of death.

Identification of Duplicates

Two or more HES records were considered to be multiple records if the patient had the same date of birth and sex, if the reports were within 3 months of each other, and if the cases were reported from the same hospital NHS trusts. However, records with different hospital NHS trusts, but with the same date of birth, same sex, and within 3 months of each other were considered duplicates when a referral pattern between NHS trusts (i.e., in the same or adjacent regions) could be identified. The earliest record was retained from each duplicate set for analysis. RLR records were considered to be duplicates if they had same date of birth and sex, same laboratory, and had been collected within 3 months of each other, whereas for ONS records, date of death and place of death were also included as criteria.

Matching and Capture-Recapture Analysis

For the incidence data, HES and RLR records between April 1996 and December 1999 were matched if the person shared the same date of birth and sex and if the date of hospital admission was within ± 30 days of the specimen date. For the mortality estimates, HES and ONS records were matched on date of birth and sex, health authority (same or adjacent), and date of death within ±2 days of date of end of episode. The number of unreported cases and the total number of cases in the population, estimated according to Hook and Regal (19), can be estimated by the following formula:

$$\hat{x} = \left(\frac{ac}{b}\right);\ \hat{N} = a + b + c + \hat{x}$$

where a is the number of cases reported to source Z only; b, the-number of matched cases; and c, the number of cases reported to source Y only; $\hat{x}$, number of unreported cases by any source and is the estimated total number of cases in a population. Incidence and mortality rate were calculated by using mid-year population estimates for each corresponding year (ONS: population Estimates Unit). The analysis was stratified by age and year to check for consistency of estimates. A sensitivity analysis was carried out by using more or less stringent matching criteria for identification of duplicates, matching on region and age. The case-fatality rate was calculated from the estimated total number of deaths and the estimated total number of cases in the population. For each source, the sensitivity of the reporting systems was calculated as the number of cases of pneumococcal meningitis reported by either source divided by the number of cases estimated from the capture-recapture analysis.

Statistical Analysis

A bootstrapping method was used to calculate approximate 95% confidence intervals (CI) for the estimated unreported cases, the estimated total number of cases, and the estimated sensitivities of the two data sources. To test-for-trend in the sensitivities across groups, weighted least squares regression was used. Sensitivities were weighted by the estimated total number in the population. Analyses were performed with Stata software (Stata Corporation, College Station, TX).

Validation of Data Sources

To validate the diagnosis and matching in the data extracts, consultant microbiologists in all PHLS laboratories in the South West Region of England were sent line-listings of RLR and HES extracts relating to their laboratory and acute NHS trust (a public agency that provides secondary healthcare services to the population of a certain geographic area in the United Kingdom), respectively.

They then verified the correctness of diagnosis and matching, according to their own laboratory records. They also obtained details from their local NHS trust of hospital episode diagnoses of pneumococcal meningitis for cross-checking against RLR and HES records.

Results

Incidence

Between April 1996 and December 1999, a total of 668 isolates of *S. pneumoniae* from patients with meningitis were documented in RLR, and 1,069 cases of pneumococcal meningitis were recorded in HES. After multiple records (20 from RLR and 332 from HES) and 2 records from the RLR in which date of birth and age were missing were excluded, 646 records in RLR and 737 in HES were retained for analysis. The mean age in RLR (55.8 years [range 16–97]) and HES datasets (55.3 years [range 16–96]) was similar, as was the sex distribution (52% in both were male) (Table 1).

Matching was possible in only 296 cases, demonstrating an overlap of fewer than half the records between the datasets, and we estimated that an additional 521 (95% CI: 477 to 568) cases were not captured by either source. The capture-recapture analysis thus showed 1,608 (95% CI 1,483 to 1,747) cases of adult pneumococcal meningitis. The estimated sensitivities of the data collection systems were 40% (95% CI 37 to 44) for RLR and 46% (95% CI: 42 to 50) for HES (Table 2). Sensitivity estimates varied by year of reporting, but no evidence of a trend was shown across the years (Table 2) or the age-groups (Table 3). The lowest sensitivities were observed in those ≥85 (16% and 19% for RLR and HES, respectively) and the sensitivities of both data sources were significantly lower in this age group than in those ≤84 ($p = 0.002$ and $p = 0.03$ for RLR and HES, respectively; Table 3). The annual incidence rate determined by capture-recapture estimates decreased from 1.36 per 100,000 per annum in 1996–1997 to 0.78 per 100,000 per annum in 1998–1999.

Matched cases did not vary significantly from cases not recaptured, when examined by patient's age or gender or by year. The mean difference between the specimen date (RLR) and date of episode (HES) for the matched cases was 0.14 days (range 10–24), with 70% of cases having the same date recorded in both datasets. Information on other covariates was largely consistent within the matched records (Table 1).

Mortality Rates

Between April 1996 and March 2000, 197 deaths from pneumococcal meningitis in England were reported by ONS, whereas 195 deaths were recorded in HES. The mean age in ONS (61.7 years [range 16–96]) and HES datasets (62.7 years [range 16–97]) was similar and had the same sex distribution (50% were male).

Capture-recapture analysis indicated 107 (95% CI 75 to 150) adult deaths from pneumococcal meningitis not reported by either source, resulting in an estimated 404 (95% CI: 350 to 466) deaths. The estimated sensitivity of ONS and HES was 49% (95% CI 42% to 56%) and 48% (95%CI 41% to 55%), respectively. The number of deaths ascertained by HES increased significantly compared to those ascertained by ONS (test-for-trend $p = 0.03$ and $p = 0.51$, respectively). Mortality rate, similarly to incidence, decreased over the study years from 0.30 to 0.15 per 100,000 per annum; thus, the case-fatality rate did not change and was estimated at 24% (95% CI 21% to 26%) (Table 4).

Table 1. Summary of data on pneumococcal meningitis for ages ≥16 years in England from the data sources used, Reconciled Laboratory Reports (RLR), Hospital Episode Statistics (HES), and the matched cases

	RLR (%)	HES (%)	Matched (%)
Year			
1996/97	133 (20.6)	239 (32.4)	51 (17.2)
1997/98	182 (28.1)	194 (26.3)	80 (27.0)
1998/99	151 (23.4)	190 (25.8)	80 (27.0)
1999[a]	180 (27.9)	114 (15.5)	85 (27.7)
Total	646	737	296
Age group			
16–19	16 (2.5)	20 (2.7)	8 (2.7)
20–24	16 (2.5)	16 (2.2)	7 (2.4)
25–44	145 (22.3)	175 (23.7)	64 (21.6)
65–74	135 (21)	140 (19)	57 (19.3)
75–84	75 (11.6)	78 (10.6)	30 (10.1)
85+	21 (0.15)	24 (3.2)	4 (1.3)
Total	646	737	296
Region			
North and York	85 (11.5)	62 (9.6)	25 (8.45)
Trent	77 (10.5)	77 (12)	44 (14.86)
West Midland	72 (9.7)	54 (8.3)	18 (6.08)
North West	101 (13.7)	69 (10.7)	41 (13.85)
Eastern	84 (11.4)	64 (10)	42 (14.19)
London	117 (15.9)	183 (28.3)	44 (14.86)
South East	113 (15.3)	47 (7.3)	43 (14.53)
South West	88 (11.9)	90 (13.9)	39 (13.18)
Total	646	737	296
Sex			
F	301 (46.6)	353 (48.0)	137 (46.3)
M	336 (52)	382 (51.8)	159 (53.7)
NR	9 (1.4)	2 (0.2)	0 (0)
Total	646	737	296
Death status			
N	42 (6.5)	597 (81)	16 (5.4)
Y	64 (10)	140 (19)	19 (6.4)
NR	540 (83.6)	0 (0)	261 (88.2)
Total	646	737	296

[a]Data not available for January–March 2000.
[b]F, female; M, male; NR, not recorded; N, no death; Y, yes, death occurred.

Sensitivity Analysis of the Incidence Data

Application of less stringent criteria, that is, matching on age instead of date of birth, yielded an additional 13

Table 2. Capture-recapture analysis for the number of cases of pneumococcal meningitis among adults (≥16 years) in England, April 1996–December 1999, by period[a]

	No. records in data sources			Capture-recapture analysis			
Period	RLR[a]	HES[b]	Matched records	Unreported cases (95% CI)	Total no. cases in population (95% CI)	Sensitivity RLR% (95% CI) p = 0.30	Sensitivity HES% (95% CI) p = 0.67
Apr 1996–Mar 1997	195	239	82	216 (156 to 300)	568 (487 to 668)	34 (28 to 40)	42 (35, 49)
Apr 1997–Mar 1998	168	194	70	174 (121 to 248)	466 (393 to 556)	36 (29 to 43)	42 (34, 49)
Apr 1998–Mar 1999	172	190	99	67 (46 to 96)	330 (286 to 379)	52 (45 to 59)	58 (50, 65)
Apr 1999–Dec 1999[b]	111	114	45	101 (64 to 158)	281 (228 to 350)	39 (31 to 49)	40 (31, 50)
All study period April 1996–Dec 1999	646	737	296	521 (434 to 625)	1,608 (1,483 to 1,747)	40 (37 to 44)	46 (42, 50)

[a]RLR, reconciled laboratory reports; HES, Hospital Episode Statistics; CI, confidence interval.
[b]RLR data not available for January through March 2000.

matched records. This gave an estimate for the total number of cases in the population of 1,541 cases, yielding a sensitivity of 42% for HES and 48% for RLR. Application of more stringent matching criteria, including matching on region, increased the estimated number of cases from 1,608 to 2,061, giving a sensitivity of 31% and 36% for HES and RLR, respectively.

Validation of Data Sources by Using Regional Data

Data were validated in 13 of the 17 laboratories, which included 76 (88%) of the 86 RLR records and 75 (86%) of the 87 HES records identified as originating in the South West Region of England. Thirty-eight cases matched between the sources, yielding an estimated capture-recapture total of 150.

Of the 38 original matches, 37 were confirmed as correct from laboratory records. Two additional matches were identified (one incorrect date of birth in HES, one in RLR). Of the remaining 35 records in HES but not in RLR, 17 had no laboratory record, 9 had laboratory evidence of pneumococcal meningitis, 8 had positive blood cultures for *S. pneumoniae*, and 1 had been incorrectly reported (meningitis due to group B streptococci). Of the 36 records in RLR but not in HES, 33 documented positive CSF cultures for *S. pneumoniae*, 1 noted septic arthritis, and 2 had no laboratory record. An additional five cases were identified (three only in laboratory records and two only in hospital trust records) that were not in the main study. When the two additional matches, the two incorrect diagnoses, and the five additional cases were taken into account, the capture-recapture estimate was unchanged at 150 cases.

Discussion

This capture-recapture analysis provides evidence of underascertainment of both incidence of and deaths from adult pneumococcal meningitis in England by the national laboratory and clinically based reporting systems. All surveillance systems compared in the study captured less than half of the estimated cases or deaths in the population. The sensitivity of these systems to capture cases occurring in those >85 years of age was significantly lower than in the younger population. Our findings are similar to capture-recapture estimates of bacterial meningitis in Italy (20), and our estimates of case-fatality rate (24%) are similar to those reported elsewhere (16%-31%) (21).

Apart from incomplete reporting in the surveillance systems, underascertainment may arise from the absence of a specific diagnosis in the severely ill, particularly the elderly (22); the absence of a confirmed microbiologic diagnosis (23) or misclassification of known pneumococcal meningitis as unspecified or unknown meningitis due to failure to collect blood or CSF samples. An active surveillance study conducted in the United States concluded that deaths due to invasive pneumococcal disease may be

Table 3. Capture-recapture analysis for the number of cases of pneumococcal meningitis among adults (≥16 years) in England, April 1996 to December 1999, by age group

	No. records in the data sources			Capture-recapture analysis			
						Sensitivity	
Age (y)	RLR	HES	Matched records	Unreported cases (95% CI)[a]	Total no. cases in population (95% CI)	RLR % (95% CI)	HES % (95% CI)
16–24	32	35	15	23 (9 to 52)	75 (52 to 110)	43 (26 to 60)	47 (29 to 65)
25–44	145	175	64	140 (96 to 205)	396 (333 to 476)	37 (29 to 44)	44 (36 to 52)
45–64	238	284	126	140 (103 to 188)	536 (474 to 607)	44 (39 to 50)	53 (47 to 59)
65–74	135	140	57	114 (75 to 170)	332 (276 to 402)	41 (33 to 49)	42 (34 to 51)
75–84	75	78	30	72 (41 to 125)	195 (151 to 257)	38 (28 to 49)	40 (29 to 51)
85+[b]	21	25	4	89 (–)	131 (–)	16 (–)	19 (–)

[a]CI, confidence intervals.
[b]Due to small numbers in the matching records cell, it was not possible to calculate the CIs for this age group.

Table 4. Capture-recapture analysis for the number of deaths from pneumococcal meningitis among adults (≥16 years) in England, April 1996 to March 2000, by period

	No. of records in the data sources[a]			Capture-recapture analysis				
Period	ONS	HES	Matched records	Unreported deaths (95% CI)	Total no. of deathsin population(95% CI)	Sensitivity ONS % (95% CI)	Sensitivity HES % (95% CI)	Case-fatality %[b] (95% CI)
Apr 1996–Mar 1997	65	55	28	36 (18 to 66)	128 (98 to 167)	51 (38 to 64)	43 (31 to 55)	23 (19 to 27)
Apr 1997–Mar 1998	51	57	20	57 (30 to 113)	145 (106 to 208)	35 (23 to 48)	40 (26 to 53)	31 (26 to 37)
Apr 1998–Mar 1999	44	45	25	15 (7 to 31)	79 (59 to 104)	56 (41 to 70)	57 (42 to 71)	24 (19 to 31)
Apr 1999–Mar 2000	37	38	22	11 (4 to 24)	64 (46 to 85)	58 (42 to 74)	58 (43 to 75)	12 (9 to 18)
All study period April 1996–March 2000	197	195	95	107 (75 to 150)	404 (350 to 466)	49 (42 to 56)	48 (41 to 55)	24 (21 to 26)[c]

[a]ONS, Office of National Statistics; HES, Hospital Episode Statistics; CI, confidence interval.
[b]Based on the number of cases in Table 1.
[c]Excluding deaths from January 2000 to March 2000 to be comparable with the number of cases in Table 1.

underestimated by 15%-45% and suggested that these missed cases could potentially be reported as unspecified deaths (24).

Our main estimates for incidence and deaths were largely supported by the sensitivity analysis. The slightly lower sensitivities estimated for 1999 HES data may be explained by the incomplete data for that year (see Methods). One possible explanation for the decreasing incidence during the study period may be a decrease in the number of diagnostic lumbar punctures performed (25,26). For pneumococcal bacteremia, PHLS reports show a small increase in incidence following the implementation of the enhanced surveillance for IPD in 1996, which (with the exception of a 1997 peak) remained relatively constant (27,28).

Strengths and Limitations of the Study

The use of capture-recapture analysis to estimate the incidence and mortality rates from pneumococcal disease has some drawbacks (29). However, in the absence of large population–based surveillance, this method can provide good estimates of disease incidence and associated deaths (30). The English national data sources used were assumed to be the most representative and complete for pneumococcal meningitis. The data were collected in each of the data sources in parallel and in a nonselective manner. The assumption that sources are independent is rarely fully met in epidemiology (31). We assume some positive dependence, in that the laboratory confirmation of a case (RLR) is likely to lead to a notification to HES and, if death occurs, notification to ONS. This dependence would have, if anything, led to an underestimation of both incidence and mortality rates (32). Negative dependence is unlikely. A third source of data for pneumococcal meningitis in England (clinician notification data) was available but did not have sufficient personal identifiers to include in the analysis. Therefore, we were unable to quantify dependency between the sources used in analysis. The capture-recapture estimates of the annual number of cases decreased during the study period. However, the sensitivities are relatively constant over time and no evidence was found for a trend over time; thus, we believe that estimates of sensitivity for the whole study period are valid.

We assume that the probability of cases being captured to all sources was not influenced by the characteristics of the case. The cases derived from the datasets were similar in terms of age, gender and outcome. Recaptured cases did not vary significantly from cases not recaptured, when examined by age, sex, or year (Table 1).

Accuracy of Diagnosis and Matching

Some diagnostic misclassification of pneumococcal meningitis may have occurred during the recording of HES and the ONS data (33,34). However, as discussed previously, that misclassification of pneumococcal meningitis as meningitis cause unspecified or cause unknown was more likely to have occurred. Nevertheless, even if misclassification occurs, capture-recapture analysis normally provides more reliable estimates than routine surveillance systems (35).

The study's matching strategy was supported by results of the validation study conducted in the South West. This study showed that matching without names for these data sources had a high degree of accuracy. An acceptable level of accuracy of recording laboratory-confirmed cases was also found for both RLR and HES datasets, with only two false-positive diagnoses being identified. The capture-recapture estimate did not change after validation.

Meningitis represents <10% of adult invasive pneumococcal disease (36). If all invasive pneumococcal positive isolates from the RLR had been included, the probability of including false cases in RLR would have greatly increased, thus increasing the probability of false matches and leading to an overestimation of the total number of cases. The inclusion of nonspecified meningitis (ICD 10 = G00.9) would have led to a similar increase in the probability of including false-positive cases in HES, and similarly of overestimating the results.

We restricted the analysis to records of pneumococcal meningitis from the primary diagnosis field of HES records

to avoid diagnoses not related to the reason for hospitalization and ensure precision of the estimates. As meningitis is commonly a serious condition, it is normally recorded as primary diagnosis in the first diagnostic field. An additional analysis including all diagnostic fields in HES gave only a marginal change in the sensitivities of the reporting systems (data not shown). Searching other diagnostic fields in the validation analysis did not identify any additional cases.

Possible Implications on Vaccine Prevention

Our capture-recapture analysis should be generalizable to other industrialized countries, with a similar epidemiology of adult pneumococcal meningitis and similar surveillance systems. Pneumococcal meningitis is associated with a high mortality rate and represents a reasonably robust indicator of the illness from invasive pneumococcal infection in the population (37). Therefore, this analysis should inform decision makers when considering prevention and control policies and orienting further research, as previous policies have been mainly based on PHLS data.

Studies suggest that rather than targeting high-risk groups, current vaccination policies for adults with pneumococcal polysaccharide vaccine (PPV) are most cost-effective if implemented for all persons >65 years (38; A. Melegaro, J. Edmunds, unpub. data). In the context of our revised estimates, the benefits of vaccination are likely to be even greater. Evaluation of the pneumococcal conjugate vaccination (PCV) indicates that population-based programs for infants and children are cost-effective compared with PPV or no vaccination (39,40). Mangtani et al. suggest that the conjugate vaccine may be more promising for preventing IPD among adults than PPV (41). Data from England and Wales show that the 7-valent PCV has about 77% of serotype coverage in adults >65 years of age and 51% in younger adults (27). Do our revised estimates of the incidence of and mortality rates from pneumococcal disease among adults justify PCV vaccination? We propose that a cost-benefit analysis of PCV vaccination of adults across a range of different ages is required. This analysis should take into account our revised estimates for incidence and deaths, the potential for immunologic boosting by further vaccination in old age, the impact of herd immunity, and the potential for a decrease in the carriage of antimicrobial-resistant strains (7,8,39–41).

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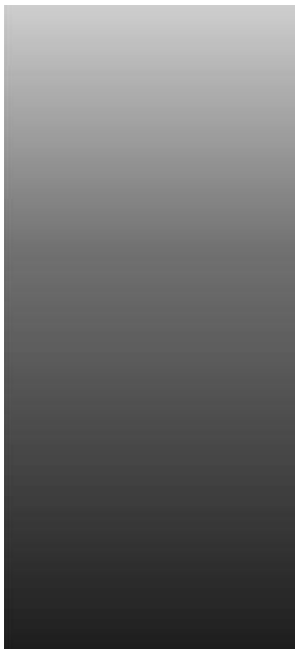

Fluoroquinolones Protective against Cephalosporin Resistance in Gram-negative Nosocomial Pathogens

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In a matched case-control study, we studied the effect of prior receipt of fluoroquinolones on isolation of three third-generation cephalosporin-resistant gram-negative nosocomial pathogens. Two hundred eighty-two cases with a third-generation cephalosporin-resistant pathogen (203 with *Enterobacter* spp., 50 with *Pseudomonas aeruginosa,* and 29 with *Klebsiella pneumoniae*) were matched on length of stay to controls in a 1:2 ratio. Case-patients and controls were similar in age (mean 62 years) and sex (54% male). Variables predicting third-generation cephalosporin resistance were surgery ($p = 0.005$); intensive care unit stay ($p < 0.001$); and receipt of a β-lactam/β-lactamase inhibitor ($p < 0.001$), a ureidopenicillin ($p = 0.002$), or a third-generation cephalosporin ($p < 0.001$). Receipt of a fluoroquinolone was protective against isolation of a third-generation cephalosporin-resistant pathogen ($p = 0.005$). Interventional studies are required to determine whether replacing third-generation cephalosporins with fluoroquinolones will be effective in reducing cephalosporin resistance and the effect of such interventions on fluoroquinolone resistance.

Resistance to third-generation cephalosporins in gram-negative nosocomial pathogens is a formidable problem, associated with adverse clinical outcomes and increased hospital costs (1–4). Measures to combat the emergence and spread of resistant nosocomial pathogens have met with varying degrees of success. Although good infection control practices are the most important measure in limiting the spread of resistance, other measures are required, including changes in antimicrobial drug–prescribing patterns through formulary modification and enhanced education of prescribers (5).

Kaye et al. reported a protective effect of fluoroquinolone use against the emergence of resistance to third-generation cephalosporins in nosocomial isolates of *Enterobacter* (6). In our study, we aimed to determine whether this protective effect is translated into an ecologic phenomenon by using individual patient-level data, i.e., whether fluoroquinolone use, in addition to lowering the likelihood of emergence of resistance in an individual patient, also results in reduced initial isolation of resistant strains in a given population. In addition, we aimed to determine whether the effect of fluoroquinolone use on *Enterobacter* spp. is applicable to other gram-negative pathogens. We conducted a matched case-control study to test the protective effect of fluoroquinolone use on the subsequent isolation of the three most common gram-negative hospital pathogens that are resistant to third-generation cephalosporins, *Enterobacter* spp., *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* (4).

Methods

Hospital Setting, Study Design, and Microbiology

During the study period, Beth Israel Deaconess Medical Center, West Campus, was a 320-bed, urban, tertiary-care teaching hospital, with 24 intensive care unit beds and approximately 12,000 admissions annually; the hospital serves a nonobstetric adult population in Boston, Massachusetts. Data were collected from administrative, laboratory, and pharmacy databases within this hospital by using relational database software (Access97, Microsoft, Redmond, WA). The microbiology database was searched to identify all cultures positive for nosocomial third-generation cephalosporin-resistant *Enterobacter* spp., *P. aeruginosa,* and *K. pneumoniae* in hospitalized patients from October 1, 1993, to June 1, 1998. To qualify for inclusion, an isolate had to grow from a culture taken no earlier than the host patient's second hospital day. For *Enterobacter* spp. and *K. pneumoniae*, third-generation cephalosporin resistance was defined as an MIC of ceftriaxone or ceftazidime of ≥16 μg/mL; resistance in *P. aeruginosa* was defined as an MIC of ceftazidime of ≥16 μg/mL.

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Patients whose clinical culture data demonstrated an isolate with the above criteria were considered case-patients. A patient could be included only once. To meet the criteria of appropriate selection of the reference group, which require that controls be derived from the same source population that gives rise to the cases (7), controls were selected randomly from hospitalized patients who did not have a positive culture for the studied organisms. Controls were matched to the cases in a 2:1 ratio on the basis of length of hospital stay until the positive culture was taken; thus at the time of matching, each control had been hospitalized as long as his or her index case-patient. This length of stay was characterized as the risk period.

Variables studied included patient demographics (age and sex), coexisting conditions (number of conditions, AIDS, diabetes mellitus, cardiovascular disease, hepatic disease, pulmonary disease, renal disease, and malignancy), hospital events during the risk period (surgery, intensive care unit stay), and receipt before the day of culture, for at least 24 hours, of an agent from any of the following antimicrobial drug classes: β-lactam/β-lactamase inhibitor combinations (mostly ampicillin/sulbactam and piperacillin/tazobactam), aminoglycosides (mostly gentamicin and tobramycin), first- or second-generation cephalosporins, third-generation cephalosporins (mostly ceftriaxone and ceftazidime), imipenem, ureidopenicillins (mostly piperacillin), and fluoroquinolones (mostly ciprofloxacin and ofloxacin). The route of administration of the antimicrobial agents was not considered, since the route was parenteral for all classes studied except fluoroquinolones. For fluoroquinolones, the nearly equivalent bioavailability between the oral and parenteral routes obviated the need to distinguish patients who received agents from this class orally from those who received them parenterally.

Statistical Analysis

Statistical analyses were performed by using SAS software (SAS Institute, Inc., Cary, NC, version 8e). Matched analyses were conducted by using a conditional logistic regression model. Variables with a p value of ≤ 0.05 on univariate matched analysis were included in a multiple conditional logistic regression model. Effect modification between factors was searched for by testing appropriate interaction terms for statistical significance. Effect estimates in the regression model were reported as hazard ratios; p values of ≤ 0.05 were considered significant.

Results

Demographics, Coexisting Conditions, and Hospital Events

Two hundred eighty-two patients with third-generation cephalosporin-resistant nosocomial target pathogens were enrolled in the study: *Enterobacter* spp. were isolated from 203 patients, *P. aeruginosa* from 50, and *K. pneumoniae* from 29. For all but two of these case-patients, two matched controls were enrolled per case; for each of the remaining two, one control was enrolled. Thus, 562 matched controls were included. Median length of stay before enrollment in the study was 12 days. Case-patients and controls were similar in age (mean 62.4 vs. 62.1 years; $p = 0.82$) and sex distribution (55.3% vs. 52.7% male; $p = 0.44$). Characteristics of the study patients and the matched univariate comparisons for case-patients and controls are summarized in Table 1. Case-patients had a significantly higher number of coexisting conditions than controls (hazard ratio [HR] 1.22; $p = 0.01$); specifically, case-patients had a higher prevalence of hepatic disease (HR 1.70; $p = 0.004$), pulmonary disease (HR 1.52; $p = 0.04$), and renal disease (HR 1.71; $p = 0.003$). Case-patients were significantly more likely than controls to have been in an intensive care unit (HR 2.65; $p < 0.001$) and to have had surgery (HR 2.03; $p < 0.001$) during the risk period.

Antimicrobial Drug Exposures

In the univariate analysis, case-patients were significantly less likely than controls to have received a fluoroquinolone (HR 0.48; $p = 0.008$). Case-patients were significantly more likely than controls to have received a β-lactam/β-lactamase inhibitor (HR 2.48; $p < 0.001$), a first- or second-generation cephalosporin (HR 1.39; $p = 0.04$), a third-generation cephalosporin (HR 2.98, $p < 0.001$), or a ureidopenicillin (HR 2.91, $p < 0.001$). There was also a trend toward greater use of aminoglycosides (HR 1.39; $p = 0.09$) and imipenem (HR 1.51; $p = 0.14$) in case-patients, but these associations did not achieve significance.

Multivariable Analysis

Results of the multivariable analysis are summarized in Table 2. Neither the total number of coexisting conditions nor the frequency of any individual condition was significantly different between cases and controls. After controlling for confounding variables, however, both hospital events examined (surgery and intensive care unit exposure) remained significantly associated with the isolation of a resistant gram-negative organism (HR 1.62; $p = 0.005$, and HR, 2.17; $p < 0.001$, respectively). Three antimicrobial drug classes remained significantly associated with isolation of a resistant pathogen: β-lactam/β-lactamase inhibitor combinations (HR, 2.52; $p < 0.001$), ureidopenicillins (HR, 2.55; $p = 0.002$), and third-generation cephalosporins (HR, 2.84; $p < 0.001$).

The only factor protective against isolation of a third-generation cephalosporin-resistant gram-negative pathogen was exposure to a fluoroquinolone. After controlling for confounding, the protective effect was even

Table 1. Characteristics of study patients and univariate analysis of outcome

Characteristic	Case-patients (n = 282) (%)	Controls (n = 562) (%)	HR (95% CI)[b]	p
Mean age (y)	62.4	62.1	1.00 (0.99 to 1.01)	0.82
Male	156 (55.3)	296 (52.7)	1.12 (0.84 to 1.50)	0.44
No. of coexisting conditions	0:14 (5.0)	0:47 (8.4)	1.22 (1.04 to 1.44)	0.01
	1:71 (25.2)	1:155 (27.6)		
	2:111 (39.4)	2:228 (40.6)		
	≥3:86 (30.5)	≥3:132 (23.5)		
AIDS	1 (0.4)	13 (2.3)	0.15 (0.02 to 1.18)	0.07
Cardiovascular disease	205 (72.7)	404 (71.9)	1.04 (0.80 to 1. 43)	0.81
Diabetes mellitus	124 (44.0)	259 (46.1)	0.92 (0.69 to 1.23)	0.57
Hepatic disease	66 (23.4)	86 (15.3)	1.70 (1.18 to 2.44)	0.004
Pulmonary disease	48 (17.0)	67 (11.9)	1.52 (1.01 to 2.28)	0.04
Renal disease	68 (24.1)	88 (15.7)	1.71 (1.20 to 2.44)	0.003
In intensive care unit during risk period	161 (57.1)	207 (36.8)	2.65 (1.91 to 3.68)	< 0.001
Malignancy	46 (16.3)	92 (16.4)	0.99 (0.67 to 1.47)	0.97
Surgery during risk period	164 (58.2)	229 (40.8)	2.03 (1.50 to 2.73)	< 0.001
Receipt of β-lactam/ β-lactamase inhibitor	111 (39.4)	125 (22.2)	2.48 (1.77 to 3.49)	< 0.001
Receipt of aminoglycoside	62 (22.0)	97 (17.3)	1.39 (0.95 to 2.04)	0.09
Receipt of 1st- or 2nd-generation cephalosporin	117 (41.5)	195 (34.7)	1.39 (1.01 to 1.92)	0.04
Receipt of 3rd-generation cephalosporin	114 (40.4)	122 (21.7)	2.98 (2.07 to 4.27)	< 0.001
Receipt of imipenem	27 (9.6)	37 (6.6)	1.51 (0.87 to 2.62)	0.14
Receipt of ureidopenicillin	42 (14.9)	32 (5.7)	2.91 (1.77 to 4.77)	< 0.001
Receipt of fluoroquinolone	23 (8.2)	79 (14.1)	0.48 (0.28 to 0.82)	0.008

[a]Outcome refers to the isolation of third-generation cephalosporin-resistant *Enterobacter* spp., *Pseudomonas aeruginosa*, or *Klebsiella pneumoniae* from a clinical specimen.
[b]HR, hazard ratio; CI, confidence interval.

more pronounced than on univariate analysis (HR, 0.4; p = 0.005). Subgroup analyses that used the same multivariable model showed a similar protective effect for fluoroquinolones against isolation of each of the three pathogens considered individually, though in the smaller two subgroups the results did not achieve significance.

Confounding by severity of illness was controlled for in the analysis by the inclusion in the final model of intensive care unit stay and surgery before culture, as both of these hospital events, particularly the former, are markers of disease severity. None of the individual coexisting conditions analyzed, nor the total number of such conditions, differed significantly between cases and controls on univariate analysis, and thus they were not included in the final model. Moreover, forcing the term for total coexisting conditions into the multivariable model expressly to control for confounding did not change the results for any of the significant terms.

Interaction terms between the following factors were analyzed: fluoroquinolone use and cephalosporin use, surgery and intensive care unit exposure, fluoroquinolone use and diabetes mellitus, and fluoroquinolone use and renal disease. None of these interaction terms achieved significance, and thus they were not included in the final model.

Discussion

Resistance to third-generation cephalosporins among gram-negative nosocomial pathogens is associated with increased mortality, length of stay, and hospital costs (1–4). Measures to reduce the extent of resistance are therefore warranted.

This study was designed to test the hypothesis that recipients of fluoroquinolones are protected against infection and colonization with the three most common third-generation cephalosporin-resistant gram-negative nosocomial pathogens, *Enterobacter* spp., *P. aeruginosa*, and *K. pneumoniae* (4). We have demonstrated a protective effect of fluoroquinolone use on infection or colonization with these resistant organisms both in crude analysis and after control for confounding variables. Moreover, subgroup analysis demonstrated this protective effect for each genus individually, though small numbers of patients with cul-

Table 2. Multivariable analysis of outcome[a]

Characteristic	HR (95% CI)[b]	p
Surgery during risk period	1.62 (1.16 to 2.25)	0.005
In intensive care unit during risk period	2.17 (1.49 to 3.16)	<0.001
Receipt of β-lactam/ β-lactamase inhibitor	2.52 (1.67 to 3.80)	<0.001
Receipt of ureidopenicillin	2.55 (1.43 to 4.53)	0.002
Receipt of 3rd-generation cephalosporin	2.84 (1.89 to 4.27)	<0.001
Receipt of fluoroquinolone	0.40 (0.21 to 0.76)	0.005

[a]Outcome refers to the isolation of third-generation cephalosporin-resistant *Enterobacter* spp., *Pseudomonas aeruginosa*, or *Klebsiella pneumoniae* from a clinical specimen.
[b]HR, hazard ratio; CI, confidence interval.

tures positive for *P. aeruginosa* and *K. pneumoniae* precluded statistical significance in these groups, due to the limited power associated with subgroup analysis. Other notable findings are that surgery, intensive care unit stay, and receipt of a β-lactam/β-lactamase inhibitor combination, a ureidopenicillin, or a third-generation cephalosporin increase the likelihood of recovery of these resistant pathogens. Although we did not match case-patients and controls based on date of admission, division of the entire study period into three time intervals showed the ratio of cases to controls to be approximately the same in each. The likelihood of spurious associations resulting from disparity between the year of hospitalization of cases and controls is therefore minimal.

Our analysis did not differentiate between infection and colonization with the pathogens studied. Since the focus of the study was the occurrence of third-generation cephalosporin-resistant nosocomial organisms in the population we studied, this distinction was not necessary. The organisms we studied are capable of causing infection in a given patient at any point after colonization. Moreover, once they have colonized a patient, they are capable of transmission to other hospitalized patients, in whom they can cause infection. Our objective, then, was not to compare rates of active disease between hospitalized groups, but rather to use the recovery of these organisms as a marker for actual or potential disease in the populations we examined.

In addition to infection control measures, such as active surveillance, hygiene, and isolation precautions, the other important strategy in checking the emergence and spread of antimicrobial resistance is the manipulation of selective antimicrobial pressure through changes in use of antimicrobial drugs (5). Previous studies exploring the effect of antibiotics on third-generation cephalosporin resistance focused on replacement of cephalosporins with other β-lactam–containing agents (8–11). No interventions involving a substitution with a fluoroquinolone have been reported.

Two main categories of β-lactamases mediate resistance to third-generation cephalosporins among the common gram-negative nosocomial pathogens: chromosomal β-lactamases and plasmid-associated extended-spectrum β-lactamases (ESBLs) (12). Enzymes that can confer resistance to most penicillins, cephalosporins, and monobactams, ESBLs belong to Bush-Jacoby-Medeiros functional group 2, whose enzymes are generally inhibited in vitro by β-lactamase inhibitors. By contrast, the chromosomal β-lactamases present in *Enterobacter* and *Pseudomonas* (which constitute 90% of the resistant isolates in our study) belong to group 1, whose enzymes are not inhibited by β-lactamase inhibitors (13).

Earlier studies described interventions carried out when plasmid-associated ESBLs were the main mechanism of resistance, so it is not surprising that replacing cephalosporins with a β-lactam/β-lactamase inhibitor combination, as was done in some of these studies (8,9,11), resulted in reduced rates of cephalosporin resistance. Our study, by contrast, found both ureidopenicillins and β-lactam/β-lactamase inhibitor combinations to be risk factors for the isolation of gram-negative organisms resistant to third-generation cephalosporins. We believe that this discrepancy relates to the fact that the predominant cause of resistance in our hospital during the study period was group 1 chromosomal β-lactamases (against which β-lactamase inhibitors are not active) and that plasmid-mediated ESBLs played only a minimal role (14).

Our findings expand on the observations of Kaye et al. regarding the protective effect of fluoroquinolones on the emergence of third-generation cephalosporin-resistant *Enterobacter* spp (6). They diverge, however, regarding risk factors. Although Kaye et al. found third-generation cephalosporin exposure to be an independent risk factor for emergence of resistance, no other antimicrobial exposure or hospital event was independently associated with this finding. We propose that the suggested discrepancy between these results and our findings that certain hospital events and antimicrobial classes confer enhanced risk for initial isolation of resistant organisms can be attributed to the difference in the design of the two studies.

Kaye et al., in examining emergence of resistance, identified clinical isolates for which β-lactamase production was induced or derepressed mutants were selected. Our study design, by contrast, detected those patients colonized or infected by an organism with preexisting third-generation cephalosporin resistance, a phenomenon made more likely by certain hospital events or antimicrobial drug exposures. Whereas Kaye's case-patients began with susceptible isolates that developed resistance after a specific exposure, our case-patients were enrolled with already resistant strains. Thus, while a particular hospital event or antimicrobial drug exposure may not induce β-lactamase production or select derepressed mutants, it may well confer enhanced susceptibility to the acquisition of a strain in which resistance mechanisms are already expressed.

Although we do not include molecular typing or epidemiologic data regarding patterns of antimicrobial drug use and colonization with resistant organisms, earlier studies conducted during our study period at the same institution have answered many of these questions (14,15). These studies showed that colonization with ceftazidime-resistant gram-negative bacilli in intensive care units during a nonoutbreak period was common, was probably acquired before admission to the unit, involved diverse strains, and was associated with prior exposure to a variety of β-lactam antimicrobial drugs.

The interventional studies performed to date replacing third-generation cephalosporins with other agents are not readily generalizable as they are prone to several possible biases: 1) they are before/after studies and are therefore prone to time effect bias; 2) they describe a group-level analysis and are therefore prone to ecologic bias (16); 3) the formulary intervention is usually coupled with improved infection control measures, causing difficulty in determining which measure is responsible for the noted effect; and 4) these studies are more likely to be reported and published when a positive effect is noted, i.e., publication bias.

Our data as well as those of Kaye et al. suggest that fluoroquinolones could be substituted for certain types of β-lactam antimicrobial drugs to prevent the emergence and lower the rates of isolation of the most common third-generation cephalosporin-resistant gram-negative nosocomial pathogens. The potential advantages of adding fluoroquinolones to the armamentarium of agents that can be used to combat third-generation cephalosporin resistance are several: they can be administered orally; they are relatively nontoxic and inexpensive; and they may allow the replacement of earlier generation cephalosporins, receipt of which has previously been identified as a risk factor for isolation of third-generation cephalosporin-resistant gram-negative organisms (15).

A limitation of retrospective analyses is the inability to prove what appear to be causal relationships. Statistical associations are interpreted as risk factors, and inverse associations as protective effects. Proof that fluoroquinolones are in fact protective against the isolation of nosocomial third-generation cephalosporin-resistant gram-negative pathogens, as suggested by the inverse association demonstrated here, will require animal models or prospective interventional studies. Such studies will also be required to determine whether reduced third-generation cephalosporin resistance will come at the cost of increased levels of fluoroquinolone resistance, a phenomenon to which Burke has referred as "squeezing the balloon" (17). Fluoroquinolone resistance, not addressed in our study, occurs primarily by means of chromosomal mutation (18), and resistant mutants could potentially be selected for by increased use of this class of antimicrobial agent. Our data, then, provide the impetus for further studies, including a prospective interventional trial to explore the overall protective efficacy of fluoroquinolones against multiresistant gram-negative pathogens.

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Evaluating Detection and Diagnostic Decision Support Systems for Bioterrorism Response

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We evaluated the usefulness of detection systems and diagnostic decision support systems for bioterrorism response. We performed a systematic review by searching relevant databases (e.g., MEDLINE) and Web sites for reports of detection systems and diagnostic decision support systems that could be used during bioterrorism responses. We reviewed over 24,000 citations and identified 55 detection systems and 23 diagnostic decision support systems. Only 35 systems have been evaluated: 4 reported both sensitivity and specificity, 13 were compared to a reference standard, and 31 were evaluated for their timeliness. Most evaluations of detection systems and some evaluations of diagnostic systems for bioterrorism responses are critically deficient. Because false-positive and false-negative rates are unknown for most systems, decision making on the basis of these systems is seriously compromised. We describe a framework for the design of future evaluations of such systems.

During the 2001 anthrax attacks, emergency response personnel, clinicians, laboratories, and public health officials were overwhelmed by requests for evaluation of suspicious powders and by calls from patients concerned about exposure to bioterrorism agents (1–4). From October through December 2001, the New York City Bioterrorism Response Laboratory processed >3,200 environmental specimens (2). In the 2 months after the discovery of anthrax in the Trenton, New Jersey, postal system, state police responded to >3,500 false alarms involving suspected anthrax (3). These services were provided at great cost (e.g., as of November 2001, Philadelphia spent $10 million to investigate and test anthrax threats) (3). Systems to detect bioterrorism agents in clinical and environmental samples and to diagnose bioterrorism-related illnesses are essential components of responses to both hoaxes and actual bioterrorism events.

First responders and public health officials require sensitive and specific detection systems that can identify bioterrorism agents early enough to take action that limits the spread of disease. Additionally, clinicians may benefit from diagnostic decision support systems, typically designed to generate a list of possible diagnoses for a given patient on the basis of clinical features, if these systems appropriately increase clinicians' consideration of bioterrorism agents.

Under the auspices of the University of California-San Francisco-Stanford Evidence-based Practice Center, we prepared a comprehensive systematic review that evaluated the ability of available information technologies and decision support systems to serve the information needs of clinicians and public health officials during a bioterrorism response (5). We describe the published evidence of evaluations of available detection systems and diagnostic decision support systems for bioterrorism-related illness. We then describe a framework that could be applied to future evaluations of these systems to determine whether they are likely to serve information needs of their users during a bioterrorism response.

Methods

We performed a systematic review of descriptions and evaluations of systems for detection of bioterrorism agents and diagnostic decision support systems that could facilitate decision making for patients with undiagnosed bioterrorism-related illness. We provide a brief overview of our methods, which are described in detail elsewhere (5).

We included reports of systems specifically designed to support the diagnosis of bioterrorism-relevant diseases or syndromes, as defined by the U.S. Department of Health and Human Services (6). We also included reports of gen-

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eral diagnostic systems (e.g., systems that provide differential diagnoses based on a patient's signs or symptoms), automated diagnostic test analysis systems, microbiologic test analysis systems for bioterrorism-specific agents, radiologic diagnostic systems that automatically make the diagnosis of pulmonary infiltrate or widened mediastinum, and rapid detection technologies. For all potentially relevant systems, reports were included if they, at a minimum, provided information about the system's purpose, hardware requirements, type of information or sample required by the system, and type of information provided by the system.

Literature Sources and Search Strategies

We searched five databases of peer-reviewed articles (e.g., MEDLINE, National Technical Information Service, GrayLIT), Web sites of relevant government agencies (e.g., U.S. Department of Energy), and relevant nongovernmental Web sites. We included terms such as bioterrorism, biological warfare, decision support system, detection, diagnosis, radiology information systems, and public health. We also reviewed conference proceedings and reference lists of included articles.

Study Selection and Data Abstraction

We screened peer-reviewed articles to determine if they met inclusion criteria. Two investigators blinded to study authors independently abstracted articles onto pretested abstraction forms. Data abstracted from each report varied, depending on the type of system described. For descriptions of detection systems, we abstracted information about the system's portability, ability to run more than one sample at a time, and ability to detect more than one bioterrorism agent. For descriptions of diagnostic decision support systems, we recorded whether bioterrorism-related illnesses were included in the system's knowledge base, how the system enabled updates of the probability of bioterrorism-related illness as an epidemic progresses, the method of reasoning used by the inference engine, and whether the system used a standard vocabulary.

Criteria for Evaluating Reports of Included Systems

A complete description of the methods used to develop our evaluation criteria for reports of detection systems and diagnostic decision support systems can be found elsewhere (5). Briefly, we reviewed reports of naturally occurring and bioterrorism-related outbreaks and solicited information from relevant experts to describe the detection and diagnostic decisions that clinicians and public health officials would have to make while responding to bioterrorism. We then described the capabilities of detection and diagnostic systems necessary to assist these decisions. We augmented this list of system characteristics with previously published standards for evaluating information technologies and diagnostic tests to develop evaluation criteria for systems designed to facilitate detection and diagnosis during a bioterrorism response (5). We did not attempt to independently evaluate detection systems and diagnostic decision support systems; rather, we relied on information provided in the published reports about these systems.

Results

We reviewed 17,510 citations of peer-reviewed articles, 6,981 Web sites of government agencies, and 1,107 nongovernmental Web sites. From these, we included 115 reports of 78 potentially relevant systems for a bioterrorism response (55 detection systems and 23 diagnostic decision support systems). We first present the evaluative data about the detection systems and then the evaluative data about the diagnostic decision support systems.

Detection Systems

We identified 55 detection systems including 4 systems that collect aerosol environmental samples; 14 particulate counters or biomass indicators that detect an increase in the number of particles in aerosol samples over baseline; 27 identification systems designed to rapidly detect bioterrorism agents collected from environmental, human, animal, or agricultural samples; and 10 systems that integrate the collection, identification, and communication of detection results (5). Other detection systems exist; however, we describe all of the systems for which we found publicly available information through the search methods described.

Only 8 of the 55 detection systems had published evaluations (Tables 1 and 2). No system was evaluated for all the evaluation criteria. Timeliness was described for 33 of the 55 detection systems. Of these systems, 20 were described in specific terms such as minutes or hours, whereas 13 systems were described in nonspecific terms such as "rapid" or "real-time". Several reports included general statements about system sensitivity or detection limits; however, studies of only 1 of the 55 detection systems specifically reported both sensitivity and specificity (Table 2) (14–18).

Of the four collection systems, we found evaluation data only for BioCapture (Meso Systems Technolgy, Inc., Albuquerque, NM), a device that has been used by fire departments in Seattle, Los Angeles, and New York among other sites to collect environmental samples for subsequent testing for bioterrorism agents (8). Although its sensitivity and specificity were not described, the BioCapture system had a collection efficiency reported to be 50% to 125% relative to reference standards (8). Reports on three other systems also included a comparison of the system under evaluation to a reference standard (Anthrax Sensor [7], MiniFlo [12], and the Fluorescence-based array [10]).

Table 1. Summary of evaluation data for detection systems and diagnostic decision support systems for a bioterrorism response

Evaluation criteria	Detection systems evaluated % (yes/total)	Diagnostic decision support systems evaluated % (yes/total)
Is the timeliness of diagnostic information described?	36 (20/55)	48 (11/23)
Are diagnostic sensitivity and specificity described?	1.8 (1/55)	13 (3/23)
Is the reference standard against which the system was compared described?	7 (4/55)	39 (9/23)
Are the system's security measures described?	0	0
Is the evaluation of the system over a range of clinical situations or patient populations described?	0	0
Is the portability of the system described?	54 (15/28)	NA[a]
Is the system's ability to run more than one sample at a time described?	10 (4/41)	NA
Is the system's ability to detect more than one bioterrorism agent described?	32 (12/37)	NA
Is the system's ability to detect either/both toxins and organisms described?	5 (2/37)	NA
Is the inclusion of all bioterrorism agents and associated illnesses in the system's knowledge base described?	NA	26 (5/19)
Is the flexibility to update the probability of bioterrorism-related illness as the epidemic progresses described?	NA	0
Is the method of reasoning used by inference engine described?	NA	26 (5/19)
Is the use of standard vocabulary described?	NA	0

[a]NA; not applicable.

Most identification systems are limited in that they can evaluate a sample for only a single bioterrorism agent in each test cycle, they often run only a limited number of samples at a time, and they cannot test for many bioterrorism agents of concern (e.g., smallpox). None of the reports of the detection systems described methods for maintaining the security of the sample or test results or evaluated the systems in different clinical settings or among different populations. We found no studies that directly compared two or more systems in any given category.

In response to the 2001 anthrax cases, considerable interest was generated in the handheld antibody-based detection tests such as the Sensitive Membrane Antigen Rapid Test (SMART) (New Horizons Diagnostic Corp., Columbia, MD) and the Antibody-based Lateral Flow Economical Recognition Ticket (ALERT) (14–18). Such systems use antibodies to recognize specific targets on the toxins, antigens, or cells of interest (13,14). Limitations of these tests include nonspecific binding of the antibodies, which may lead to false-positive results and degradation of the antibodies over time, which may lead to false-negative results (13,14). Additionally, these tests are limited by the availability of antibodies. Given concerns about the diagnostic sensitivity and specificity of hand-held, antibody-based tests when used during the anthrax attacks, the Federal Bureau of Investigation and Centers for Disease Control and Prevention performed an independent evaluation of these tests (19). Although these results are not yet publicly available, the July 2002 Statement by the U.S. Department of Health and Human Services regarding hand-held assays for identification of *Bacillus anthracis* spores stated, "These studies confirm the low sensitivity of such assays and their potential to produce false-positive results with non-anthrax bacteria and chemicals. The performance of handheld assays for the detection of biological agents other than *B. anthracis* has not been evaluated and their use is also not recommended at this time" (20). Instead, law enforcement should transport samples quickly to a Laboratory Response Network facility, where cultures will be performed and preliminary results made available within 12 to 24 hours (20).

Several detection systems were designed in part, if not fully, by the military, and battlefield evaluations may have been performed. However, the paucity of publicly available information about such evaluations prevents conclusions about whether these systems will serve the detection needs of first responders and clinicians. Moreover, even if battlefield evaluation data were available, these systems would require additional study to confirm their utility for civilian users.

Diagnostic Systems

We identified 23 diagnostic decision support systems that may enhance clinician consideration of bioterrorism-related illness. We found six general diagnostic systems, four systems designed to improve radiologic diagnoses, four telemedicine systems, and nine systems for other diagnostic purposes (5). None has been formally evaluated with respect to a bioterrorism response; however, 15 diagnostic decision support systems had published evaluations for potentially analogous situations (Table 3).

The general diagnostic decision support systems are typically designed to assist clinicians develop a differential diagnosis list on the basis of patient-specific signs and symptoms. The included general decision support systems require manual entry of patient information by clinicians (Table 3). They use probabilistic or rules-based inference to compare patient information with a knowledge base to

Table 2. Evaluation data for detection systems for bioterrorism agents

System name	Purpose	Evaluation data[b]
Anthrax Sensor (7)	A portable detection system for "highly sensitive detection of biological agents within seconds" (7).	Reported to be capable of detecting endotoxins at a level that is "20 times lower than previously achieved by similar devices" (7).[c]
BioCapture (8)	A portable collection system for use by first responders.	Was compared to an All Glass Impinger (AGI) that collects samples into liquid and a Slit Sampler that impacts bacteria directly onto growth media and found to have a collection efficiency of 50%-80% relative to the AGI and 60%-125% relative to the Slit Sampler (8).[c]
Digital Smell/Electronic Nose (9)	To detect and classify microorganisms according to the volatile gases given off during metabolism.	An array of 15 sensors was able to correctly classify 68 of 90 colonies containing 0 or 1 of 5 test organisms and an uninoculated control; however, it registered 22 of 90 as false-positives (9).
Fluorescence-based array immuno-sensor (10)	To provide simultaneous, antibody-based detection of bioactive analytes in clinical fluids.	Bioterrorism agents intended to be detected include *Staphylococcus* enterotoxin B and F1 antigen from *Yersinia pestis*. It was unable to detect *S.* enterotoxin B levels (<125 ng/mL) in experimentally spiked urine, saliva, and blood products; sensitivity for F1 antigen from *Y. pestis* was reported at 25 ng/mL (10).
LightCycler; Ruggedized Advanced Pathogen Identification Device (RAPID) (11)	LightCycler uses a PCR cycler for "real-time" quantification of DNA samples. RAPID is a rugged, portable system that uses LightCycler technology for field detection of bioterrorism agents.	RAPID is reported by the manufacturer to be **99.9% specific** (11). For each assay, the sensitivity is set to half the infective dose (for example, the infectious dose of foot and mouth disease is 10 virus particles; RAPID's sensitivity is set to detect 5 virus particles [11]).[c]
MiniFlo (12)	For rapid, portable detection of multiple biological agents using flow cytometry.	Detected 87% of unknown biological agent simulants, including agents similar to anthrax and plague, with a false-positive rate of 0.4% (12). Bioterrorism agents identifiable: *Y. pestis* and *Bacillus anthracis*, as well as other viruses, bacteria and proteins (12).
Model 3312A Ultraviolet Aerodynamic Particle Sizer (UV-APS) and Fluorescence Aerodynamic Particle Sizer-2 (FLAPS-2) (13)	To detect living organisms in aerosols and nonvolatile liquids.	FLAPS-2 was able to detect 39 of 40 blind releases of stimulant aerosols (of particle ranging in size from 0.5 to 15 μm) at a distance of about 1 km with no false alarms during a 3-week period. In another trial, it was able to detect as few as 10 agent-containing particles per liter of air (13,4).
Sensitive Membrane Antigen Rapid Test (SMART) and the Antibody-based Lateral Flow Economical Recognition Ticket (ALERT) (14–17)	A handheld antigen/antibody test for the rapid detection of bioterrorism agents.	When field tested during the Gulf War, the SMART system had an "alarmingly" high false-positive rate thought secondary to contamination (14). SMART tests are reported per the manufacturer to have a **96% to 99% sensitivity** and **94% to 99% specificity** for *Vibrio cholerae* O139 and O1) (14–17)

[a]PCR; polymerase chain reaction.
[b]Where possible, we report sensitivity and specificity data (and highlight them in bold); if the published reports did not provide these values directly but did provide sufficient data for them to be calculated, we performed these calculations.
[c]Denotes systems for which available evaluation data were from manufacturers' Web sites only.

generate a differential diagnosis list that is typically ranked in descending order of likelihood. Some of the systems provide additional information about the suspected diseases and suggest appropriate diagnostic tests if clinicians choose to pursue these diagnoses.

Three diagnostic decision support systems (Columbia–Presbyterian Medical Center Natural Language Processor, Neural Network for Diagnosing Tuberculosis, and SymText) were specifically evaluated for both sensitivity and specificity and typically performed better than physicians-in-training but not as well as experienced clinicians (22,32,35,36). Also, the accuracy of the decision support systems decreased for difficult cases. The need for clinicians to manually enter patients' data into diagnostic decision support systems, a laborious step that may be a barrier to the use of these systems and increases interuser variability, is eliminated by the few systems that automatically collect patient data from an electronic medical record (21,22,35,36). For example, diagnostic decision support systems currently available in hospitals with electronic medical records provide clinicians with an estimate of the likelihood of community-acquired pneumonia or active pulmonary tuberculosis based exclusively on data collected from the medical record (21,32,33,35,36).

Two infectious disease diagnostic decision support systems, The Computer Program for Diagnosing and Teaching Geographic Medicine and GIDEON, included most of the bioterrorism-related organisms in their knowledge bases (23,28). In an evaluation of The Computer Program for Diagnosing and Teaching Geographic Medicine, the system correctly identified 222 (75%) of 295 cases and 128 (64%) of 200 hypothetical cases (23). The clinical diagnosis was included in the computer differential diagnosis list in 95% of cases. Several cases included in this evaluation were for the causative agents of anthrax, brucellosis, cholera, Lassa fever, plague, Q fever, and tularemia.

An evaluation of GIDEON compared its diagnostic accuracy to that of medical house officers admitting 86 febrile adults to the hospital (28). The house officers listed

Table 3. Evaluation data for diagnostic decision support systems for bioterrorism-related illness

System name	Purpose	Evaluation data[b]
Clinical decision support system for detection and respiratory isolation of tuberculosis patients (21)	To automate the detection and respiratory isolation of patients with positive cultures and chest x-rays suspicious for TB.	In a retrospective analysis, the system increased the proportion of appropriate TB isolations in inpatients from 51% to 75% but falsely recommended isolation of 27 of 171 patients. In a prospective analysis, the system correctly identified 30 of 43 of patients with TB but not identify 21 of these patients (false-negatives). However, the decision support system identified 4 patients not identified by the clinicians (21).
Columbia–Presbyterian Medical Center Natural Language Processor (22)	To automate the identification of 6 pulmonary diseases (including pneumonia) through analysis of radiology reports.	The system had a **sensitivity of 81%** (95% confidence interval [CI] 73% to 87%) and a **specificity of 98%** (95% CI 97% to 99%) compared to physicians who had an average sensitivity of 85% and specificity of 98% (22).
Computer Program for Diagnosing and Teaching Geographic Medicine (23)	To provide a differential diagnosis of infectious diseases matched to 22 clinical parameters for a patient; also to provide general information about infectious diseases, anti-infective agents, and vaccines.	The computer program correctly identified 75% (222 of 295) of the actual cases and 64% (128 of 200) of the hypothetical cases of patients with infectious diseases (23). The clinical diagnosis was included in the computer differential diagnosis list in 94.7% of cases. Among the cases included in this evaluation, several were for bioterrorism diseases (23).
DERMIS (24,25)	To provide a differential diagnosis of skin lesions.	The system correctly diagnosed lesions 51% to 80% of the time and included the correct diagnosis among its top 3 choices 70% to 95% of the time (out of a total of 5,203 cases) (24,25). The system was more accurate for dermatologist users than general practitioners.
Dxplain (26)	To provide a differential diagnosis based on clinician-entered signs and symptoms. The system includes descriptions and findings for potential bioterrorism agents, and is updated weekly to account for potential outbreaks.	In an evaluation of 103 consecutive internal medicine cases, Dxplain correctly identified the diagnosis in 73% of cases, with an average rank of 10.7 (the rank of a diagnosis refers to its position on the differential diagnosis—for example, the diagnosis with the greatest likelihood of being the actual disease is ranked first and the next most likely diagnosis is ranked second) (26).
Fuzzy logic program to predict source of bacterial infection (27)	To use age, blood type, gender, and race to predict the cause of bacterial infections.	The program was able to correctly classify 27 of 32 patients into 1 of 4 groups based on demographic data alone (27).
Global Infectious Disease and Epidemiology Network (GIDEON) (28)	To provide differential diagnoses for patients with diseases of infectious etiology. All potential bioterrorism agents as specified by CDC are included in the GIDEON knowledge base (28).	Whereas medical house officers listed the correct diagnosis first in their admission note 87% of the time (for 75 of 86 patients), GIDEON provided the correct diagnosis for 33% (28 of 86 patients) (28).
Iliad (and Medical HouseCall which is a system for consumers derived from Iliad) (29–31)	To provide a differential diagnosis based on clinician-entered signs and symptoms. The knowledge base is focused in internal medicine and was last updated in 1997.	In a multicenter evaluation, each of 33 users analyzed 9 diagnostically difficult cases. On average, Iliad included the correct diagnosis in its list of possible diagnoses for 4 of the 9 cases, and included the correct diagnosis within its top 6 diagnoses for 2 of the 9 cases. The differential diagnosis generated by Iliad is not dependent upon the level of training of the user (29–31).
Neural Network for Diagnosing Tuberculosis (32)	To predict active pulmonary TB (using clinical and radiographic information) so that patients may be appropriately isolated at the time of admission.	The neural network correctly identified 11 of 11 patients with active TB (**100% sensitivity, 69% specificity)** compared with clinicians who correctly diagnosed 7 of 11 patients (64% sensitivity, 79% specificity) (32).
PNEUMON-IA (33)	To diagnose community-acquired pneumonia from clinical, radiologic and laboratory data.	The decision support system correctly identified pneumonia in 4 of 10 cases, compared with between 3 and 6 cases for the clinician experts (33).
Quick Medical Reference (QMR) (34)	To provide a differential diagnosis based on clinician-entered signs and symptoms.	One prospective study used QMR to assist in the management of 31 patients for which the anticipated diagnoses were known to exist in the QMR knowledge base. In the 20 cases for which a diagnosis was ultimately made, QMR included the correct diagnosis in its differential in 17 cases (85%) and listed the correct diagnosis as most likely in 12 cases (60%) (34).
SymText (35,36)	To analyze radiology reports for specific clinical concepts such as identifying patients with pneumonia.	Average **sensitivity and specificity** for assessing the location and extension of pneumonia was 94% and 96% for physicians and **34% and 95%** for SymText. In selecting patients who are eligible for the pneumonia guideline, the area under the ROC curves was 89.7% for SymText and 93.3% for physicians (35,36).
Texas Infectious Disease Diagnostic Decision Support System (37)	To provide a weighted differential diagnosis based on manually entered patient information.	The system was compared to a reference standard that missed the diagnosis of 98 of 342 cases of brucellosis. In 86 of the 98 patients, this system listed brucellosis in the top 5 diagnoses on the differential diagnosis list, and in 69 of these 98 patients, brucellosis was the only disease suggested by the system. The system missed the diagnosis in 12 of 98 patients. On average, without the system it took 17.9 days versus 4.5 days with the system to suspect the correct diagnosis (37).

Table 3 continued. Evaluation data for diagnostic decision support systems for bioterrorism-related illness

System name	Purpose	Evaluation data[b]
University of Chicago – Artificial Neural Network for Interstitial Lung Disease (38)	To help radiologists differentiate among 11 interstitial lung diseases by using clinical parameters and radiographic findings to develop a differential diagnosis.	Areas under the ROC curve obtained with and without the system output were 0.911 and 0.826 ($p < 0.0001$), respectively (38).
University of Chicago – Computer Aided Diagnosis of Interstitial Lung Disease (39)	To aid in the detection of interstitial lung disease in digitized chest radiographs.	Areas under the ROC curve obtained with and without computer-aided diagnostic output were 0.970 and 0.948 ($p = 0.0002$), respectively (39).

[a]TB, tuberculosis; CDC, Centers for Disease Control and Prevention; ROC, receiver-operating characteristic curve.
[b]Where possible, we report sensitivity and specificity data (and highlight them in bold); if the published reports did not provide these values directly but did provide sufficient data for them to be calculated, we performed these calculations.

the correct diagnosis first in their admission note 87% (75/86) of the time compared with 33% (28/86) for GIDEON (28). To limit the differential diagnosis provided by the system, users enter the geographic area where the outbreak occurred. This geographic information is compared with the known areas of natural occurrence. Adding this geographic information could falsely decrease the probability of disease if a bioterrorism agent were used in a region that had little naturally occurring disease from that organism.

Many diagnostic decision support systems use probabilistic information about the likelihood of disease. Because bioterrorism-related illness is relatively rare, in the event of bioterrorism these systems will have inappropriately low pretest probabilities for bioterrorism agents. Only Dxplain was described as being able to change the probability of disease based on information about suspected bioterrorism events to improve the system's performance (26). Additionally, no report specifically described restricting access to the system by user type or other security measures.

Discussion

We systematically examined the 115 published reports of 55 detection and 23 diagnostic systems for bioterrorism responses. We found that technologies are increasingly available to assist detection and diagnostic tasks involved in a bioterrorism response but that only 23 systems were evaluated according to one or more evaluation criteria. Of these, 13 were compared to a reference standard test, none was evaluated in a range of clinical situations or in different populations, and only 4 reported both sensitivity and specificity. This remarkable lack of published evaluation data markedly affects both purchasers and users of such technologies. Decision makers will find it difficult to choose systems for purchase as they make resource allocations for bioterrorism preparedness. Users of these technologies may find it difficult to interpret the detection and diagnostic information provided by these systems. For example, if a first responder were asked to determine the presence or absence of a bioterrorism agent in a suspicious powder using a detection system with a high false-positive rate, he may cause unnecessary evacuation of environments suspected to be contaminated, work stoppages, and anxiety. In contrast, if a first responder used a system with high false-negative rate, he may have missed a bioterrorism agent, thereby risking excessive disease and death. Thus, for detecting and diagnosing bioterrorism-related illness, users require systems that are both highly sensitive and specific. Because ideal systems with near perfect sensitivity and specificity do not currently exist, and may be very difficult to produce for use in the field, users of available systems are faced with substantial challenges when interpreting the results from diagnostic tests.

We can illustrate the critical importance of sensitivity and specificity of detection systems by considering the anthrax attacks of fall 2001. The Trenton, New Jersey, state police evaluated >3,500 samples of suspicious powders, and none contained anthrax (3). For the purpose of illustration, let us assume that, before testing, 5 of these 3,500 samples were estimated to contain anthrax (i.e., pretest probability equals 0.0014). If a detection test had a sensitivity of 96% and specificity of 94% (i.e., the lower range reported for SMART/ALERT), we can calculate the posttest probability of anthrax with both positive and negative test results by using Bayes' theorem (40). If such a detection system indicated a positive result, the probability that the sample contained anthrax would be approximately 2%. That is, 98% of the positive results would be false-positives. If the system indicated a negative result, the probability of anthrax in the sample would be 0.006%. Thus, the test would be useful when negative, but provide little help if positive. If the sensitivity and specificity of the detection systems were both 99% (i.e., the upper range reported for SMART/ALERT), the posttest probability after a positive test would be 12%, and after a negative test, virtually 0. Thus, even with a specificity of 99%, only 12% of samples indicated as positive would contain anthrax, and 88% would be false-positive results. This relationship between a diagnostic test's sensitivity and specificity and the pretest probability of disease is depicted in Figure 1.

This example illustrates the challenges for bioterrorism detection systems. Testing will often be done at very low pretest probabilities. Thus, a bioterrorism detection system must have very high specificity or the vast majority of pos-

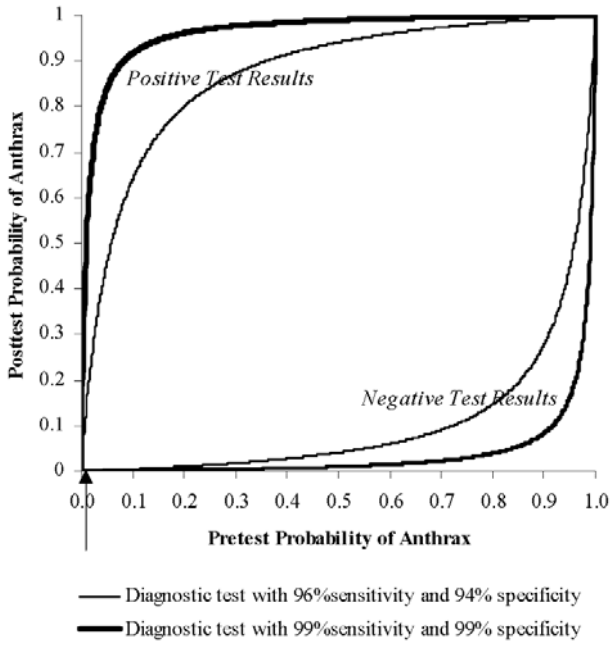

Figure 1. Effect of sensitivity, specificity, and pretest probability on posttest probability of anthrax's being present. Upper curves show the posttest probability of anthrax's being present after a positive detection or diagnostic test result. Lower curves show the posttest probability of anthrax's being present after a negative detection or diagnostic test result. Separate curves are drawn for two diagnostic tests described in the text: one with 99% sensitivity and 99% specificity (thick) and another with 96% sensitivity and 94% specificity (thin). The arrow marks a pretest probability of disease of 0.0014, which relates to the example described in the text.

itive results will be false-positives. In contrast, under circumstances when testing is performed at relatively high pretest probability (for example, in a heavily contaminated building), a negative test result will only be convincing if the sensitivity of the system is very high. Thus, interpretation of diagnostic test results requires ongoing evaluation of the pretest probability of a bioterrorist attack.

A common approach to minimize false-negative and false-positive results is to perform confirmatory tests after initial tests are completed. Such use of tests in sequence creates additional difficulties interpreting their results. Under ideal conditions for sequential tests, we can use the posttest probability of the first test as the pretest probability of the second test to calculate the posttest probability after the confirmatory test. This calculation is only accurate, however, if the sensitivity and specificity of the confirmatory test are the same regardless of whether the initial test was positive or negative. If this circumstance is not met, investigators must measure the sensitivity and specificity of the confirmatory test in samples or populations with negative and positive results on the initial test. This information is rarely available.

Sensitivity and specificity are defined only for a test with two outcomes, such as positive or negative. For tests with multiple outcomes, such as a detection system that identifies multiple agents, investigators can characterize the performance of the test with likelihood ratios (40). Users can calculate the posttest probability for such a test with the likelihood ratio form of Bayes' theorem (40).

Evaluation of diagnostic decision support systems is more complex because the purpose of these systems is typically to generate a differential diagnosis. Thus, the evaluation determines the appropriateness of the differential diagnosis, and perhaps, if the diseases in the differential diagnosis are ranked, how high the correct disease is ranked. Specific recommendations for evaluation of decision support systems have been published elsewhere (5). The studies of the diagnostic decision support systems included in Table 3 use a variety of approaches to assess the performance of the systems. However, only two have been evaluated specifically for capture of diseases caused by bioterrorism agents in the differential diagnosis list. Many of the systems require manual entry of patient data, and none are in widespread use. Based on the available evidence, we conclude that the available diagnostic decision support systems will be of limited usefulness in response to a bioterrorism event.

Recommendations for Study Design of Detection Systems

For the purpose of evaluation, detection systems have much in common with diagnostic tests. Published guidelines for evaluating diagnostic tests are well established and promote study designs that provide unbiased estimates of both sensitivity and specificity (or likelihood ratios) relative to an acceptable reference standard, in the appropriate clinical population or setting.

The first important design consideration is that both sensitivity and specificity (or likelihood ratios) must be measured relative to an appropriate reference standard. Many of the studies included in our review measured only sensitivity or specificity. Because sensitivity and specificity are jointly determined by the choice of threshold for a positive (or abnormal) test, either sensitivity or specificity can be made arbitrarily high at the expense of the other. Thus, reporting one without the other is not informative. Reporting both sensitivity and specificity for a variety of thresholds for abnormal tests as a receiver operating characteristic (ROC) curve (Figure 2) is preferable. ROC curves are useful because differences in sensitivity and specificity of two tests could be due either to real differences in the accuracy of the test or to the use of a different threshold for an abnormal test. When results are reported as an ROC curve, no such confounding will occur.

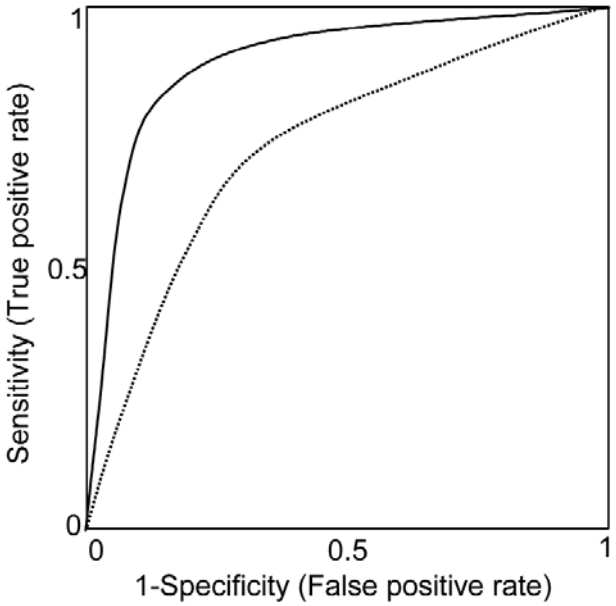

Figure 2. Receiver-operating characteristic curves (ROC). Each point along a ROC represents the trade-off in sensitivity and specificity, depending on the threshold for an abnormal test. Here, two hypothetical diagnostic tests are compared. The diagnostic test represented by the unbroken ROC curve is a better test than that represented by the broken ROC curve, as demonstrated by its greater sensitivity for any given specificity (and thus, greater area under the curve).

To develop unbiased estimates of sensitivity and specificity, studies of detection systems should use an appropriate reference standard test, the reference standard should be applied to all samples, the tests should be interpreted while blinded to results of the reference standard, and the samples or patient population should resemble as closely as possible the populations in which the system will be used (40). The reference standard should be used for all positive and negative samples. Selective use of the reference standard, for example, using the reference standard only on samples that are positive on the test under consideration, creates so-called test referral bias which can produce overestimates of sensitivity and underestimates of specificity (40). Test-interpretation bias may occur if the result of the detection system is not determined while blinded to the reference test (and vice versa). This bias causes an artificial concordance between the detection system and reference test, which results in overestimates of both sensitivity and specificity. Finally, the detection system should be evaluated under the most realistic conditions possible, which may be difficult to implement for bioterrorism agents given the range of conditions from hoaxes with no cases to real situations with a number of cases.

Evaluations of detection systems are ongoing (19). We expect with the heightened attention to bioterrorism preparedness planning that the systems for both detection and diagnosis will improve, as will their evaluations. Evaluations that adhere to the principles for design of studies of diagnostic tests will provide substantially more information than is now available and will help users interpret the results provided by these systems. Our review of 78 detection and diagnostic systems found that many of the evaluations performed to date are critically deficient. Further evaluative studies will delineate the usefulness of these systems.

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Respiratory and Urinary Tract Infections, Arthritis, and Asthma Associated with HTLV-I and HTLV-II Infection

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Human T-lymphotropic virus types I and II (HTLV-I and -II) cause myelopathy; HTLV-I, but not HTLV-II, causes adult T-cell leukemia. Whether HTLV-II is associated with other diseases is unknown. Using survival analysis, we studied medical history data from a prospective cohort of HTLV-I– and HTLV-II–infected and –uninfected blood donors, all HIV seronegative. A total of 152 HTLV-I, 387 HTLV-II, and 799 uninfected donors were enrolled and followed for a median of 4.4, 4.3, and 4.4 years, respectively. HTLV-II participants had significantly increased incidences of acute bronchitis (incidence ratio [IR] = 1.68), bladder or kidney infection (IR = 1.55), arthritis (IR = 2.66), and asthma (IR = 3.28), and a borderline increase in pneumonia (IR = 1.82, 95% confidence interval [CI] 0.98 to 3.38). HTLV-I participants had significantly increased incidences of bladder or kidney infection (IR = 1.82), and arthritis (IR = 2.84). We conclude that HTLV-II infection may inhibit immunologic responses to respiratory infections and that both HTLV-I and -II may induce inflammatory or autoimmune reactions.

Human T-lymphotropic virus types I and II (HTLV-I and -II) are presumed to have derived from primate T-lymphotropic viruses with which they share significant nucleotide sequence homology (1). They are transmitted by sexual intercourse; by parenteral modes such as unscreened blood or shared injection equipment; and from mother to child, predominantly by breast feeding (2–4). HTLV-I has been causally associated with adult T-cell leukemia and HTLV-associated myelopathy. HTLV-II has also been associated with HTLV-associated myelopathy, but not with leukemia (5).

Other possible health outcomes of chronic HTLV-I and -II infection have not yet been adequately investigated. Patients with adult T-cell leukemia may develop opportunistic infections such as *Pneumocystis carinii* pneumonia (6) or *Strongyloides* superinfection (7), but clinical immunodeficiency does not appear to develop in most persons with chronic HTLV-I or -II infection. On the contrary, syndromes suggestive of increased immunologic response such as uveitis (8), pneumonitis (9,10), and rarely, cases of lymphocytic arthritis (11,12) have been reported, although only uveitis has been epidemiologically associated with HTLV-I (8). Investigators in Japan have linked HTLV-I to a higher occurrence of various medical conditions (13) and virus-associated malignancies (14). Other investigators have reported an association between HTLV-II and pneumonia among injection drug users (15).

Case series and cross-sectional studies of HTLV-I and -II disease outcomes are vulnerable to potential bias and confounding. We have prospectively followed a large cohort of former blood donors with well-documented HTLV-I and -II infection at enrollment, and a similar group of uninfected donors, all of whom are HIV seronegative. We report on the occurrence of various disease outcomes in this cohort after a median follow-up of 4.3 years.

Methods

Study Design and Participants

This study is a prospective, multicenter cohort of persons with HTLV-I and -II infections, which were detected at the time of attempted blood donation at five U.S. blood centers and comparable HTLV–seronegative donors.

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Details of the cohort enrollment and follow-up procedures have been published previously (16,17). The study protocol was approved by the UCSF Committee on Human Research and by IRB at other participating institutions.

We determined HTLV serostatus by obtaining enzyme immunoassay test results followed by confirmatory Western blot. A central laboratory performed HTLV-I versus -II typing with a type-specific serologic assay, polymerase chain reaction (PCR), or both, as previously described (18). Unequivocal results from the type-specific serologic assay correlated well with those from the polymerase chain reaction assay. All participants were seronegative for HIV when baseline test were performed. For most participants, hepatitis C virus antibody status was not available at the time of enrollment.

Disease Endpoints

Each visit with a study nurse consisted of an interviewer-administered health history questionnaire and phlebotomy of blood for complete blood count and other studies. Selected diagnoses (cancer, neurologic and autoimmune conditions) reported on the questionnaire triggered requests for confirmatory medical records. We included nine conditions or diseases (pneumonia, acute bronchitis, bladder or kidney infection, arthritis, hypertension, asthma, cancer, diabetes, and thyroid disease) and eight symptoms (trouble walking, climbing, or rising from chair; incontinence; pre- or post-void urgency; lymphadenopathy; night sweats; weight loss; foot paresthesias; and impotence [males]) in the data analysis.

Statistical Analysis

We used the Kaplan-Meier product limit method to calculate the unadjusted probability of disease-free survival during the study period for each disease outcome by HTLV status. Survival time was defined as the number of days from the baseline visit until the date that an adverse health outcome was first diagnosed or the end of observation. We performed the log-rank test to assess the differences in disease-free survival time (days) between HTLV-seronegative participants and HTLV-I– or HTLV-II–infected participants, respectively.

To adjust for possible confounding factors, we performed multivariable analysis with HTLV status as an independent variable, survival time as a dependent variable, and possible confounding factors as covariates. In constructing the survival analysis models, we considered a number of covariates, which are described as follows: demographic variables (forced into all models), education, smoking history (pack-years, forced into the models for bronchitis and pneumonia), alcohol consumption, blood center, community versus autologous donation type, injection drug use (except in models for arthritis, hypertension, cancer, neurologic and urologic symptoms), parity (in models for urinary symptoms, bladder and kidney infections, and in females only), and number of sex partners (in model for bladder and kidney infections only). Using a backward selection process, all these covariates were added to the initial model, but only covariates with significant independent associations themselves ($p < 0.05$), or which substantively modified HTLV incidence ratio, were retained in the final model. We did not include interaction terms because similar analyses of data from prior cohort visits had indicated the absence of significant interaction.

To examine the differences in the cumulative number of episodes of pneumonia, bronchitis, and bladder or kidney infection by HTLV status, we used the negative binomial model, a generalization of the Poisson model, to compute incidence rate ratios (RR) with 95% confidence interval (CI) for each of these outcomes. This model took into account that the recurrence of the disease in a participant may be associated with both the overall disease incidence and its previous occurrence in that participant. We also adjusted for possible confounding factors using the same modeling strategy as for the survival analyses.

For the analysis of symptoms, we calculated unadjusted and adjusted odds ratio (OR) with 95% CI for any occurrence of each symptom by HTLV status by using logistic regression models. To adjust for possible confounding, we added other possible confounding factors as covariates to the models by using the same approach as in the survival analyses. All analyses were performed using Statistical Analysis System (SAS) software (19).

Results

Study Population and Follow-up

During the initial visits in 1990 through 1992, we enrolled 154 HTLV-I, 387 HTLV-II, and 799 HTLV seronegative persons. Two HTLV-I participants were excluded from this analysis because they did not complete the screening physical examination at the initial visit. The baseline characteristics of the study population are given in Table 1. The HTLV groups and seronegative participants were comparable with respect to age, sex, race or ethnicity, blood center visited, and type of blood donation (autologous versus allogeneic), except for slightly higher proportions of blacks among the HTLV-I group and Hispanics among the HTLV-II group. The HTLV-II group had the lowest socioeconomic status, as indicated by educational attainment and income, and the HTLV-I group had intermediate status. Pack-years of cigarette smoking and amount of alcohol intake were higher in the HTLV-II group. Consistent with the recognized epidemiologic risk factors for HTLV-I and -II infection (3,4), the HTLV-I and -II groups had more lifetime sexual partners than seroneg-

Table 1. Characteristics of the multicenter, prospective human T-lymphotropic virus (HTLV) cohort study population[a]

Characteristics	HTLV-I (n = 152)	HTLV-II (n = 387)	HTLV negative (n = 799)
Age (y)	No. (%)	No. (%)	No. (%)
18–29	5 (3)	11 (3)	34 (4)
30–39	28 (18)	104 (27)	171 (21)
40–49	55 (36)	168 (43)	288 (36)
50–59	32 (21)	73 (19)	175 (22)
≥60	32 (21)	31 (8)	131 (16)
Sex			
Male	43 (28)	102 (26)	257 (32)
Female	109 (72)	285 (74)	542 (68)
Race/ethnicity			
Asian	20 (13)	8 (2)	60 (8)
Black	61 (40)	125 (32)	248 (31)
Hispanic	9 (6)	104 (27)	150 (19)
Other	1 (1)	7 (2)	30 (4)
White	59 (39)	140 (36)	309 (39)
Unknown	2 (1)	3 (1)	2 (0)
Education			
High school or less	45 (30)	135 (35)	129 (16)
Some college	66 (43)	195 (51)	363 (46)
College	30 (20)	45 (12)	181 (23)
College (>4 years)	11 (7)	11 (3)	123 (15)
Income			
<$30,000	46 (30)	144 (38)	167 (21)
$30,000–49,999	51 (34)	120 (32)	221 (28)
≥$50,000	55 (36)	113 (30)	401 (51)
Center			
1	32 (21)	51 (13)	122 (15)
2	29 (19)	39 (10)	102 (13)
3	44 (29)	206 (53)	345 (43)
4	31 (20)	68 (18)	156 (20)
5	16 (11)	23 (6)	74 (9)
Blood donor type			
Autologous	28 (18)	39 (10)	111 (14)
Allogeneic	124 (82)	348 (90)	688 (86)
Smoking history (pack/y)			
Nonsmoker	74 (52)	125 (36)	413 (54)
0–13	24 (17)	117 (33)	184 (24)
>13	43 (31)	109 (31)	174 (23)
Alcohol intake (average drinks per wk)			
Nondrinker	19 (13)	20 (6)	70 (9)
0–1	58 (41)	134 (38)	352 (46)
>1	64 (45)	200 (57)	339 (45)
Lifetime sex partners			
<6	56 (38)	87 (23)	381 (49)
≥6	92 (62)	292 (77)	403 (51)
Injection drug use			
Ever	148 (98)	294 (76)	787 (99)
Ex-injection drug user	2 (1)	75 (19)	9 (1)
Current injection drug user	1 (1)	17 (4)	1 (0)

[a]Missing data (up to 6%, depending upon the variable) were excluded from the calculation of percentages.

ative participants, and almost 24% of the HTLV-II participants had a lifetime history of injection drug use, although only 4.4% of the HTLV-II group admitted to current injection drug use.

We present data through the third study visit in 1995 through 1996. Median follow-up time was 4.3 years for all 1,338 participants, including those with no follow-up. Median follow-up did not differ by HTLV status and was 4.4 years for the HTLV-I group, 4.3 years for the HTLV-II group, and 4.4 years for the HTLV-seronegative group.

HTLV-I Findings

Compared to seronegative persons, HTLV-I–infected persons were more likely to have a new diagnosis of bladder or kidney infection ($p = 0.009$) and arthritis ($p = 0.0002$) (Figure). Among the HTLV-I–infected persons, two had asthma; an insufficient number to test the difference relative to seronegative persons. The HTLV-I participants showed no statistically significant differences in the incidence of pneumonia, acute bronchitis, and hypertension, cancer, diabetes, and thyroid disease (data not shown).

The number of incident cases diagnosed (limited to one case per person) and the unadjusted and adjusted incidence ratios (IR) for several diagnoses are given in Table 2. Compared with results for seronegative persons, and after multivariable adjustment for relevant confounding variables, HTLV-I infection was associated with bladder or kidney infection (IR 1.82, 95% CI 1.19 to 2.77) and arthritis (IR 2.84, 95% CI 1.51 to 5.33). The risks of developing pneumonia, acute bronchitis, hypertension, and cancer were not significantly increased. Too few cases of asthma (n = 2), thyroid disease (n = 1), and diabetes mellitus (n = 5) existed among the HTLV-I participants to perform survival analysis.

To further investigate the occurrence of infectious diseases, we also analyzed the incidence density of three infectious diseases by HTLV status, whether or not each diagnosis was a first or recurrent case (Table 3). In the incidence density analysis (Table 3), an average of 1.75 cases of bladder or kidney infection occurred per HTLV-I participant over the 4.4-year median follow-up time, compared with 0.63 per seronegative participant over the same period. The unadjusted and adjusted RRs for the HTLV-I group were significantly greater than unity for bladder or kidney infection. HTLV-I participants had increased prevalence rates of neurologic symptoms, self-reported lymphadenopathy, and night sweats, but they reported weight loss no more frequently than did HTLV-seronegative persons (Table 4).

HTLV-II Findings

Disease-free survival curves for selected medical diagnoses, by HTLV status, are represented in the Figure. Compared to seronegative persons, HTLV-II–infected participants were more likely to experience acute bronchitis ($p < 0.0001$), bladder or kidney infection ($p = 0.0008$), arthritis ($p = 0.0003$), and asthma ($p = 0.0007$); the likelihood of acquiring pneumonia was increased but not significantly ($p = 0.08$). Differences between the HTLV-II and HTLV-seronegative participants in the incidence of cancer, hyper-

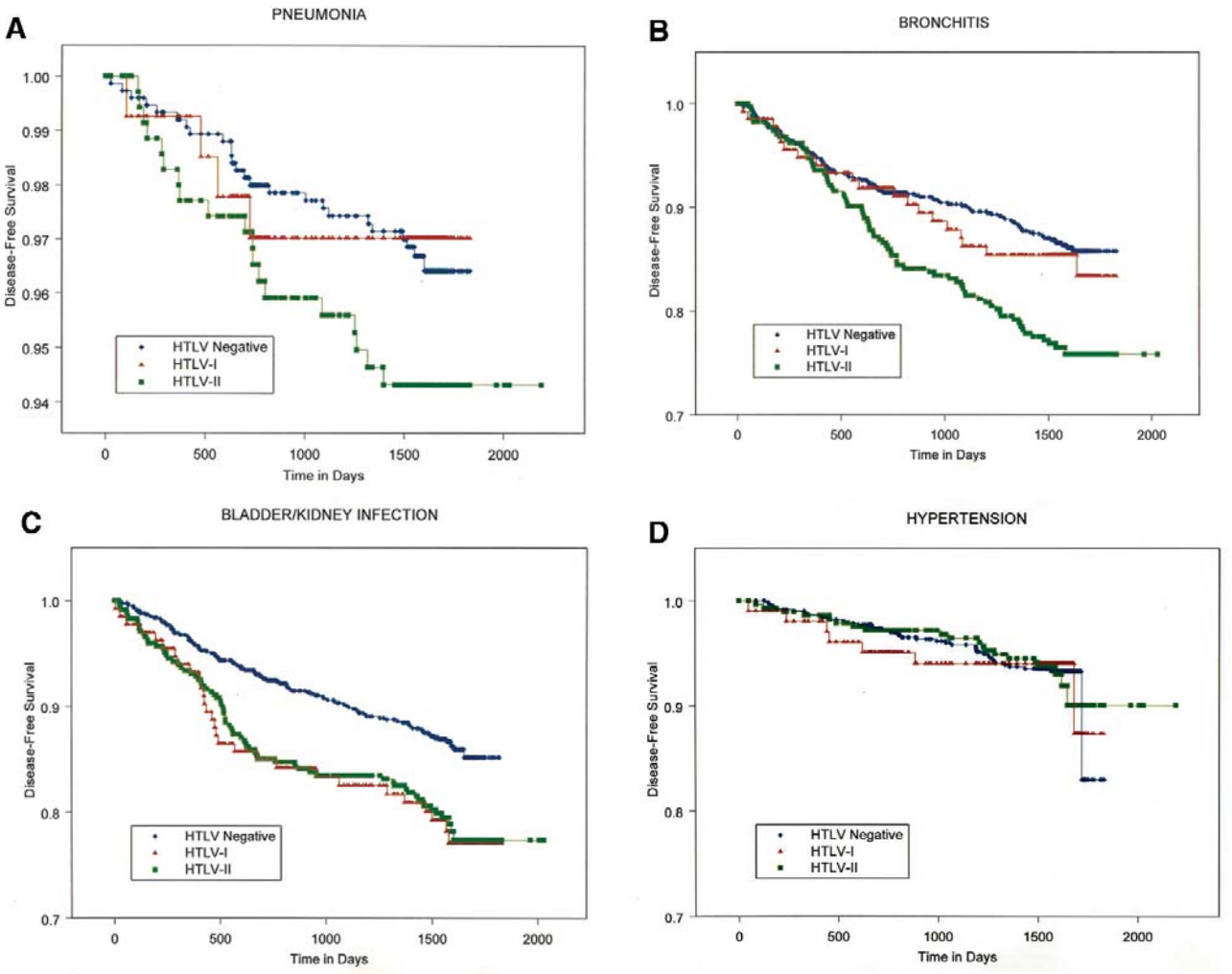

Figure. Kaplan-Meier survival curves showing disease-free survival for one noninfectious and three infectious diseases, by human T-lymphotropic virus (HTLV) status, through visits 2 and 3 of prospective observation. HTLV-I–infected (red triangles) and HTLV-II–infected (green squares) participants are compared to HLTV-seronegative participants (blue diamonds), respectively. Panels are as follows: A) pneumonia; B) acute bronchitis; C) bladder or kidney infection; and D) hypertension. The vertical axis scale has been compressed in panel A because of the lower overall incidence of pneumonia.

tension, diabetes, or thyroid disease were not statistically significant.

The number of incident cases diagnosed (limited to one case per participant), and the unadjusted and adjusted IRs, for several diagnoses are given in Table 2. Compared with results for seronegative participants, and after adjusting for confounding variables, HTLV-II was associated with acute bronchitis (OR 1.68, 95% CI 1.24 to 2.29), bladder or kidney infection (OR 1.55, 95% CI 1.14 to 2.11), arthritis (OR 2.66, 95% CI 1.58 to 4.45), and asthma (OR 3.28, 95% CI 1.57 to 6.84). The association between HTLV-II infection and pneumonia was of borderline statistical significance (IR 1.82 , 95% CI 0.98 to 3.38). IRs for hypertension and cancer were not increased for HTLV-II participants. Too few cases of thyroid disease (n = 7) and diabetes mellitus (n = 7) were found among the HTLV-II participants to perform survival analysis.

In the incidence density analysis of recurrent infections, an average of 0.21 cases of pneumonia, 1.10 cases of acute bronchitis, and 1.25 cases of bladder or kidney infection occurred among HTLV-II participants during their 4.3-year median follow-up time (Table 3). The corresponding incidence densities for the seronegative group were 0.08, 0.59, and 0.63, respectively, over their 4.4 year median follow-up time. Both unadjusted and adjusted RRs, calculated by using negative binomial modeling, were significantly greater than unity for all three diseases (Table 3). Compared with seronegative participants, HTLV-II participants had increased prevalence rates of several neurologic, lymphatic, and constitutional symptoms, but the prevalence of impotence (males) was not significantly increased (Table 4).

Table 2. Incidence of medically diagnosed conditions and selected unadjusted and adjusted incidence ratios among human T-lymphotropic virus (HTLV)-I– and HTLV-II– infected participants and HTLV-seronegative participants, visits 2 and 3[a]

	HTLV seronegative (N = 799)	HTLV-I (N = 152)			HTLV-II (N = 387)		
Diagnosis	Cases (%[b])	Cases (%[b])	IR[c]	Adj. IR (95% CI)[d]	Cases (%[b])	IR[c]	Adj. IR (95% CI)[d]
Pneumonia	25 (3)	5 (4)	0.89	0.79 (0.27 to 2.29)	19 (5)	1.70	1.82 (0.98 to 3.38)
Acute bronchitis	103 (14)	21 (15)	1.12	1.10 (0.68 to 1.79)	81 (23)	1.83	1.68 (1.24 to 2.29)
Bladder or kidney infection	105 (14)	31 (23)	1.74	1.82 (1.19 to 2.77)	73 (21)	1.68	1.55 (1.14 to 2.11)
Arthritis	32 (5)	16 (16)	3.19	2.84 (1.51 to 5.33)	32 (12)	2.51	2.66 (1.58 to 4.45)
Hypertension	40 (7)	7 (7)	1.06	0.99 (0.44 to 2.22)	20 (7)	1.08	1.09 (0.63 to 1.89)
Asthma	15 (2)	2 (2)	—	—	20 (6)	3.38	3.28 (1.57 to 6.84)
Cancer	21 (3)	3 (2)	0.81	0.72 (0.21 to 2.43)	8 (2)	0.87	1.10 (0.44 to 2.32)

[a]Only the first diagnosis of each condition is considered for each participant. IR, incidence ratio derived from survival analysis; CI, confidence interval.
[b]Denominator for percentage calculation varied according to the number of participants included in each disease-specific analysis.
[c]Unadjusted incidence ratio derived from survival analysis.
[d]After a backward selection process, including all potential confounding variables, the final survival analysis model contained these variables: age, gender, race/ethnicity, and (for pneumonia, bronchitis, and asthma) smoking history (see Methods for details of the statistical analysis).

Discussion

In summary, HTLV-II–infected participants had a higher incidence of acute bronchitis, bladder or kidney infection, arthritis and asthma, and a higher incidence of pneumonia than did HTLV-seronegative participants followed concurrently. The finding of a higher rate of these infectious diseases among HTLV-II participants was supported both by survival analysis, which considered only the first diagnosis, and by negative binomial modeling, which considered both first and recurrent infections. HTLV-I participants had a higher incidence of bladder or kidney infection and arthritis. Cancer incidence was not higher in either the HTLV-I or HTLV-II participants compared to its incidence in seronegative participants, although the number of cases was small.

Our finding of a higher incidence of respiratory tract infections among HTLV-II–infected persons is consistent with most of the small number of other published clinical studies on this topic. Robert-Guroff et al. found higher rates of HTLV-II infection among injection drug users with abscess than in those without abscess (20). In another cross-sectional analysis, Modahl et al. found that injection drug users with HTLV-II infection were more likely to have been diagnosed with pneumonia, abscess, or lymphadenopathy (15), although a subsequent case-control study did not find that either HTLV-II or HIV are risk factors for skin and soft tissue abscess among injection drug users (21). LaGrenade et al. have documented an association between HTLV-I infection and *Staphylococcus*- and *Streptococcus*-related infective dermatitis among Jamaican children (22). An independent nested case-control study of pneumonia, abscess, and endocarditis among Baltimore injection drug users found no association between these infections and HTLV-II seropositivity (23). A number of opportunistic co-infections have been reported in conjunction with HTLV-I infection, including *Strongyloides* hyperinfection (7), *P. carinii* pneumonia (in patients with HTLV-I–related adult T-cell leukemia) (6), and leprosy (24).

The biologic basis for a putative increased susceptibility to certain infections in humans with chronic HTLV-II infection is not well described. In contrast to the predominant CD4+ lymphotropism of HTLV-I, HTLV-II provirus in vivo is integrated at highest levels into CD8+ lymphocytes but may also be demonstrated in CD4+, both CD45RO+ and CD45RO-, and even non-T (CD14, CD16, and CD19) lymphocytes (25). Delayed hypersensitivity response to mumps virus and *Candida* antigens is normal among HTLV-II participants, suggesting intact cell-mediated or T-helper 1-type immunity (26). Although subtle differences may exist, the overall distribution of lymphocyte subsets is not perturbed in persons with HTLV-II (27,28). However, total immunoglobulin G levels are high-

Table 3. Incidence density (ID)[a] and standard deviation (SD) of medically diagnosed infectious diseases, and selected crude and adjusted rate ratios (RR), among human T-lymphotropic virus (HTLV)-I– and HTLV-II–infected participants and HTLV-seronegative participants, visits 2 and 3

	HTLV seronegative (N = 799)	HTLV-I (N = 152)			HTLV-II (N = 387)		
Diagnosis	ID (SD)	ID (SD)	RR[b]	Adj. RR (95% CI)[c]	ID (SD)	RR[b]	Adj. RR (95% CI)[c]
Pneumonia	0.08 (0.37)	0.11 (0.47)	1.49	1.33 (0.66 to 2.66)	0.21 (0.96)	2.82	2.65 (1.67 to 4.21)
Acute bronchitis	0.59 (1.78)	0.82 (2.25)	1.38	1.33 (0.84 to 2.12)	1.10 (2.75)	1.83	1.53 (1.10 to 2.14)
Bladder or kidney infection	0.63 (2.04)	1.75 (4.61)	2.73	2.32 (1.50 to 3.59)	1.25 (3.48)	1.94	1.94 (1.40 to 2.68)

[a]Defined as the mean of the total number of infectious disease diagnoses divided by the number of participants (with or without the infection) in each group at baseline. The period of observation was 4.4 years (HTLV-I), 4.3 years (HTLV-II), and 4.4 years (HTLV seronegative), and each participant may have multiple diagnoses of each condition.
[b]Unadjusted.
[c]Adjusted. Adjusted models included age, gender, race/ethnicity and duration of follow-up for all infections, and injection drug use (for pneumonia), smoking (for acute bronchitis), and community versus autologous blood donation (for bladder/kidney infection).

Table 4. Prevalence of medically diagnosed symptoms, and unadjusted and adjusted odds ratios (OR) derived from logistic regression models, in human T-lymphotropic virus (HTLV)-I– and HTLV-II–infected participants and HTLV-seronegative participants, visits 2 and 3[a]

	HTLV seronegative (N = 799)	HTLV-I (N = 152)			HTLV-II (N = 387)		
Symptoms	Cases (%)[b]	Cases (%)[b]	OR[c]	Adj. OR (95% CI)[d]	Cases (%)[b]	OR[c]	Adj. OR (95% CI)[d]
Trouble walking, climbing or rising from chair	147 (21)	52 (42)	2.71	2.67 (1.74 to 4.09)	133 (42)	2.78	3.44 (2.52 to 4.71)
Incontinence, pre- or post-void urgency	175 (25)	50 (40)	2.03	2.02 (1.33 to 33.07)	134 (43)	2.25	2.59 (1.92 to 3.49)
Lymphadenopathy	29 (4)	11 (9)	2.26	2.39 (1.14 to 5.03)	40 (13)	3.40	3.08 (1.85 to 5.13)
Night sweats	20 (3)	15 (12)	4.68	4.73 (2.31 to 9.69)	47 (15)	6.02	4.97 (2.77 to 8.94
Weight loss	40 (6)	9 (7)	1.29	1.10 (0.51 to 2.37)	40 (13)	2.43	2.10 (1.22 to 3.60)
Foot paresthesias	57 (8)	22 (18)	2.44	2.46 (1.41 to 4.28	66 (21)	3.02	3.27 (2.19 to 4.88)
Impotence (males only)	33 (5)	10 (8)	2.24	2.05 (0.77 to 5.49)	13 (4)	1.10	1.27 (0.56 to 2.91)

[a]Only the first diagnosis of each symptom is considered for each participant.
[b]Denominator for percentage calculation varied according to the number of participants included in each disease-specific analysis.
[c]Unadjusted.
[d]Adjusted. Models were adjusted for age, race and ethnicity, and duration of follow-up (all symptoms), and gender (all except impotence). In addition, specific models included community versus autologous donation (for trouble walking and incontinence, weight loss and impotence) and injection drug use [night sweats and weight loss]).

er in HTLV-II-infected persons (29), in vitro cell proliferation in response to pokeweed mitogen is suppressed in HTLV-II infection (28), and HTLV-II may induce expression of interferon-γ, granulocyte macrophage-colony-stimulating factor, and other cytokines (30,31). Although HTLV-II provirus has also been demonstrated in macrophages (32), whether such infection influences macrophage regulation or function to a clinically notable degree is not known. Finally, lymphocytic pneumonitis has been reported in association with HTLV-I infection (9,10), and clinically diagnosed cases of pneumonia and acute bronchitis in HTLV-II- infected persons could conceivably represent autoimmune rather than infectious disease.

Our finding of an association of both HTLV-II and HTLV-I with bladder or kidney infection is consistent with a previous report of unspecified renal disease in a prospective cohort study of HTLV-I-infected persons in Japan (33). However, such associations must be interpreted cautiously in light of the known association of both retroviruses with HTLV-associated myelopathy (5,34,35). Since urinary frequency and urgency are among the first symptoms of bladder hyperreactivity due to the underlying myelopathy, HTLV-I- or -II-infected persons might seek medical care for these symptoms. Urinary tract infection or kidney disease secondary to unrecognized neurogenic bladder dysfunction might be diagnosed, or they may be treated presumptively for urinary tract infection on the basis of their bladder symptoms. In either case, an increased incidence of diagnosed bladder or kidney infection may not necessarily indicate that HTLV-I or -II infection is the cause of these urinary infections. Finally, although we controlled for the number of sexual partners, residual confounding by sexual activity could have influenced our bladder or kidney infection finding (36).

The increased incidence of arthritis observed for both persons infected with HTLV-I and HTLV-II supports reports of possible autoimmune syndromes with HTLV infection. HTLV-I has been epidemiologically associated with uveitis (8). Several previous reports of HTLV-I in case series of arthritis have been limited by the lack of appropriate controls (11,12). Nonetheless, high numbers of HTLV-I-infected lymphocytes have been demonstrated in synovial fluid from some of these case-patients. Although we obtained medical records to verify arthritis and other diagnoses, the records did not give sufficient information to classify the type of arthritis and diagnostic evaluations were limited in most cases. Although an association between HTLV-I infection and asthma has been reported among Japanese men (13), we are unaware of previous reports of an increased incidence of asthma in association with HTLV-II infection. Also, a few cases of lymphocytic pneumonitis have been reported in patients with HTLV-I infection, particularly those with myelopathy (9,10). We plan a more intensive diagnostic evaluation of the HTLV-II participants in this study with recurrent pneumonia or asthma to explore the possible contribution of undiagnosed lymphocytic pneumonitis to the observed clinical signs and symptoms.

We have previously reported a single case of adult T-cell leukemia which was diagnosed between the first and second visits of the patient in this cohort study (17); no additional cases have been diagnosed to date. That neither HTLV-I nor -II participants had an increased incidence of nonhematologic cancer in our current analysis is potentially reassuring to persons infected with these retroviruses. However, an increased incidence of some cancers, especially those thought to be induced by viruses, has been reported in a Japanese HTLV-I cohort (37). We might not have detected a small increase in IR because of the relatively small number of cases detected during our 4.3-year follow-up period. Alternatively, we might not have had enough HTLV-I or -II participants who were co-infected

with other oncogenic viruses such as hepatitis C virus to detect a synergistic effect between HTLV-I or -II and these viruses (38).

The two- to three-fold higher prevalence of self-reported neurologic symptoms, including trouble walking, climbing stairs, or rising from a chair, and bladder symptoms may represent early spinal cord injury due to HTLV-I or -II. As follow-up of the cohort continues, we shall be able to determine whether those reporting such symptoms in earlier visits have a higher incidence of overt myelopathy than asymptomatic HTLV-infected participants. On the other hand, the frequency of self-reported lymphadenopathy and night sweats is unlikely to be caused by preclinical hematologic malignancy, given the rarity of that disorder in HTLV-I–infected persons and its lack of association with HTLV-II. These symptoms might be related to known effects of HTLV-I and -II on lymphocytic proliferation and cytokine expression, or they might simply reflect reporting bias.

Strengths of the current study include its controlled, prospective, cohort design, stringent confirmation of HTLV-I– and -II–infection status at baseline, and systematic ascertainment of disease outcomes. One potential weakness is that differences in socioeconomic status and risk behaviors could have confounded disease associations between HTLV–infected and uninfected previous blood donors, even though we selected the uninfected participants in strata defined by the age, sex, race or ethnicity, center, and blood donation type of the HTLV groups. We controlled for the socioeconomic and behavioral factors using multivariate analyses, but residual confounding could affect the magnitude of the associations we observed. Second, recall bias may exist in that participants with HTLV infection might differentially report more diagnoses because of heightened concern about their own health. Our questionnaire requested only medically confirmed diagnoses, and the absence of associations with noninfectious disease, such as hypertension, diabetes, and thyroid disease, suggests that generalized overreporting of illness was not a problem. Additionally, infectious disease associations with HTLV have not been widely reported, so we do not think that recall bias specific to these diagnoses was a serious concern. Finally, follow-up time to date is modest for a chronic infection such as HTLV, and our findings may change with longer observation.

In conclusion, HTLV-II infection is associated with an increased incidence of respiratory and urinary tract infections and asthma, and both HTLV-I and -II are associated with increased incidence rates of arthritis, compared with results for seronegative persons. These findings suggest that chronic infection with HTLV-II may inhibit host immunologic responses to infection, or more specifically, to respiratory infections. The arthritis and asthma results, and possibly the respiratory tract diagnoses, suggest that other inflammatory or autoimmune reactions may be induced by HTLV-I or HTLV-II infection. Additional in vitro and in vivo research on the immunologic consequences of HTLV infection is needed.

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Bacillus anthracis Incident, Kameido, Tokyo, 1993

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In July 1993, a liquid suspension of *Bacillus anthracis* was aerosolized from the roof of an eight-story building in Kameido, Tokyo, Japan, by the religious group Aum Shinrikyo. During 1999 to 2001, microbiologic tests were conducted on a liquid environmental sample originally collected during the 1993 incident. Nonencapsulated isolates of *B. anthracis* were cultured from the liquid. Multiple-locus, variable-number tandem repeat analysis found all isolates to be identical to a strain used in Japan to vaccinate animals against anthrax, which was consistent with the Aum Shinrikyo members' testimony about the strain source. In 1999, a retrospective case-detection survey was conducted to identify potential human anthrax cases associated with the incident, but none were found. The use of an attenuated *B. anthracis* strain, low spore concentrations, ineffective dispersal, a clogged spray device, and inactivation of the spores by sunlight are all likely contributing factors to the lack of human cases.

Incident

On June 29, 1993, five residents of Kameido, Koto-ward, an eastern area of Tokyo, reported foul odors to local environmental health authorities. On investigation, officials found that the odors originated from the eight-story headquarters building of the religious group Aum Shinrikyo. The group was suspected of abducting several escaped members and anti–Aum Shinrikyo activists; however, lacking strong evidence of criminal activity, national security and law enforcement authorities had not restricted Aum Shinrikyo's activities.

On June 30, the local environmental health authority registered 41 complaints that foul odors were causing appetite loss, nausea, and vomiting in some exposed persons. Because of the complaints, officials requested permission to inspect the building's interior, but Aum Shinrikyo members refused. Officials checked the building's surroundings, collected air samples, and began to survey activity at the building, but other than the nuisance posed by the odor, no readily apparent risk to human health could be found.

On the morning of July 1, neighbors reported loud noises and an intermittent mist emanating from one of two cooling towers on the building's roof (Figure 1). As the day progressed, residents (mostly living south of the building) lodged 118 complaints about the foul odors with the environmental health office. Light rain fell early in the day (a total of 7 mm, 1 mm each hour from 1:00–7:00 a.m.). Wind (2–4 m/sec) blew from north-northeast to northeast in the morning and northeast to east-northeast in the afternoon. The minimum and maximum temperatures were 16.9°C at 3:00 a.m. and 4:00 a.m. and 19.9°C at 3:00 p.m., respectively. The day was rainy and cloudy, with no direct sunlight.

The same day, residents in the neighborhood reported a "gelatin-like, oily, gray-to-black" fluid from the mist from the cooling towers collecting on the side of the building. Environmental officials collected samples of this fluid and stored them in a refrigerator (4°C) for later testing.

Intermittent misting continued until demands from local residents forced Shoko Asahara, founder of Aum Shinrikyo, to agree on the morning of July 2 to cease using the rooftop device and to clean and vacate the building. No equipment remained when officials inspected the building on July 16, although they noted black stains on the walls.

This incident was largely forgotten until, in the aftermath of the March 1995 sarin gas attack on the Tokyo subway system, police investigations uncovered evidence that Aum Shinrikyo was involved in bioterrorism. The true nature of the Kameido incident was not revealed to the public until Asahara was arraigned on May 23, 1996. Aum Shinrikyo members testified that the odors were caused by their efforts to aerosolize a liquid suspension of *Bacillus anthracis* in an attempt to cause an inhalational anthrax epidemic. They believed this epidemic would trigger a world war and lead to Asahara's ruling the world.

At the time of the incident, the illnesses reportedly associated with the release were not well studied. In particular, no one sought evidence of inhalational anthrax or

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Figure 1. Spraying scenes from the Aum Shinrikyo headquarters building (photographs taken July 1, 1993, by the Department of Environment, Koto-ward).

other syndromes caused by the anthrax bacillus, since the true nature of the mist was not recognized. Reports of short-term loss of appetite, nausea, and vomiting (symptoms not typical of *B. anthracis* infection) among some residents were the only contemporary evidence of human illness associated with the incident. Vague reports of illness in birds and pets were also noted in local media (1), but the exact nature of these illnesses remained unclear.

Laboratory Findings

In November 1999, after long negotiation, local environmental authorities agreed that the one remaining fluid sample, collected as part of the 1993 investigation, could be tested for microbiologic pathogens. The test tube, which contained 2.6 mL of a turbid, gray-to-black fluid, was transferred to Northern Arizona University in Flagstaff, Arizona, for testing to identify and characterize its microbial flora.

Provisional microscopy examination of the fluid stained by malachite green/safranin showed bacterial spores, a large amount of debris, and vegetative bacterial cells. Aliquots of the fluid were streaked on sheep blood agar plates and incubated aerobically at 37°C. After overnight growth, the plates were found to contain mixed bacterial flora; approximately 10% of the colonies were similar in appearance to *B. anthracis*. Suspect colonies were nonhemolytic and had the "gray ground glass" appearance typical of *B. anthracis* (Figure 2). Based on the number of colonies on the plates, the original liquid suspension had about 4 x 10^3 colony-forming units (CFU) of the *B. anthracis*–like agent per milliliter.

A representative selection of 48 colonies of the *B. anthracis*–like agent was purified by single colony streaking and then subjected to multiple-locus, variable-number tandem repeat analysis (MLVA). The MLVA polymerase chain reaction (PCR) primers are specific for eight amplicon sites unique to *B. anthracis* (2), two of which are plasmids carrying genes for anthrax toxin (pX01) and capsule (pX02). Amplicon-size patterns are diagnostic for particular diversity groups and strains within *B. anthracis* (3).

Analysis of the 48 suspect colonies confirmed them to be *B. anthracis*, and all had the same genotype. Of the eight marker sites, one—the locus for the pX02 plasmid coding for the anthrax capsule—consistently failed to amplify (3). All colonies contained the pX01 plasmid (coding for anthrax toxin) but lacked the pX02 plasmid (Figure 3). This genotype was identical to that of the Sterne 34F2 strain, used commercially in Japan to vaccinate animals against anthrax.

Epidemiologic Findings

In Japan, culture-confirmed human anthrax is on the national notifiable disease list, and physicians are required to report all cases to the government. During the 1990s, only four human anthrax cases were reported (4). One of these cases, in Tokyo in August 1994, was in a man in his eighties from Sumida-ward, adjacent to Koto-ward (the location of Kameido); however, this case had no apparent association with the July 1993 incident.

A retrospective case-detection survey was conducted to assess the possibility that some anthrax cases might have gone unrecognized or unreported. Using the official "foul odor" complaints as a guide, residences of the 118 complainants from July 1, 1993, were mapped to identify the area with the presumed highest risk for infection (Figure 4). The high-risk area included a 7-digit zip code area (0.33 km^2) in Kameido, containing approximately 3,400 households and 7,000 residents. In 1999, physicians at 39 medical facilities (15 internal medicine, 7 dermatology, and 17 other specialties) serving the high-risk area were surveyed by telephone. None of these physicians reported having seen cases of anthrax, unexplained serious respira-

Figure 2. Fluid collected from the Kameido site cultured on Petri dishes to identify potential *Bacillus anthracis* isolates.

tory illnesses, or hemorrhagic meningitis, which is often a complication of systemic anthrax (5,6) in residents of the high-risk area.

Discussion

The Kameido incident is the first documented instance of bioterrorism with an aerosol containing *B. anthracis*. Aum Shinrikyo members testified in 1995 that they were working with *B. anthracis*, but 6 years passed before the strain was isolated and characterized (3).

Why the Kameido incident failed to produce any documented cases of anthrax has not been fully explained, but the basis may be multifactorial. A virulent strain of *B. anthracis*, a sufficient concentration to cause disease, effective aerosolization, and favorable weather conditions would all have been necessary to produce the anthrax epidemic Aum Shinrikyo members said they wanted.

Molecular subtype analysis results demonstrate that the strain used in Kameido was a vaccine strain of *B. anthracis* without the ability to produce a protective capsule (3). This strain is generally regarded as nonpathogenic for immunocompetent people and is widely used in livestock without adverse consequences. Even if the strain had been virulent, however, the concentration of spores in the liquid suspension (10^4/mL) was significantly less than the 10^9 to 10^{10} organisms/mL considered to be optimal in a liquid-based biologic weapon.

The viscosity of the suspension was also greater than desirable. Successfully weaponizing anthrax spores requires creating a fine-particle cloud with a sufficiently high concentration of *B. anthracis*. The human respiratory infectious dose 50 (dose that will produce an infection in 50% of exposed persons) is unknown but has been estimated to be 8,000 to 10,000 spore-bearing particles <5 mm in diameter (7). Kameido residents described a gelatinous substance, suggesting the suspension would be poorly dispersed and droplets would be too large to form particles <5 mm in diameter. Additionally, the effectiveness of the spray system (code named "Water Mach" by the Aum Shinrikyo) was questionable; it apparently broke down repeatedly, and hydrostatic back pressure caused the suspension to leak from tubing used to transport it up eight stories. The spray head may have clogged with the high-viscosity fluid, contributing to back pressure.

Climate could have been another mitigating factor. While *B. anthracis* spores resist many environmental influences, they are killed by sunlight (7), with an estimated survival time in direct sunlight in July of <2.5 h. In Kameido, survival time may have been longer since the weather was overcast on July 1 (the day the mist was reported), but spore inactivation by solar radiation would still have reduced the already-low potential for infection.

Because of the associated foul odor, residents quickly detected a problem, but local officials did not suspect Aum Shinrikyo of developing biologic weapons, and they conducted no microbiologic examination at the time. The actual cause of the foul odor remains undetermined, but it

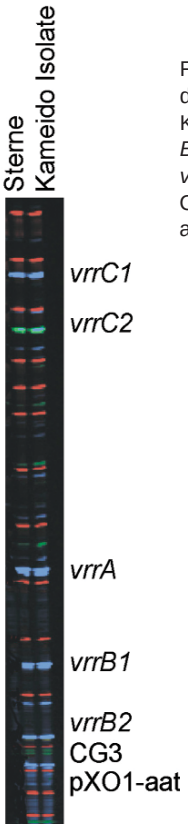

Figure 3. Multiple-locus, variable-number tandem repeat analysis genotype of all 48 Kameido isolates and the Sterne strain of *Bacillus anthracis*: *vrr*A, 313 bp; *vrr*B1, 229 bp; *vrr*B2, 162 bp; *vrr*C1, 583 bp; *vrr*C2, 532 bp; CG3, 158, bp; pX01-att, 129 bp; pX02, no amplification.

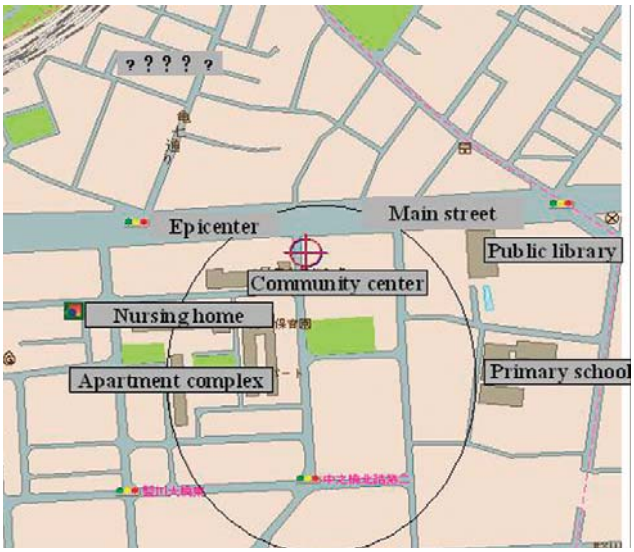

Figure 4. High-risk area for infection, based on foul odor complaints.

may have been caused by heating the medium used to grow *B. anthracis* or failing to wash the medium from the suspension before dispersal. Geographic distribution of complaints about the odor corresponded with expected dispersal patterns of the aerosol under prevailing weather conditions. Infection risk could theoretically extend beyond the area of the foul odor complaints; however, focusing the telephone survey on this "high-risk" area provided the greatest chance of finding related cases of anthrax. Routine disease reporting by Tokyo-area medical association members did not provide evidence of potentially related cases outside the high-risk area.

Conclusions

The Kameido investigation first showed the value of a high-resolution subtyping system for *B. anthracis* in forensic investigations. Its value was confirmed during investigations of the "anthrax letters" mailed to several persons in the United States in 2001 (6).

The details of Aum Shinrikyo activities led to a wider appreciation that subnational organizations may use biologic agents as weapons. Awareness is especially important in being prepared for a bioterrorist attack, since recognizing its nature early can substantially reduce associated sickness and death (8,9). Early recognition, however, requires training health professionals to recognize these diseases, having laboratories available to rapidly confirm clinical suspicions, and developing an active national surveillance program. Countries must also be able to rapidly deploy trained medical personnel, medical materials, and epidemiologists to affected communities. Most countries will need coordination among government agencies and private facilities with expertise relevant to the agents involved. To be effective, these measures require ongoing planning, preparation, and practice.

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Panton-Valentine Leukocidin and Staphyloccoccal Skin Infections in Schoolchildren[1]

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The Panton-Valentine leukocidin is associated with staphylococcal skin and pulmonary infections. We describe a school outbreak of skin infections and the public health response to it. Nasal carriage of a Panton-Valentine leukocidin–positive *Staphylococcus aureus* clone was detected only in previously ill classmates and their family members.

Staphylococcus aureus colonizes approximately 30% of the general population and up to 50% of persons who are intravenous drug users, diabetics, or healthcare workers. The organism is mainly transmitted between persons by close contact (1,2). In addition to its increasing ability to resist antimicrobial agents, *S. aureus* displays a wide array of virulence factors that render it capable of causing a larger spectrum of infections than any other bacteria (1).

Exotoxins constitute essential components of the virulence mechanisms of *S. aureus*. Nearly all strains secrete hemolysins, nucleases, proteases, lipases, hyaluronidase, and collagenase, which convert host tissues into nutrients required for bacterial growth (3). Some strains produce additional exoproteins that may be responsible for particular clinical manifestations, including the staphylococcal enterotoxins, the toxic-shock syndrome toxin-1, the exfoliative toxins, and the Panton-Valentine leukocidin (PVL) (3). PVL, a bicomponent cytotoxin encoded by two contiguous and cotranscribed genes carried on a bacteriophage, causes leukocyte destruction and tissue necrosis. This cytotoxin is produced by <5% of *S. aureus* isolates and has been associated with necrotic lesions involving the skin and with severe necrotizing pneumonia (4,5).

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The Study

From September 1999 to November 2000, 6 of the 22 students from a single third-grade classroom in a town of 12,000 in western Switzerland had 13 episodes of skin infections, including furuncles, abscesses, and cellulitis. The outbreak peaked in October and November 2000, with three new cases and four relapses (Figure 1). Two children were hospitalized. Most patients were treated with systemic antimicrobial agents. Some needed surgical incisions and drainage. Six cultures were performed and consistently grew *S. aureus* resistant to penicillin and amoxicillin (PRSA) and susceptible to flucloxacillin, amoxicillin/clavulanic acid, cephalosporins, erythromycin, doxycycline, clindamycin, trimethroprim/sulfamethoxazole, ciprofloxacin, and rifampin.

At the end of November 2000, after health authorities were notified, nasal screening for carriage of *S. aureus* was performed first in the 22 classmates and their 2 teachers and next in the families of those who had had skin infections or who had been found to be carriers of PRSA. Nasal cultures were collected by using rayon swabs moistened with 5% NaCl, rotated five times in both anterior nares. The samples were carried within 2 hours to the laboratory and kept overnight at 35°C in an enrichment Mueller-Hinton broth with 5% NaCl. They were then plated onto mannitol-salt agar and sheep blood agar. The plates were incubated at 35°C with 5% CO_2 for 24 hours. Persons with mannitol-fermenting, coagulase-positive colonies were considered carriers of *S. aureus* after species were confirmed and antimicrobial susceptibility was determined in an automated system (Vitek 2, bioMérieux, France). PRSA were saved and subsequently typed by pulsed-field gel electrophoresis (PFGE) after digestion by *Sma*I. Three isolates representative of the three clones were forwarded to the French Reference Center for Staphylococcal Toxemia, where polymerase chain reaction (PCR)-based methods were used to detect genetic sequences encoding enterotoxins, exfoliative toxins, toxic-shock syndrome toxin-1, β-hemolysin, LukE-LukD leukotoxin, and PVL (4).

Ten of the 22 classmates and 1 of the 2 teachers (46%) were colonized with *S. aureus.* None harbored methicillin-resistant *S. aureus* (MRSA). Nine carried PRSA, including four of the six previously infected children (the other two had negative cultures). Two carried penicillin-susceptible isolates. The nine PRSA carriers and the two previously infected students with no nasal *S. aureus* had 36 family members, 3 of whom had recently also had skin infections. These three persons belonged to two families that included formerly infected classmates. Thirty-five family mem-

[1]This report was presented in part at the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), September 27–30, 2002, San Diego, California.

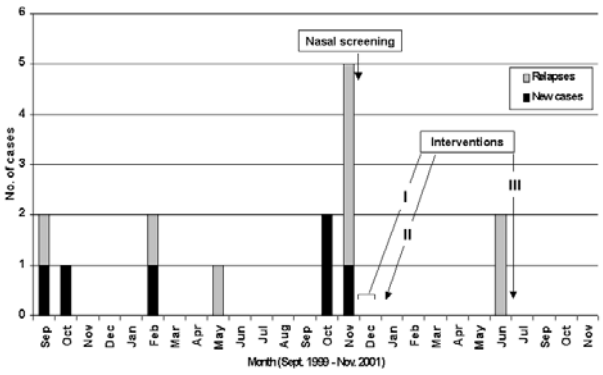

Figure 1. Cases of skin infections among schoolchildren, Switzerland, September 1999–November 2001. I: Nasal mupirocin twice a day, chlorhexidine showers once a day for carriers of penicillin-resistant *Staphylococcus aureus* and their family members (5 days); alcoholic hand rubs in the classroom and at home (3–4 weeks). II: Repeated measures (5 days) in those still found to be carriers and in their family members. III: Repeated measures limited to the two relapsing children and their family members.

bers were screened. Cultures grew *S. aureus* in 15 (43%), of whom 12 harbored PRSA and 3, penicillin-susceptible isolates. The results of PFGE, including PRSA isolates still found after the first set of preventive measures had been taken (see next paragraph), are shown in Figure 2. Genes encoding for PVL were found only in clone A (Table), which was harbored by three of the six previously infected classmates and six of their family members. The three clones showed the same susceptibility profile, which also corresponded to the profile found previously in the infecting isolates.

While waiting for PFGE and PCR results, we implemented measures derived from those in use for MRSA in hospitals (6). After information and teaching had been provided to the students and their parents, nasal mupirocin twice a day and chlorhexidine showers once a day for 5 days were prescribed for all the members of the nine families that included ≥1 PRSA carrier, regardless of clonal type. These measures were repeated a second time in five families because ≥1 of their members still harbored PRSA 1–2 weeks after the first set of measures was implemented. Moreover, alcohol-based hand rubs (500-mL multiuse containers) were used several times a day for 3 to 4 weeks in the nine families and by every student and teacher when entering and leaving the classroom.

One year later, no new case of skin infection had been detected among this population through active surveillance by the school nurse or periodic enquiries to the local pediatricians. Two children had relapsed 6 months later, in June 2001 (no culture available). Nasal mupirocin and chlorhexidine showers were repeated for 5 days in their families.

Conclusions

PVL has been associated with staphylococcal skin and pulmonary infections by means of retrospective and prospective studies comparing the clinical features of patients infected with PVL gene-positive or negative staphylococci (4,5). The intrafamilial spread of such strains (e.g., from mother to infant through breastfeeding) has been reported (7–9). To our knowledge, this report is the first on an outbreak attributable to a PVL-positive *S. aureus* clone that spread among schoolchildren and their families.

Lina et al. found that 50%-93% of *S. aureus* responsible for cutaneous abscess, cellulites, or furunculosis and 85% of those responsible for community-acquired pneumonia harbored the genes encoding for PVL compared with none of those causing diseases such as nosocomial pneumonia, infective endocarditis, urinary tract infection, enterocolitis, or toxic-shock syndrome (4). Gillet et al. found that 16 patients with community-acquired pneumona attributable to PVL-positive *S. aureus* were younger (median age 14.8 years), had less underlying disorders, and had more often had influenza-like syndromes or furuncles than 36 patients with community-acquired pneumonia due to *S. aureus* without PVL genes. The patients in the first group also had a more severe disease course and 75% of them died; in the other group, 47% died (5). Dufour et al. reported on 14 cases of community-acquired infections due to PVL-positive MRSA, suggesting the community emergence of a new superadapted *S. aureus* strain (9).

In our study, nine persons belonging to a population of classmates, teachers, and their family members were found to be nasal carriers of a single clone of PVL gene–positive, methicillin-susceptible *S. aureus* after six students and three of their relatives had had relapsing episodes of skin

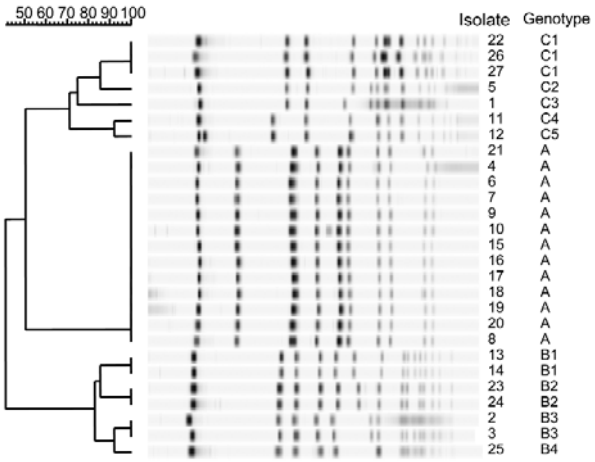

Figure 2. Band patterns of the *Staphylococcus aureus* isolates cultured from nasal swabs (pulsed-field gel electrophoresis). Some persons had two consecutive positive cultures (see text).

Table. Detection of genes encoding for various exotoxins, by clone

	Clone A	Clone B	Clone C
β-hemolysin	–	–	–
Enterotoxins	G, I, K, L, M	G, I, K, L, M	H, M, O
Exfoliative toxins	–	–	–
LukD–LukE	+	–	–
Panton-Valentine leukocidin	+	–	–
Toxic-shock syndrome toxin-1	–	–	+

[a]Multiplex polymerase chain reaction, one isolate representative of each clone.

infections in 13 months. Overall, the 44% prevalence of *S. aureus* carriage in this population was higher than the expected rate of 30% (2) but close to it without taking the isolates belonging to the PVL-positive clone (A) into account. Although the numbers were small and this difference could be due to chance, this finding suggests an addition to the usual rate of colonization caused by the introduction of clone A into the studied population, an event that seemed to have occurred recently, as suggested by its very homogenous PFGE profile as compared with the others.

Even though the *S. aureus* responsible for infections had not been saved and could therefore not be tested, the nasal recovery of a single PVL-positive clone only from persons who had had skin infections or from their family members strongly suggests pathogenicity of this particular clone. Indeed, Prevost et al. found identical PFGE profiles in nasal and furuncle PVL-positive isolates from patients with skin infections (10). Although only one isolate representative of each clone was tested for PVL genes, we believe that, given the large size of the bacteriophage carrying these genes, it is very unlikely that isolates showing the same PFGE pattern could differ regarding their PVL status. In addition, the *S. aureus* found in subsequent nasal swabs from the same persons in our study belonged consistently to the same clone as the first cultured isolate, suggesting that these persons were colonized with a single clone each and that the hazard of picking one among several strains of *S. aureus* for PFGE typing was low. Similarly, Peacock et al. found that 29 of 31 patients with >1 positive nasal culture for *S. aureus* within a 1-month period harbored the same clone over time (11). The presence of LukD and LukE genes in addition to genes encoding for PVL was considered trivial since these toxins do not appear to be linked with specific infections (3).

To prevent new cases or more severe diseases, the decision was made to implement MRSA measures derived from those in use in many hospitals (6). These measures consisted of the repeated application of nasal mupirocin and chlorhexidine showers for those considered at risk for infection or transmission and the simultaneous use of alcohol-based hand rubs for the same persons, plus all of their classmates and teachers. Given the absence of new cases and the occurrence of only two relapses 1 year after these measures were implemented, they may have played a role in controlling this outbreak.

In summary, PVL-positive *S. aureus* may spread between persons in close contact and cause disease, mainly among otherwise healthy children or young adults. The clinical manifestations may initially correspond to skin infections and then progress to severe necrotizing pneumonia with a high death rate. Despite its limitations, this study suggests that future research should investigate whether the early identification of such strains, coupled with timely measures aimed at decolonizing carriers and interrupting person-to-person transmission, could prevent or control potentially lethal outbreaks.

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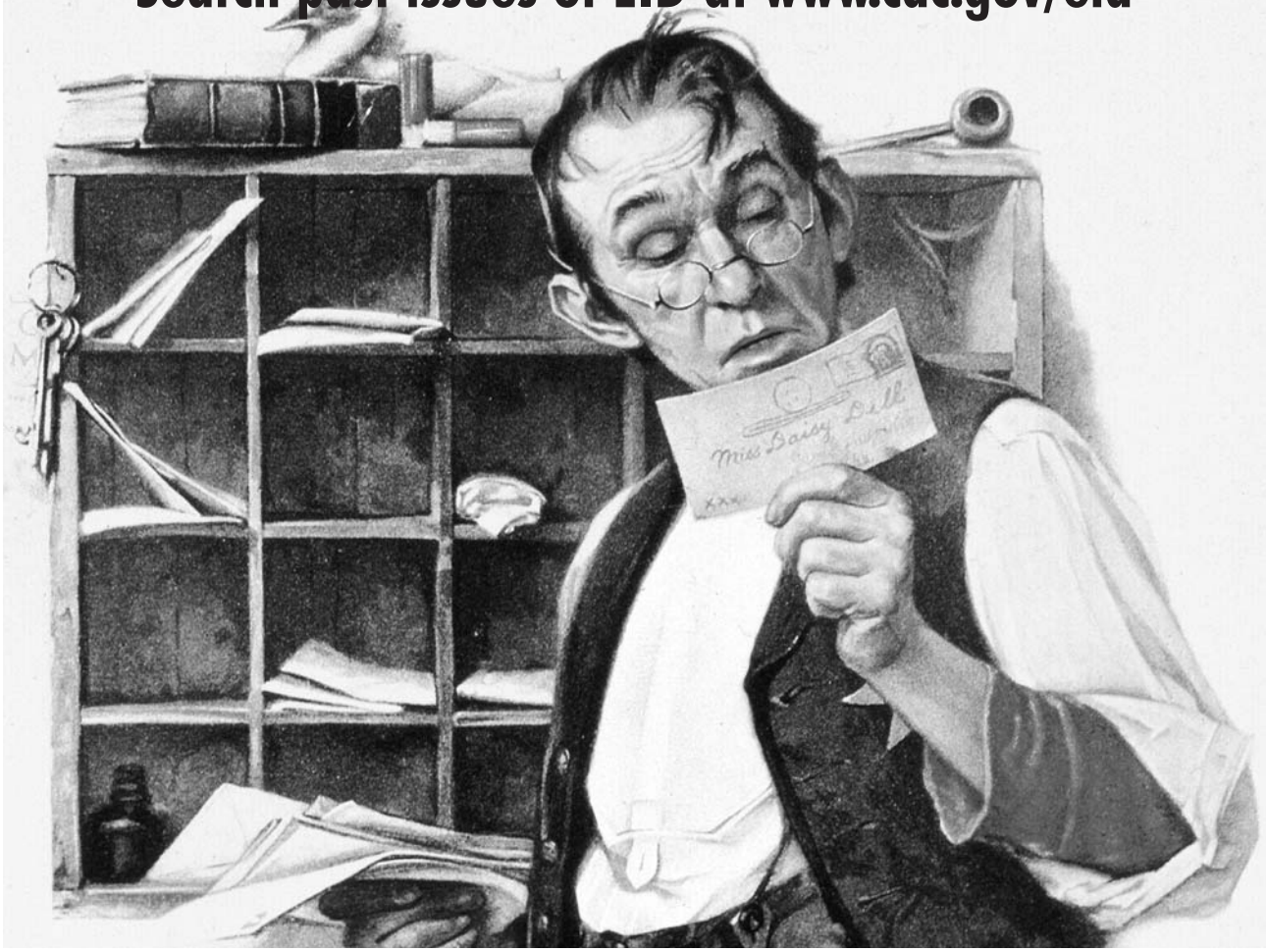

Rickettsia mongolotimonae Infection in South Africa

Anne-Marié Pretorius* and Richard J. Birtles†

We report the first laboratory-confirmed case of *Rickettsia mongolotimonae* infection in Africa. The patient sought treatment for an eschar on his toe; lymphangitis, severe headaches, and fever subsequently developed. After a regimen of doxycycline, symptoms rapidly resolved. *R. mongolotimonae* infection was diagnosed retrospectively by serologic tests and molecular-based detection of the organism in biopsy specimens of eschar material.

Rickettsioses are infections of emerging medical importance, particularly in southern Africa, where an increasing number of cases are being encountered among both residents and tourists (1). Three *Rickettsia* species have been associated with human disease in South Africa to date. *Rickettsia conorii* has long been recognized as the agent of Mediterranean spotted fever, and more recently, a newly recognized species, *R. africae*, has been identified as the agent of African tick-bite fever. In 2002, the first case report of a patient infected with *R. aeschlimannii* was published (2). In addition to these recognized pathogens, *Rickettsia* species, including *R. mongolotimonae*, have been detected in human-biting arthropods in Africa. This species (3) was first encountered in *Hyalomma asiaticum* ticks in Inner Mongolia in 1991 (4) but has subsequently been associated with human infections in southern France (5) and, perhaps of most relevance to this report, has been detected in *H. truncatum* ticks collected from cattle in Niger (6). This species of tick, which at least during its immature life stages parasitizes migratory birds, is widely distributed in many African countries, including South Africa (7).

The Study

In September 2002, a 34-year-old (HIV-seronegative) construction worker, working near Ellisras in South Africa's Northern Province, discovered a lesion on the inside of the second toe on his right foot (Figure); subsequently, severe headaches and high fever developed. He was examined at a local hospital and found to have lymphangitis extending pretibially from the lesion; as a result of his other symptoms, he was treated for blood poisoning with ceftriaxone sodium, 1,000 mg once daily. During the next 3 days, the lesion at the bite site (noted by the examining physician) remained very sore, and the patient's right inguinal lymph node became enlarged and very painful. The patient then decided to return to his hometown and sought treatment from his general practitioner (on day 5 after discovery of the lesion). On examination, the lesion and lymphangitis were clearly visible on the patient's toe, although cellulitis and edema were not observed. His inguinal lymph node had swollen to 3 cm in diameter, and he was still febrile (38.5°C). Blood samples were then obtained as well as a biopsy specimen from the lesion. A regimen of doxycycline, 100 mg per day orally, for 5 days was prescribed and 1 day's dosage was administered. The next day, the patient was afebrile, and the lymphangitis had completely resolved.

In the laboratory, a Giemsa stain of a smear prepared from the patient's blood showed activated lymphocytes. A complete blood count showed thrombocytosis (632 x $10^3/\mu L$), but all other hematologic parameters were within the normal range. Biochemical findings showed elevated levels of alanine transaminase (66 IU/L), blood urea nitrogen (7.2 g/L), and triglycerides (2.2 mmol/L); and decreased levels of chloride (96.3 mmol/L) and albumin (38g/L); all other tests yielded results within the normal range. Testing of the patient's serum with the Weil-Felix test demonstrated an antibody titer (80) only against the OX2 *Proteus* antigen, giving presumptive evidence of a rickettsial infection. As a result, antirickettsial microimmunofluorescence testing was performed (8). The serum did not yield significant immunoglobulin (Ig) M titers against *R. conorii* or *R. africae* antigens, but IgG titers of

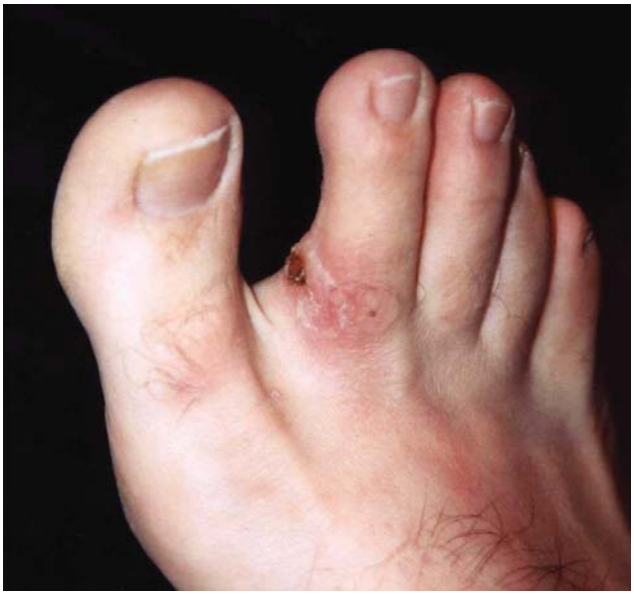

Figure. Eschar with lymphangitis.

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64 were found by using both antigens. DNA was extracted from the eschar biopsy specimen by using the QIAamp Tissue kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. This DNA extract was used as template in a previously described polymerase chain reaction assay targeting a *Rickettsia* spp. *rOmpA* fragment (9). An amplification product was obtained from this extract but not from any concurrently processed control materials. The amplification product was purified; the nucleic acid sequences of both strands were then determined. The sequence obtained from these efforts was found to share >99% similarity with the corresponding *rOmpA* fragment of *R. mongolotimonae*.

Conclusions

The combination of clinical and laboratory data yielded strong evidence that the case described here was an infection of *R. mongolotimonae*, the first reported in southern Africa. A single eschar is also typical of *R. conorii* infections, but these are characterized by rash (Mediterranean spotted fever), which the current patient did not have. Although *R. africae* infections manifest only rarely as a rash, they are typified by multiple eschars (10). This case description is also very similar to that relating to a French patient infected with *R. mongolotimonae*, who had lymphangitis and inguinal lymphadenopathy. The serologic findings indicate exposure to a spotted fever group rickettsia rather than to a specific species within this group, but the near identity of the *rOmp*A sequence obtained from the patient's eschar to that of *R. mongolotimonae* provides a clear indication that this species, rather than other spotted fever group rickettsiae, was present at the site of the tick bite. Although no tick was found in association with the patient's eschar, his infection may have been acquired from a *H. truncatum*, as this species is abundant in the region of the bushveld where the patient had been working and is known to feed on humans (11).

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Domestically Acquired *Campylobacter* Infections in Finland

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Campylobacter jejuni isolates (n = 533) from domestic cases diagnosed in Finland during a 3-month peak period were studied. The highest rate was observed among those 70–74 years of age. Domestic *C. jejuni* isolates were especially frequent in the eastern districts. Six serotypes covered 61% of all *C. jejuni* isolates.

In developed countries, campylobacters are the most common culture-confirmed bacterial causes of gastroenteritis (1,2). The most common species identified in patients is *Campylobacter jejuni*. Since 1998, campylobacters also have been the most common bacterial enteropathogens detected in Finnish patients. A similar increasing trend has been recognized in many other European countries, such as Sweden, Denmark, and the United Kingdom (3–5). The epidemiology and modes of transmission are not well known, but risk factors for acquiring *Campylobacter* infection include handling or eating chicken, barbecuing, drinking unpasteurized milk or contaminated water, and traveling abroad (1,6–8).

Some 30%–40% of *Campylobacter* infections detected in Swedish persons have been acquired in Sweden (3). Yet, in Denmark, approximately 80% of human infections are of domestic origin (4), suggesting that differences in risk factors may exist in these neighboring countries. In Finland, since 1994, all clinical microbiology laboratories are required to notify the National Infectious Disease Register (NIDR) of all *Campylobacter* findings based on culture, but no data on the distribution of domestically acquired and imported *Campylobacter* infections are collected.

We attempted to collect all *Campylobacter* isolates cultured in Finland from clinical stool samples of patients with domestically acquired infections during the seasonal peak of *Campylobacter* infections in 1999 and to analyze the heat-stable serotypes of *C. jejuni* strains.

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The Study

All clinical microbiology laboratories culturing campylobacters in Finland were asked to collect domestic *Campylobacter* isolates detected from human clinical fecal samples. Isolates collected on July 1 through September 30, 1999, from patients who had not been abroad for 2 weeks before becoming ill, were considered of domestic origin and were included in the study. Information on foreign travel, received by the physicians, was collected by the local clinical microbiology laboratories when culture results were reported to the clinical unit. The isolates were sent to Helsinki University Central Hospital (HUCH) Laboratory Diagnostics along with information about the patient (date of birth, sex, recent travel history) and the isolate (date of stool sampling, hippurate hydrolysis result). Consecutive isolates from the same patient were excluded. Isolates were stored at –70°C before serotyping. Hippurate-positive (*C. jejuni*) isolates were subsequently serotyped based on heat-stable Penner's (Pen) antigens by passive hemagglutination using a serotyping set including 25 antisera (Campylobacter Antisera Seiken Set, Denka Seiken Co., Tokyo) as earlier described (9).

A total of 3,303 *Campylobacter* cases in Finland in 1999 were reported to NIDR; of these, 1,412 (43%) cases were diagnosed during our study period. In the present study, a total of 551 *Campylobacter* isolates were collected from patients who had presumably acquired their infections in Finland. The absence of case linkage between the two data sources prevents exact correlation between them; however, the number of domestic cases from the isolate collection is approximately 40% of the number of cases in NIDR for the same period. Of the strains collected, 533 (97%) were *C. jejuni* and 18 (3%) were *C. coli*. (Consecutive isolates related to outbreaks were not detected.)

The 533 case-samples of domestic *C. jejuni* infection collected within a population of 5.17 million yields a rate of 41.2 domestic *C. jejuni* cases per 100,000 inhabitants for the 3-month period. A higher proportion of patients were male (304 males, 57%). The rate of domestic *C. jejuni* infections by age group varied from 19.6 to 72.8 per 100,000 inhabitants for the 3-month period, with the highest rates observed among those ≥60 years old, among young adults (20–34 years of age), and among children <5 years of age (Figure 1).

Based on the municipality of the clinical unit from which the stool culture had been sent, several eastern hospital districts had high rates of domestic *Campylobacter* cases during the study period; the 95 % confidence intervals for these cases did not overlap those

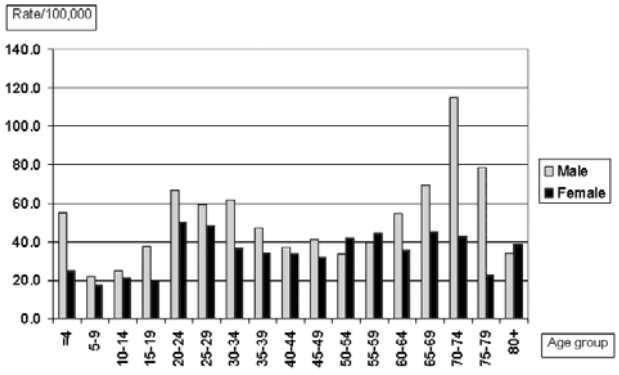

Figure 1. Domestically acquired *Campylobacter jejuni* infections in Finland by age and sex, July–September 1999.

in several southern and western hospital districts with low rates (Figure 2). In some eastern districts, domestically acquired *Campylobacter* infections comprised even more than 80% of all cases reported to NIDR in July. In the other hospital districts, a peak was also demonstrated in July; then the number of domestic *C. jejuni* cases declined in August, and the number of isolates collected in September comprised only 10% of all strains isolated during the 3-month study period.

The seasonal distribution of the different serotypes is presented in the Table. The predominant serotypes (Pen 1,44, Pen 2, Pen 4-cluster, Pen 6,7, Pen 12, and Pen 27) comprised 61% of the isolates (Table). No clear geographic differences in the distribution of serotypes were found. None of the strains reacted with the antisera against serotypes Pen 10, 32, 38, or 45. Reactions with serotype Pen 3 and Pen 8 antisera were only present in complex serotypes. Serotypes Pen 5, 11, 18, 19, 31, 52, and 55 (not included in the Table) were so uncommon that they together represented approximately 5% of all the isolates.

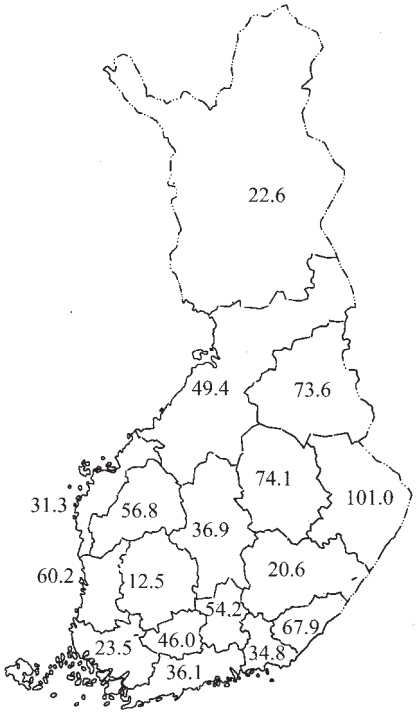

Figure 2. Rate of domestically acquired *Campylobacter jejuni* infections in Finland per 100,000 inhabitants, July–September 1999.

Table. Penner serotypes of *Campylobacter jejuni* isolates detected from human fecal samples of patients in Finland with domestically acquired infections, July–September 1999

	No. (%)[a]			
Penner serotype	July	August	September	3-month period
1,44	17 (5)	4 (3)	3 (6)	24 (5)
2	7 (2)	29 (21)	10 (19)	46 (9)
4-cluster[b]	34 (10)	19 (14)	6 (11)	59 (11)
6,7	75 (22)	11 (8)	1 (2)	87 (16)
12	32 (9)	23 (17)	16 (30)	71 (13)
15	4 (1)	1 (1)	1 (2)	6 (1)
21	8 (2)	0 (0)	2 (4)	10 (2)
23, 36, 53	2 (1)	2 (1)	3 (6)	7 (1)
27	30 (9)	9 (7)	0 (0)	39 (7)
37	2 (1)	7 (5)	0 (0)	9 (2)
41	6 (2)	0 (0)	0 (0)	6 (1)
57	16 (5)	3 (2)	0 (0)	19 (4)
Untypeable	36 (11)	19 (14)	5 (9)	60 (11)
Complex	64 (19)	7 (5)	4 (7)	75 (14)
Total	342	137	54	533

[a]Uncommon serotypes Pen 5, 11, 18, 19, 31, 52, and 55 not included.
[b]4-cluster, serotypes 4, 13, 16, 43, 50.

The mean age of all case-patients was 41.4 years. Patients infected with *C. jejuni* serotype Pen 15 (mean age 23.5 years, 95% CI 6.0, 41.0) or serotype Pen 2 (mean age 32.7 years, 95% CI 26.3, 39.0) were significantly younger than those infected with serotype Pen 27 (mean age 49.6 years, 95% CI 42.7, 56.4) or serotype Pen 6,7 (mean age 46.8 years, 95% CI 42.2, 51.4). The age distribution of serotypes Pen 2 and Pen 27 is shown in Figure 3. Differences between the age distributions of case-patients infected with other serotypes were not significant.

Conclusions

All hospital districts in Finland participated in this study, and the rate of domestically acquired *C. jejuni* infections, as measured by the number of isolates sent, was highest in several eastern hospital districts. The higher rates cannot be explained by urban versus rural lifestyle alone since the western districts in the study included rural and urbanized areas in an approximately similar proportion as in the eastern districts.

The six most commonly found serotypes accounted for approximately 60% of the cases. We have studied human domestic *Campylobacter* infections in Finland since 1995 (9–11) and found the same serotypes to be common among *C. jejuni* isolates collected during July through September,

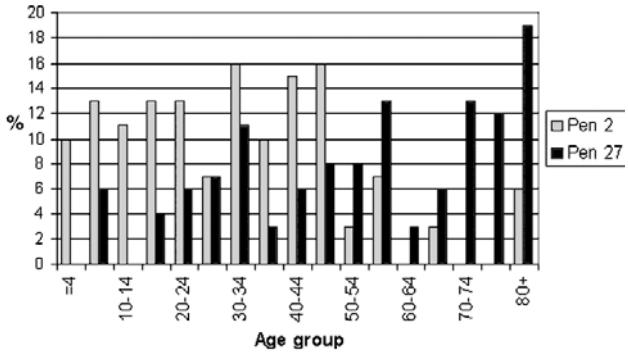

Figure 3. Pen 2 and Pen 27 serotypes among domestically acquired *Campylobacter jejuni* infections in Finland by age, July–September 1999.

although annual variation in the relative proportion of predominant serotypes is evident. Serotypes Pen 1,44, Pen 2, and Pen 4-cluster have been found relatively often in human fecal samples in Denmark (4,12), England (13), and New Zealand (14), but serotypes Pen 6,7, Pen 12, and Pen 27 have been less common (<3% each) in those studies.

From May to September 1999, a total of 1,132 chicken flocks, representing most of the chicken meat produced and consumed in Finland, were monitored for campylobacters. Thirty-one *C. jejuni*–positive flocks were detected; the most common Penner serotypes identified were Pen 6,7 and Pen 12, found mainly from July through August (15). These particular serotypes were also commonly found in human infections in the present study in July and in August. Serotypes Pen 27 and Pen 4-cluster were also found in the chickens (15). These results indicate some overlapping between human and chicken strains at the serotype level. Humans and chicken may share a common source for *C. jejuni*, or humans may acquire the infection from contaminated chicken meat.

The highest rate of domestic *C. jejuni* infection was found among patients in the age group of 70–74 years, accompanied by an above average rate among patients 65–69 years old. This is the first report of such an age peak for *C. jejuni* (1,3,4). Furthermore, the age distribution of patients infected with isolates of certain serotypes suggests that older people in Finland may have somewhat different sources of infection than younger people. Serotypes Pen 6,7 and Pen 27, in particular, appeared to be relatively common among the elderly patients. In our study, serotype Pen 2 was more common than Pen 27 in all age groups <50 years of age (excluding the age group 25–29 years) but less common in all older groups. Since serotypes Pen 6,7 and Pen 27 are also common in chicken (15), there may be a link between raw poultry handling or chicken consumption practices and *C. jejuni* in the elderly.

During the 1999 seasonal peak, domestically acquired cases of *C. jejuni* in Finland were caused by the same serotypes that are most commonly found in other developed countries, yet some common serotypes in our study seem to be less common elsewhere. High rates were observed among elderly men and in the east of Finland. The six most common serotypes covered 61% of all isolates. We recommend further study to determine if the results were specific to the time period or representative of a persistent phenomenon.

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Salmonella Serovars from Humans and Other Sources in Thailand, 1993–2002

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We serotyped 44,087 *Salmonella* isolates from humans and 26,148 from other sources from 1993 through 2002. The most common serovar causing human salmonellosis in Thailand was *Salmonella enterica* Weltevreden. Serovars causing human infections in Thailand differ from those in other countries and seem to be related to *Salmonella* serovars in different food products and reservoirs.

Salmonella enterica is one of the most common causes of human gastroenteritis. The infection is caused primarily by improper handling and digestion of uncooked food; a large number of food animal sources have been identified as reservoirs of the bacteria (1). More than 2,500 serovars of *S. enterica* have been identified; most have been described as the cause of human infections, but only a limited number of serovars are of public health importance. *S. enterica* serovars Typhimurium and Enteritidis have been reported to be the most common causes of human salmonellosis (1,2). However, in some regions other serovars are of greater importance (3,4).

Different serovars in one country can be of global importance because of travel and animal and food product trade. Knowledge about the occurrence and epidemiology of different serovars in different countries and geographic regions may assist in the recognition and tracing of new emerging pathogens. We review the trends in serovars of *Salmonella* causing infections in humans and potential reservoirs in Thailand during 1993 to 2002.

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The Study

The World Health Organization (WHO) National Salmonella and Shigella Centre in Bangkok receives all isolates suspected to be *Salmonella* from all diagnostic laboratories throughout Thailand. From1993 to 2002, these have included 62 governmental general hospitals; 5 private hospitals; 12 regional medical centers; 3 laboratories within the Department of Livestock Development; 6 laboratories within the Fisheries Department, the Laboratory of Bangkok Health Center, and U.S. Embassy; and 28 food industry laboratories. All laboratories are encouraged to use both direct plating and enrichment broth to isolate *Salmonella*. For enrichment the laboratories use Selenite, Tetrathionate or Rappaport-Vassiliadis Soya Peptone broth. For direct plating they use Salmonella/Shigella, Xylose Lysine Desoxycholate, Brilliant Green or Modified Semi-solid Rappaport-Vassiliadis agar. Accepted brands are Difco, Oxoid, MAST, BBL, and Merck. For shipment the isolates are stabbed in nutrient agar sticks.

On arrival, all isolates were purified and confirmed to be *Salmonella* on the basis of reactions on triple sugar iron agar and lysine indol motility agar. All strains identified as *S. enterica* were serotyped according to the Kauffman-White serotyping scheme (5). *Salmonella* antisera (S & A Reagent Laboratory LMT, Bangkok, Thailand) were used for serotyping. From 1993 through 2002, a total of 70,235 isolates received were confirmed as *S. enterica* and serotyped.

A total of 118 serovars were identified among the 44,087 isolates from humans. The 25 most common serovars accounted for 86% of the isolates, the 10 most common for 64.7%, and the 5 most common serovars (*S.* Weltevreden, *S.* Enteritidis, *S.* Anatum, *S.* Derby, *S.* 1,4,5,12:i) for 44.3% of the isolates (Table 1). The proportion of *S.* Weltevreden isolates decreased from 13.5% in 1993 to 9.3% in 1996 and has since increased to 18% in 1999, 15.9% in 2001, and 7.9% in 2002. The proportion of *S.* Enteritidis isolates has decreased during the period from 14% to 9% in 2001 and 12.6% in 2002. The proportion of *S.* Anatum has varied from 4% to 10%. *Salmonella* (1,4,5,12:i) peaked in 1996 at 10% but has otherwise been 6%-8%. The proportion of *S.* Typhimurium isolates peaked in 1997 at 9%, but was 4% in 2002. An increase has been observed for *S.* Rissen (2% to 8%), *S.* Stanley (2% to 6%), *S.* Panama (1% to 6%), and *S.* Schwarzengrund (0% to 2%), while a decrease has been observed for *S.* Derby (11% to 3%) and *S.* Krefeld (4.5% to 1%). The trends of the most common serovars are shown in Figure 1.

Samples have not been systematically taken from the different reservoirs for *Salmonella* infections in humans. However, data from samples were available from chicken, seafood, other food products, and water for all 10 years. Data from ducks were only available from 1998 to 2002.

Table 1. The 25 most common serovars of *Salmonella* isolates from humans reported annually, 1993–2002, Thailand

Serovar	Y and no. of isolates (%)										
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	Total
Weltevreden	443	574	816	337	335	485	862	660	657	322	5,491
	(13.5)	(9.9)	(12.3)	(9.3)	(9.7)	(11.6)	(18.0)	(16.1)	(15.9)	(7.9)	(12.5)
Enteritidis	471	833	877	489	365	396	401	306	357	515	5,010
	(14.3)	(14.4)	(13.2)	(13.4)	(10.5)	(9.5)	(8.4)	(7.5)	(8.6)	(12.6)	(11.4)
Anatum	146	397	568	229	298	320	235	412	340	318	3,263
	(4.4)	(6.9)	(8.5)	(6.3)	(8.6)	(7.6)	(4.9)	(10.1)	(8.2)	(7.8)	(7.4)
Derby	368	650	576	277	252	251	141	156	111	107	2,889
	(11.2)	(11.3)	(8.7)	(7.6)	(7.3)	(6.0)	(3.0)	(3.8)	(2.7)	(2.6)	(6.6)
1, 4, 5, 12:i:-ssp.I	193	272	422	355	212	228	248	248	336	290	2,804
	(5.9)	(4.7)	(6.3)	(9.8)	(6.1)	(5.4)	(5.2)	(6.1)	(8.1)	(7.1)	(6.4)
Typhimurium	154	216	326	238	305	278	258	205	175	167	2,322
	(4.7)	(3.7)	(4.9)	(6.5)	(8.8)	(6.6)	(5.4)	(5.0)	(4.2)	(4.1)	(5.3)
Rissen	54	162	222	143	295	246	317	287	259	334	2,319
	(1.6)	(2.8)	(3.3)	(3.9)	(8.5)	(5.9)	(6.6)	(7.0)	(6.3)	(8.2)	(5.3)
Stanley	64	147	186	85	99	147	245	210	242	263	1,688
	(1.9)	(2.5)	(2.8)	(2.3)	(2.9)	(3.5)	(5.1)	(5.1)	(5.9)	(6.4)	(3.8)
Panama	31	64	9	80	173	172	264	209	160	230	1,474
	(0.9)	(1.1)	(1.4)	(2.2)	(5.0)	(4.1)	(5.5)	(5.1)	(3.9)	(5.6)	(3.3)
Agona	118	215	236	103	102	76	95	76	75	90	1,096
	(3.6)	(3.7)	(3.6)	(2.8)	(2.9)	(1.8)	(2.0)	(1.9)	(1.8)	(2.2)	(2.7)
Choleraesuis	99	87	139	122	68	118	92	69	85	186	1,065
	(3.0)	(1.5)	(2.1)	(3.4)	(2.0)	(2.8)	(1.9)	(1.7)	(2.1)	(4.5)	(2.4)
Hadar	64	8	198	67	80	8	96	106	136	112	1,023
	(1.9)	(1.4)	(3.0)	(1.8)	(2.3)	(2.0)	(2.0)	(2.6)	(3.3)	(2.7)	(2.3)
Paratyphi A	76	107	134	330	47	157	108	—	15	7	981
	(2.3)	(1.9)	(2.0)	(9.1)	(1.4)	(3.8)	(2.3)		(0.4)	(1.7)	(2.2)
Krefeld	149	129	135	52	74	67	72	36	32	39	785
	(4.5)	(2.2)	(2.0)	(1.4)	(2.1)	(1.6)	(1.5)	(0.9)	(0.8)	(1.0)	(1.8)
Paratyphi B var Java	31	40	66	46	61	56	113	120	117	48	698
	(0.9)	(0.7)	(1.0)	(1.3)	(1.8)	(1.3)	(2.4)	(2.9)	(2.8)	(1.2)	(1.6)
Typhi	61	53	41	42	43	64	68	—	213	82	667
	(1.9)	(0.9)	(0.6)	(1.2)	(1.2)	(1.5)	(1.4)		(5.2)	(2.0)	(1.5)
Virchow	52	69	77	28	35	45	89	70	102	79	646
	(1.6)	(1.2)	(1.2)	(0.7)	(1.0)	(1.1)	(1.9)	(1.7)	(2.5)	(1.9)	(1.5)
Lexington	40	67	66	35	45	60	68	56	88	52	577
	(1.2)	(1.2)	(1.0)	(1.0)	(1.3)	(1.4)	(1.4)	(1.4)	(2.1)	(1.3)	(1.3)
Blockley	82	78	53	27	20	49	45	56	47	41	498
	(2.5)	(1.4)	(0.8)	(0.7)	(0.6)	(1.2)	(0.9)	(1.4)	(1.1)	(1.0)	(1.1)
Hvittingfoss	12	94	125	27	12	16	66	41	33	35	461
	(0.4)	(1.6)	(1.9)	(0.7)	(0.3)	(0.4)	(1.4)	(1.0)	(0.8)	(0.9)	(1.0)
Senftenberg	62	126	64	16	28	37	29	20	26	44	452
	(1.9)	(2.2)	(1.0)	(0.4)	(0.8)	(0.9)	(0.6)	(0.5)	(0.6)	(1.1)	(1.0)
Bovismorbificans	32	54	87	16	37	42	56	30	29	56	439
	(1.0)	(0.9)	(1.3)	(0.4)	(1.1)	(1.0)	(1.2)	(0.7)	(0.7)	(1.4)	(1.0)
London	27	92	72	45	67	71	24	15	8	0	421
	(0.8)	(1.6)	(1.1)	(1.2)	(1.9)	(1.7)	(0.5)	(0.4)	(0.2)	(0.0)	(1.0)
Schwarzengrund	0	9	3	3	6	26	76	99	98	52	372
	(0.0)	(0.2)	(0.0)	(0.1)	(0.2)	(0.6)	(1.6)	(2.4)	(2.4)	(1.3)	(0.8)
Emek	31	38	56	29	29	51	30	26	27	30	347
	(0.9)	(0.7)	(0.8)	(0.8)	(0.8)	(1.2)	(0.6)	(0.7)	(0.7)	(0.7)	(0.8)
Other	424	1,116	1,011	415	380	643	679	577	366	598	6,299
	(12.9)	(19.3)	(15.2)	(11.4)	(11.0)	(15.4)	(14.2)	(14.1)	(8.9)	(14.6)	(14.3)
Total	3,284	5,770	6,647	3,636	3,468	4,184	4,777	4,090	4,134	4,097	44,087

The 10 most common serovars from all sources are given in Table 2. All serovars that were represented with >6% of the isolates are given in Figure 2.

S. Enteritidis (19.9%) was the most common serovar among the 14,559 *Salmonella* isolates from chicken, followed by *S.* Hadar (9.3%) and *S.* Paratyphi B var Java (7.1%). The most common serovar among the 1,007 isolates from seafood was *S.* Weltevreden (26%); among the 2,670 isolates from duck, the most commonly isolated serovars were, *S.* Weltevreden (12%), *S.* Hadar (9.9%), *S.* Stanley (10.4%), and *S.* Chester (6.4%). Among the 6,928 isolates from other food products, *S.* Anatum (17.0%), *S.* Rissen (10.3%), *S.* Hadar (6.3%), and *S.* Weltevreden (6.6%) were the predominant serovars; among the 984 isolates from water, they were *S.* Weltevreden (14.5%), *S.* Anatum (11.5%), *S.* Rissen (9.5%), and *S.* Derby (7.2%).

Similar trends were detected for some of the serovars among isolates causing infections in humans and contami-

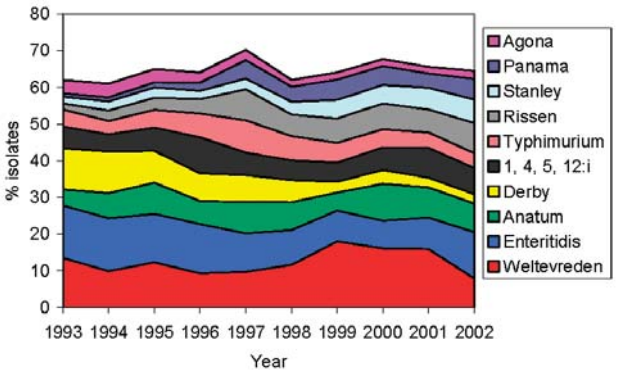

Figure 1. Trends over time for the 10 most common *Salmonella* serovars causing infections in humans between 1993 and 2002.

nation in some of the food products (Tables 3 and 4). In chicken, the relative incidence of *S.* Enteritidis decreased from 17.1% in 1993 and 33.8% in 1994 to 6.6% in 2001 and increased to 14.2% in 2002. Similarly, *S.* Derby decreased from 6.7% in 1993 to 1.1% in 2002. In contrast, *S.* Schwarzengrund increased from 0.3% in 1993 to 26.2% in 2001, with a decrease to 7.2% in 2002. *S.* Rissen in other food products increased from 4.7% (1993) to 14.7% (2002); *S.* Panama was found in <3% (1993) to >4% (2002); *S.* Stanley was found in 1% (1993) to 7.3% (2002); and *S.* Schwarzengrund was found in 0% (1993) to 3% (2001), followed by a decrease to 1% (2002).

Conclusions

S. enterica continues to be one of the most important causes of foodborne gastrointestinal infections in humans. During the last few decades *S.* Enteritidis and *S.* Typhimurium have emerged as the two predominant serovars in most Western countries. The epidemiology of these serovars has been studied, and different programs have been established to limit the spread of these serovars. However, other serovars may have a different epidemiology, and conditions are optimal for spread between reservoirs in some countries.

Our report indicates that *S.* Weltevreden was the most common serovar isolated from humans in Thailand. A similar finding has been reported from Malaysia (3), and *S.* Weltevreden was the most common serovar to cause human infections in India during the early 1970s (6). Before 1970, this serovar constituted <4% of total human salmonellosis. The number of reported infections caused by *S.* Weltevreden increased in the early 70s; in 1972, this serovar constituted 29.1% of all *Salmonella* infections in India. Thong et al. (7) found the same types of *S.* Weltevreden among isolates infecting humans and those in

Table 2. Distribution of the 10 most common serovars from the different reservoirs, Thailand[a]

	Reservoir and no. of isolates (%)					
Serovar	Humans	Frozen chicken	Frozen seafood	Frozen duck	Other food products	Water
Weltevreden	5,491 (12.5)	—	265 (26.3)	320 (12.0)	457 (6.6)	143 (14.5)
Enteritidis	5,010 (11.4)	2,901 (19.9)	14 (1.4)	—	309 (4.5)	22 (2.2)
Anatum	3,263 (7.4)	423 (2.9)	20 (2.0)	—	1,177 (17.0)	113 (11.5)
Derby	2,889 (6.6)	—	20 (2.0)	—	370 (5.3)	71 (7.2)
1, 4, 5, 12:i:-ssp.I	2,804 (6.4)	—	—	—	—	—
Typhimurium	2,322 (5.3)	—	12 (1.2)	—	198 (2.9)	—
Rissen	2,319 (5.3)	—	21 (2.1)	—	712 (10.3)	93 (9.5)
Stanley	1,688 (3.8)	—	20 (2.0)	279 (10.4)	—	—
Panama	1,474 (3.3)	—	—	41 (1.5)	254 (3.7)	47 (4.8)
Agona	1,096 (2.7)	452 (3.1)	—	80 (3.0)	273 (3.9)	39 (4.0)
Paratyphi B var Java	—	1037 (7.1)	—	—	—	—
Hadar	—	1,357 (9.3)	21 (2.1)	263 (9.9)	439 (6.3)	—
Virchow	—	863 (5.9)	—	—	249 (3.6)	27 (2.7)
Schwarzengrund	—	565 (3.9)	—	—	—	—
Emek	—	359 (2.5)	—	—	—	—
Blockley	—	676 (4.6)	—	—	—	—
Amsterdam	—	368 (2.5)	—	103 (3.9)	—	—
Seftenberg	—	—	49 (4.9)	86 (3.2)	—	—
Lexington	—	—	47 (4.7)	—	—	35 (3.6)
Newport	—	—	—	100 (3.7)	—	—
Tennessee	—	—	—	77 (2.9)	—	—
Chester	—	—	—	171 (6.4)	—	—
London	—	—	—	—	—	22 (2.2)
Other	15,824 (35.9)	5,558 (38.2)	518 (51.4)	1,150 (43.1)	2,490 (35.9)	372 (37.8)
Total	44,087	14,559	1,007	2,670	6,928	984

[a]—, not among the top 10 serovars.

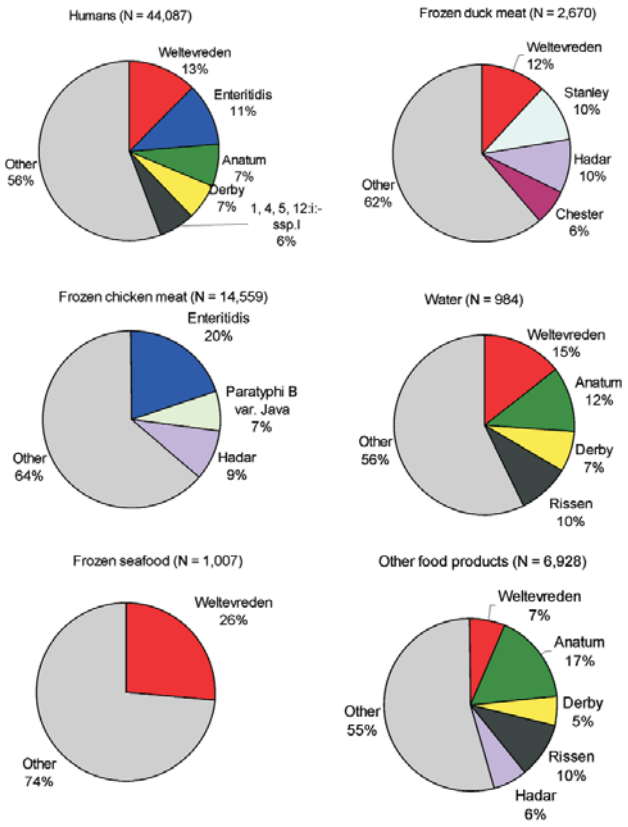

Figure 2. Distribution of the most common *Salmonella* serovars among the different reservoirs. Only serovars accounting for more than 6% of the isolates were included.

raw vegetables, suggesting that this is a potential reservoir of this serovar in Malaysia. Raw vegetables may, however, be contaminated by both feces and water. In a recent study from the United States, *S.* Weltevreden was the most common serovar found in seafood mainly imported from Thailand and Malaysia (8). These observations could point to a water-related source for *S.* Weltevreden. *S.* Weltevreden was the most common serovar in isolates from seafood, water, and duck, which suggests a water-related source for this serovar in Thailand.

S. Enteritidis infections in humans in Thailand increased from 1.3% in 1990 to 14% in 1993 to 1994 (4). Thus, Thailand has also been part of the global pandemic of *S.* Enteritidis observed in the late 1980s (9). The 1995 global survey conducted by WHO showed that the global pandemic has continued and expanded (2). The *S.* Enteritidis pandemic appears to have ended in 1997; this finding is similar to the decrease observed in Thailand, where *S.* Enteritidis has decreased during the last decade. However, this serovar is still an important cause of human infections, reflected in the increase in 2002. The frequent occurrence of this serovar in chickens suggests that poultry may be an important reservoir, a finding that is consistent with almost all other studies in other countries (10). Eggs have also been found to be important reservoirs in other countries (10) but were not examined in this study.

S. Anatum has consistently been one of the most important causes of salmonellosis in Thailand. The main reservoirs seem to be other food products and water. This serovar has previously been isolated from a large number of different animal sources.

The importance of *S.* Derby has decreased in Thailand. *S.* Derby has been associated with pigs (11,12). Pork and other swine products were not sampled in this report, but a frequent occurrence of the serovar was observed among unspecified other food products, which could include pork.

Salmonella isolates of serovar (1,4,5,12:I) were frequently found in isolates from humans, but infrequently in isolates from the different food reservoirs. Thirty isolates from Thailand were examined by phage typing and susceptibility testing (data not shown). A variable resistance pattern was observed, and five (17%) had a resistance pattern and phage reaction in agreement with *S.* Typhimurium U302. Twenty (67%) did not react with any phages. Some of these isolates from Thailand might be *S.* Typhimurium, but a large number might belong to other serovars.

S. Typhimurium is among the most prevalent serovars in Europe and America and of growing importance in Southeast Asia, Africa, and the Western Pacific (2). In Thailand, the importance of this serovar has not increased and continues to account for 5% of all human infections. *S.* Typhimurium can be found among a large number of different animal reservoirs; no specific source has been found.

S. Rissen has been isolated infrequently as a cause of human infections, and limited information about the potential reservoirs are available. The importance of this serovar seems to be increasing in Thailand. A specific reservoir for

Table 3. Annual number of reported *Salmonella* isolates from chicken in which changes in number of infections in humans were observed

Salmonella serovar	Trend in human isolates (%)	% of isolates from chicken									
		1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Enteritidis	14 to 13	17.1	33.8	29.5	15.0	18.5	15.3	14.2	12.0	6.6	14.2
Derby	11 to 3	6.7	0.9	2.6	4.3	1.2	0.4	1.3	1.1	1.6	1.1
Schwarzengrund	0 to 1	0.3	0.1	0.0	0.2	1.6	1.4	3.5	15.0	26.2	7.2
No. of isolates		1,909	2,370	2,010	1,005	1,534	1,414	908	952	836	1,621

Table 4. Annual number of reported *Salmonella* isolates from other food products in which changes in number of infections in humans were observed

Salmonella serovar	Trend in human isolates (%)	% of isolates from other food products									
		1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Rissen	2 to 8	4.7	11.0	8.9	10.0	3.6	7.0	8.6	15.3	14.2	14.7
Panama	1 to 6	2.8	1.6	1.6	2.9	2.1	0.6	4.2	6.7	5.3	4.2
Stanley	2 to 6	0.9	1.1	1.6	1.8	0.9	1.9	3.0	1.9	1.9	7.3
Schwarzengrund	0 to 1	0.0	0.0	0.0	0.0	0.4	0.6	1.7	3.8	2.9	1.0
No. of isolates		107	182	258	450	1,498	483	999	946	697	1,308

S. Rissen has not been identified, but the frequent occurrence of this serovar in *Salmonella* from water and other food products indicates that a foodborne or waterborne reservoir is a possibility.

S. Stanley infections were among the 15 most common serovars in 12 of 104 countries (2) in 1995. The relative importance of this serovar seems to be increasing in Thailand, and the only reservoir where the serovar was found in high frequency was duck.

S. Panama has been the cause of a number of outbreaks in different countries (13) and among the 15 most common serovars in 10 of 104 countries in a recent WHO survey (2). To date, no specific reservoir has been identified. The importance of this serovar seems to be increasing in Thailand and may be correlated to a simultaneous increase among food products.

S. Schwarzengrund has only been isolated sporadically from infections in humans and from animal sources. The number of infections caused by this serovar in Thailand is still very low. However, the proportion seems to be increasing in isolates from humans and chicken. From 1993 to 1997, this serovar constituted <0.2% of all reported humans salmonellosis, a proportion that increased from 1% to 2% in 2001 to 2002. During the same period, the proportion among isolates from chicken has increased from a similar figure to 26% of all isolates in 2001 and 7.2% in 2002. Thus, some evidence exists that this serovar could be increasing in importance in the chicken population and subsequently in humans in Thailand. *S.* Schwarzengrund has also been found in chickens in other studies, suggesting that poultry could be the most common reservoir (14,15).

A large number of other serovars were also isolated from humans and nonhuman sources. However, some serovars most commonly found in the 1995 WHO survey were only infrequently observed in Thailand. *S.* Hadar was the 12th most common serotype and *S.* Typhi was the 16th, while other commonly observed serovars such as *S.* Infantis and *S.* Newport were not observed among the 25 most common serovars. *S.* Hadar has been associated with poultry (15). This finding was also observed in this study; *S.* Hadar was frequently isolated from chicken and duck. However, this finding does not seem to have a major impact on the number of infections in humans.

The results from this report show that serovars can differ largely between countries and regions, which is likely related to the available reservoirs for persistence and spread of *Salmonella* infections. The distribution of serovars causing infections in Thailand differs markedly from those reported in other countries and seems to be related to the *Salmonella* serovars in the different food products and other reservoirs for infections. Of particular interest is the frequent occurrence of *S.* Weltevreden and recent increase in occurrence of *S.* Rissen, *S.* Stanley, and *S.* Schwarzengrund.

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Myiasis during Adventure Sports Race

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Travelers who have visited tropical areas may exhibit aggressive forms of obligatory myiases, in which the larvae (maggots) invasively feed on living tissue. The risk of a traveler's acquiring a screwworm infestation has been considered negligible, but with the increasing popularity of adventure sports and wildlife travel, this risk may need to be reassessed.

Case Report

In November 2001, a 41-year-old Finnish man, who was participating in an international adventure sports race in Para (a jungle area in Brazil), tripped at night over a loose rock while he was riding a bicycle. He received a bruised, lacerated wound on his left dorsal antebrachium, which he quickly wiped with paper. The remaining gravel, dirt, and three unidentified winged insects were removed, and the wound was thoroughly rinsed 3 hours later by medical personnel. Petrolatum was applied topically and the wound bandaged. The patient continued the jungle race for the next 108 hours, during which the wound was hastily cleansed and rebandaged twice. The patient repeatedly swam in the Amazon Basin. At the race's finish, the wound appeared purulent and was cleansed with tap water, soap, and a brush. Numerous attached ticks were removed from his skin.

The arm became tender, and the patient had bouts of intensified local pain. A possible, round entry wound, about 1 mm in diameter, was noted, at the bottom of which the patient observed motion. Five days after the accident, a physician explored the wound with scissors at a breakfast table. No larvae were found. Sugar was applied topically and cefalexine prescribed. Twenty hours later, the bouts of pain recurred with increasing frequency. The patient believed that something left in the wound "ate his flesh." Nine days after the accident, at the Frankfurt Airport, Germany, the patient found a larva (Figure, part B) and an exit site wound (Figure, part A) under the bandage. The bouts of tenderness subsided. The patient's wound was reexamined 1 day later and was found to be largely healed; the forming scar remained somewhat tender and itchy for 2 months. The maggot was sent to the Finnish Museum of Natural History, Helsinki, Finland, and identified as a third-stage larva of *Cochliomyia hominivorax* (Coquerel), the New World screwworm fly. In addition to the New World screwworm fly, an important Old World species, *Chrysoimya bezziana*, is also found in tropical Africa and Asia.

Conclusions

Myiasis is the infestation of live humans and vertebrate animals by fly larvae. These feed on a host's dead or living tissue and body fluids or on ingested food. In accidental or facultative wound myiasis, the larvae feed on decaying tissue and do not generally invade the surrounding healthy tissue (1). Sterile facultative *Lucilia* larvae have even been used for wound debridement as "maggot therapy." Myiasis is often perceived as harmless if no secondary infections are contracted. However, the obligatory myiases caused by more invasive species, like screwworms, may be fatal (2). The screwworms are capable of penetrating through minor cracks in the skin, entering body openings, and migrating from wounds to living tissue. Most often this is initiated when the flies, attracted by the wounds, lay their eggs in

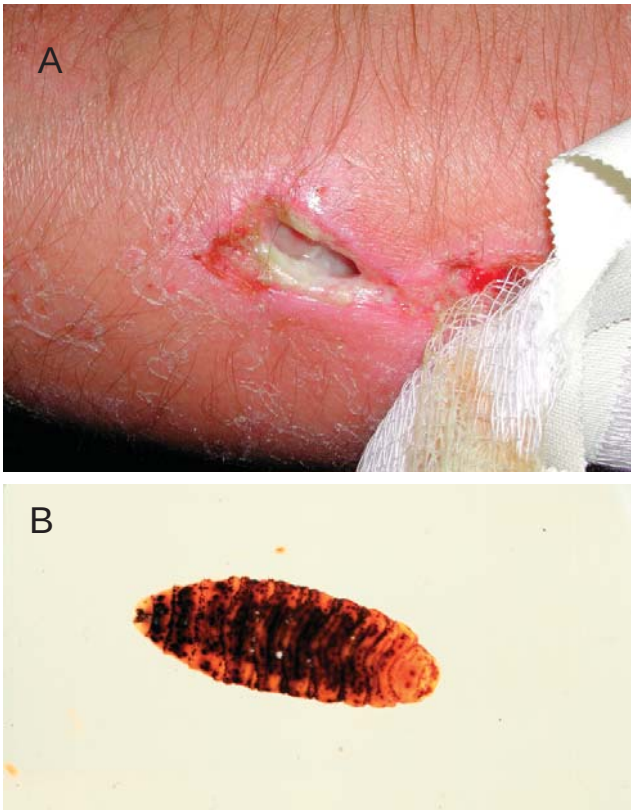

Figure. Exit site wound on patient's arm (A) and a maggot, measuring 16 mm in length, from patient's wound (B).

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necrotic, hemorrhagic, or pus-filled lesions. Infestation and penetration of the ocular, nasopharyngeal, paranasal, and auricular cavities are frequently described (3). The female flies colonize the wounds with bacteria like *Providencia* spp., which in turn attract more flies by the distinct odor of the bacterial mass. Secondary bacterial infections are common. All conditions compromising the integrity of skin (like tick bites and skin diseases) predispose to the infestation (4). The eggs of *Cochliomyia hominivorax* are laid in batches. Hundreds of maggots of both sexes can be found in a wound. Screwworms can thus be introduced to new areas by just one index patient. Considerable risk exists of reintroducing *C. hominivorax* into areas from which it has been eradicated. Within 24 hours of hatching, the larvae begin feeding and cause extensive tissue destruction, pain, and even death. The larvae possess powerful oral hooks and can invade cartilage and bone (5). After feeding for 4 to 8 days, the larva leaves the wound to pupate in the soil (6,7). The pest is viable year round in areas with temperatures constantly >16°C.

C. hominivorax was first found to be infesting people in the penal colony of Devil's Island in French Guiana in 1858. It was originally distributed from the southern United States to Argentina between the 35° north latitude and the 35° south latitude. Due to a massive sterile male fly release program**,** *C. hominivorax* was eliminated from North America. *C. hominivorax* is an important insect pest of livestock in the neotropical regions and has caused substantial losses to the livestock industries of the Americas (7). It is classified as a restricted animal pathogen in the United States. At present, populations of *C. hominivorax* are found in Central and South America and in certain Caribbean Islands (4,6,7). Human infestations remain an important health problem, primarily affecting the severely debilitated or persons with no access to healthcare. In the largest published study, the fatality rate was 2.8% (7). Isolated reports of infestations in the United States and Mexico are often traced to the importation of infested animals. Between 1969 and 1988, 39 human cases were reported (7). The potential for the introduction of *C. hominivorax* into new areas is ever present. In 1988, the screwworm appeared in Libya, most likely imported by infested livestock. It overwintered and became for the first time established outside the Western Hemisphere, where it infected 3,000 livestock and >200 persons. From Tripoli, it spread rapidly approximately 200 km and threatened to spread further to the savannas of sub-Saharan Africa. This possibility presented a potential threat of enormous proportions to the livestock, wildlife, and human populations of a large part of the Eastern Hemisphere. After a large-scale campaign, the screwworm was successfully eliminated from the area in 1991 (7,8).

In humans, the most common sites of *C. hominivorax* infestation are the nose, eyes, and skin. The manifestations depend on the anatomic region affected but are usually characterized by local pain, intense pruritus, cutaneous nodules, and larvae emerging from wounds or cavities (7,9). Massive infestations can result in the death of the host, usually attributed to "massive toxic shock" or to penetration of viscera or cavities, especially in the head and neck area (6,8). Infestation of paranasal sinuses often goes undetected for long periods. When infestation is suspected, a careful search for a larval infestation of eyes, nose, paranasal sinuses, or wounds should be performed, if necessary with the help of computed tomography or magnetic resonance imaging (7). Radiographic studies may only show edema. No effective antimicrobial therapy is available, although doramectin and ivermectin have been investigated for prophylactic use in cattle. Treatment involves removal of the larvae (reviewed in [5] and [6]). Irrigation with either chloroform or ether is advocated. Surgery is often required. For identification, the larvae should be hatched to adult flies, or first killed by immersion in nearly boiling water, then cooled and preserved in 80% ethanol. Secondary bacterial infections should be treated with local wound care and administration of antimicrobial agents.

In industrialized countries wound myiasis is a sign of neglected wound care, with mostly facultative myiases seen. The patients are often debilitated, of lower economic status, homeless, or substance abusers. The main focus is in treating secondary bacterial infections and in proper debridement. The risk of a traveler's acquiring *C. hominivorax* is thought to be negligible (3). With the increasing popularity of adventure and wildlife travel, the risk may need to be reassessed. Travelers can contract aggressive obligatory myiases. This is the third report of *C. hominivorax* infestation in a tourist in 3 years (4,9). Also, a previous case was reported in a U.S. Army ranger who was wounded in action in Panama (5). In particular, medical personnel who treat patients who have participated in adventure sports events should recognize the intensity of the exposure to even the most exotic infectious diseases.

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International Conference on Women and Infectious Diseases: from Science to Action

The International Conference on Women's Health and Infectious Diseases, sponsored by the Centers for Disease Control and Prevention (CDC) and partners, will be held at the Marriott Marquis, Atlanta, Georgia, February 27–28, 2004. Intended for clinicians, scientists, women's health advocates, health educators, public health workers, academicians, and representatives from all levels of government and from community-based, nonprofit, philanthropic, and international organizations, the conference will promote prevention and control of infectious diseases among women worldwide.

Featured sessions will include women and HIV/AIDS, perinatal infectious diseases, immunizations, links between infectious and chronic diseases, and the impact of globalization. Other topics include infectious disease disparities, gender-appropriate interventions, and effective health communication.

Speakers will include, Julie L. Gerberding, CDC director, who will speak about the impact of infectious diseases on women; Carol Bellamy, executive director, United Nations Children's Fund (UNICEF), who will speak about globalization and its effect on infectious diseases among women; and Mirta Roses Periago, director, Pan American Health Organization (PAHO), will speak about prevention of infectious diseases among women globally.

For information, about cost and registration, contact the Office of Minority and Women's Health, National Center for Infectious Diseases, CDC, at Web site: www.womenshealthconf.org; email: omwh@cdc.gov; or phone: BeJaye Roberts, 404-371-5492.

Estimating the Public Health Impact of Rabies

Paul G. Coleman,* Eric M. Fèvre,† and Sarah Cleaveland†

Rabies is a fatal, preventable zoonosis, but it is not effectively controlled throughout much of the developing world. The impetus for control is hampered by a lack of awareness of its true impact. We estimate a disability-adjusted life year (DALY) score for rabies to quantify the disease impact relative to other diseases to set priorities for public health interventions.

Rabies is a fatal disease that is considered a reemerging zoonosis throughout much of the world (1,2). Rabies satisfies all the World Health Organization (WHO) criteria for diseases that are a priority for control (3) and, unlike many other emerging zoonoses (such as West Nile virus), safe and effective animal and human vaccines are widely available for its prevention and control. Despite this, rabies remains a neglected disease that is poorly controlled throughout much of the developing world, particularly Africa and Asia, where most human rabies deaths occur (3,4). A major factor in the failure of rabies control is the low level of political commitment, partly arising from a lack of quantitative data on the true public health impact of the disease (3) and the cost-effectiveness and cost benefits of controlling it (5).

The disability-adjusted life year (DALY) is a standardized, comparative measure of disease impact developed to assess the relative impact of different diseases across different settings and at different stages of economic and public health development (6). The DALY is a combination of the years of life lost (YLL) due to premature death and the years of life lived with a disability (YLD). DALYs have been used to organize disease control in the health sector (7) because interventions can be prioritized on the basis of their impact in reducing disease and on the cost-effectiveness of the intervention. Most emerging human diseases are zoonotic (2); while DALYs have been estimated for some of these, such as leishmaniasis and trypanosomiasis, a DALY score has never been determined for rabies, which has failed to be considered in any of the annual global disease burden estimates made by WHO (8).

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Country-Level Estimates

A DALY estimate, which can be used to rank diseases globally, can also be used to prioritize health interventions at a country level. As a result of widespread problems of data quality and underreporting of rabies, a new approach has recently been adopted in Tanzania to estimate human rabies deaths by using a decision-tree method based on the incidence of human dog-bite injuries. Such bites are reported routinely and more reliably than rabies cases themselves (9). Age-specific human rabies incidence figures calculated from detailed data collected in the Mara Region (9), northern Tanzania, were extrapolated to provide a country-level rabies DALY estimate of 42,669 for all of Tanzania in 2000 (Table 1).

This example demonstrates how a country-specific mortality and DALY estimate can be calculated by using quality data collected from a specific study site. Indeed, the same method used to estimate the annual number of human rabies cases (9), and thus DALY impact, in Tanzania may be applied across sub-Saharan Africa to estimate the regional level of underreporting relative to officially reported figures. However, care needs to be taken when extrapolating from small-scale studies to regional and national levels. For example, in Tanzania, country-level estimates of human rabies deaths are likely to be affected by regional variations in rabies incidence in different dog populations (which are the main source of human rabies exposures), availability of postexposure treatment, and levels of knowledge about rabies, which will affect the probability of seeking treatment in hospitals. In addition, knowing the scale of DALYs lost due to a single disease in isolation is not helpful to decision makers prioritizing interventions with limited funds. Better country-level estimates for other diseases also need to be determined. However, this study is a first step.

Global Estimate

We calculated the global DALY for rabies based on annual WHO estimates of 35,000 deaths (10) and using a standard method (6) to allow comparison with the most recent estimates (8) for the diseases identified for the United Nations Development Program/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, known as TDR (11). The figure of 35,000 deaths per year may be expressed in terms of DALYs if certain assumptions about the age and sex distribution of rabies patients are made. Data on the age-related exposure to rabies were obtained from Eng et al. (12), a detailed study of human rabies in Mexico. Analysis of dog bite injuries showed a ratio of male:female cases of 0.53:0.47. The age distribution of persons bitten was skewed towards the younger ages (median age 9 years, range <1–84 years) a common pattern seen across developing country settings;

Table 1. Estimates of the DALY impact of human rabies in Tanzania in 2000[a,b]

Age group (y)	Rabies cases
0–4	10,986
5–14	14,504
15–44	13,876
45–59	1,497
60+	1,807
All ages	42,669

[a]DALY, disability-adjusted life year.
[b]The DALY estimates were based on the estimated incidence of human deaths for Tanzania as reported by Cleaveland et al. (9).

60% of cases occurred in the 0- to 12-year age range, 10% in the 13- to 19-year range, and 30% in the >20 age range.

When these age and sex distributions of patients are used, an annual impact of 35,000 human rabies deaths equates to approximately 1.16 million DALYs. This estimated DALY impact is conservative because it considers only the YLL component and does not takes into account YLDs resulting from the illness associated with the trauma of animal bites and postexposure therapy, if available.

A total of 1.16 million DALYs places rabies just behind trachoma, slightly above onchocerciasis, and well above dengue (Table 2). This estimate shows rabies to be an important disease in terms of DALYs if the WHO figures reflect the true public health situation. However, unlike other zoonoses in the DALY ranking system**,** human rabies is fully preventable by disease control aimed at the animal reservoir. All 1.16 million DALYs could, in theory, be averted through veterinary interventions.

Although the above DALY figure gives a useful indication of the global DALY for rabies, the true global incidence (and hence DALY) of human rabies is difficult to assess because rabies is often inconsistently reported. For example, the 1996 World Survey of Rabies (10) recorded a total of 33,212 rabies deaths worldwide (of which 30,000 were reported by India), while only 1,326 were reported in 1991 (when India reported only 34) (13). Although rabies is known to be grossly underreported in most developing countries, the degree of underreporting is difficult to assess. However, recent studies from Tanzania indicate that human rabies deaths may be up to 100 times higher than officially reported (9), with an estimated incidence of human rabies similar to that recorded during active surveillance studies (14). More country-level estimates of underreporting, using methods similar to that developed for Tanzania (9), need to be conducted to provide more reliable figures of the true global scale of human rabies. However, even if the 35,000 estimated human rabies cases were more than double the true global figure, the DALY impact attributable to rabies would still be comparable to that of dengue fever, which is recognized by TDR as a major public health threat throughout the tropics.

Table 2. The global DALY scores for rabies and other selected diseases[a,b]

Disease	Total DALYs lost (x 1,000)
Malaria	42,280
Tuberculosis	36,040
Lymphatic filariasis	5,644
Leishmaniasis	2,357
Schistosomiasis	1,760
Trypanosomiasis	1,598
Rabies	1,160
Onchocerciasis	987
Chagas	649
Dengue	653
Leprosy	177

[a]DALY, disability-adjusted life year.
[b]The DALY score for rabies was based on official World Health Organization (WHO) figures (10). The other listed diseases constitute the official DALY score in 2001 for the priority diseases in the TDR (United Nations Development Program/World Bank/WHO Special Programme for Research and Training in Tropical Diseases) portfolio (8).

Conclusions

The value of providing a quantitative estimate of disease impact due to rabies, even with the inaccuracies of existing case data, should not be underestimated. Rabies is often perceived as a rare or insignificant disease of humans in developing countries; this perception has been a major factor hampering the development of disease control initiatives. Furthermore, control of rabies is often seen as the responsibility of veterinary authorities, but demonstration of the public health importance of rabies and the benefits of disease control to the public health authorities (both in terms of DALYs saved and reduced costs of postexposure treatment) will encourage involvement of the health sector in control efforts. Integration of medical and veterinary sectors is likely to be crucial for effective disease control, as shown by the success of recent rabies control programs in Central and South America, where medical authorities have taken a lead role in implementing mass dog vaccination programs (15).

This first estimate of a global DALY score for rabies, together with the Tanzania-specific example, indicates that the disease exerts a considerable public health impact, exceeding other prominent diseases that currently achieve a higher priority for disease control. Furthermore, the human disease effects of rabies could be eliminated through vaccination of animal reservoirs by using technologies and methods that are available and accessible.

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Antifungal Susceptibilities of *Cryptococcus neoformans*

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Susceptibility profiles of medically important fungi in less-developed countries remain uncharacterized. We measured the MICs of amphotericin B, 5-flucytosine, fluconazole, itraconazole, and ketoconazole for *Cryptococcus neoformans* clinical isolates from Thailand, Malawi, and the United States and found no evidence of resistance or MIC profile differences among the countries.

Prompt identification of agents associated with emerging infectious diseases and documentation of resistance among these agents to available antimicrobial drugs depend on existing surveillance activities for emerging pathogens and antimicrobial resistance. Although the World Health Organization has undertaken initiatives (1) in these areas, surveillance of antimicrobial resistance in developing countries is lacking or has been generally ignored (2). Natural selective pressures exerted on microorganisms by routine, inappropriate, or excessive use of antimicrobial drugs are factors in the development of antimicrobial resistance. In tropical developing countries, unrestricted availability of antimicrobial drugs without prescriptions, suboptimal therapeutic regimens, blind empiric prescribing practices that are not epidemiologically directed, and lack of laboratory capacity or skilled personnel for susceptibility testing contribute to the spread of antimicrobial resistance (2). Although numerous studies have examined bacterial and mycobacterial resistance in the tropics, less is known about the susceptibility profiles of medically important fungi to antifungal agents (3–5). Given that only a few antimicrobial drugs may be available in developing countries because of limited resources or cost restrictions, the surveillance for resistance among common pathogens to available drug treatment is essential for appropriate patient care and improved patient outcome.

Cryptococcus neoformans, an opportunistic fungal pathogen that causes disease predominantly in immunocompromised patients, is a frequent cause of fatal mycotic infections among patients with AIDS (6). In sub-Saharan Africa, cryptococcal meningitis occurs in 30% of AIDS patients and is likely to remain a substantial cause of death in these patients unless highly active antiretroviral therapy becomes available (6–8). Until such a time, treatment with antifungal agents, including long-term, suppressive antifungal regimens, remains the only recourse.

The Study

We sought to determine if substantial differences in susceptibility profiles to common antifungal agents existed among clinical isolates of *C. neoformans* from three geographically diverse areas. Sixty-five clinical isolates of *C. neoformans* from Malawi, Thailand, and the United States were available for study. The 16 isolates from Malawi and 29 isolates from Thailand were recovered from the bloodstream of febrile, adult inpatients during previous bloodstream infection studies in these regions (9,10). The 20 isolates from the United States were recovered from the bloodstream, lung tissue, cerebrospinal fluid, and other sterile sites in routine clinical practice in the clinical microbiology laboratories of the Cleveland Clinic Foundation and Duke University Medical Center. The yeast isolates from all of the countries were shipped to Duke University Medical Center for testing and maintained in frozen stock vials at –70°C. Sixty-five yeast isolates were recovered from the frozen stock vials on potato dextrose agar and incubated at 30°C for 48 hours. The antifungal susceptibilities of the isolates were determined by using the Sensititre YeastOne system (Trek Diagnostic Systems Ltd., West Sussex, England), which includes amphotericin B, 5-flucytosine, fluconazole, itraconazole, and ketoconazole. All isolates were incubated for 72 hours, according to the manufacturer's instructions. Inoculum assessments were performed on all trays and were within acceptable limits. The trays were visually inspected, and the MICs were determined according to the manufacturer's guidelines. Interpretive guidelines and breakpoints for susceptibility testing of *C. neoformans* are not yet available from the National Committee for Clinical Laboratory Standards (NCCLS); therefore, only MIC comparisons were performed (11).

For isolates from each country, we recorded the MIC at

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which 50% of the isolates were inhibited (MIC_{50}) and the MIC at which 90% of the isolates were inhibited (MIC_{90}) and determined the MIC geometric mean for each therapeutic agent. We compared the MIC geometric means for the three countries with a one-way analysis of variance (ANOVA) to determine if significant differences existed. Additional comparisons between the MIC_{50} and MIC_{90} were not undertaken, since these were within one dilution of one another.

The *C. neoformans* isolates from the United States, Thailand, and Malawi demonstrated similar susceptibility profiles to the common antifungal agents against which they were tested (Table 1). The percentage of isolates inhibited at each concentration of antifungal agent over the full dilution series is summarized in Table 2. The isolates from the three countries did not differ significantly in their susceptibility to fluconazole ($p = 0.198$), itraconazole ($p = 0.163$), 5-flucytosine ($p = 0.713$), or ketoconazole ($p = 0.531$). The geometric mean of the MIC values for amphotericin B in Thailand, the United States, and Malawi was 1.2 μg/mL, 1.4 μg/mL, and 1.6 μg/mL, respectively. These mean values were significantly ($p = 0.019$) different.

Conclusions

Resistance to antifungal drugs is rare among clinical isolates of *C. neoformans* but has been reported (4,12). The use of antifungal agents, particularly in long-term suppressive regimens, has raised concern about the development of drug resistance in *C. neoformans*. However, an extensive survey of the susceptibility profiles of clinical isolates of *C. neoformans* at a university hospital during 1987 to 1994 helped to allay these fears by indicating no emergence of resistance (13).

This study also demonstrates no evidence of resistance among clinical isolates of *C. neoformans* from Thailand, Malawi, and the United States. For each country, the MIC_{50} and MIC_{90} of isolates to commonly used antifungal agents were within one dilution from each other. In addition, the MIC ranges were similar. Statistical comparison of the MIC geometric means confirmed that no significant differences existed between the three regions for fluconazole, itraconazole, 5-flucytosine, or ketoconazole. The only statistically significant differences were observed for amphotericin B susceptibilities; however, this difference was believed to be clinically irrelevant since the MIC geometric means for amphotericin B were 1– 2 μg/mL, or within one dilution. Our documentation of the absence of resistance among *C. neoformans* isolates from the United States is consistent with data published by the Centers for Disease Control and Prevention, which showed in vitro resistance to antifungal agents to be uncommon and unchanged among *C. neoformans* isolates from 1992 to 1998 (14).

The similarity between the MICs of *C. neoformans* isolates from Malawi and the United States concurs with data from a previous study of 164 African and 402 North American clinical isolates of *C. neoformans* isolates that were tested and found to be susceptible to fluconazole and other triazoles, with over 99% inhibited by concentrations of fluconazole ≤32 μg/mL (5). The MIC_{50} and MIC_{90} in that study were lower than those in this study, although the YeastOne trays have been found to agree well with the NCCLS reference method for itraconazole and the other azoles (15). Also, the MICs of fluconazole documented in our study are similar to those previously reported for isolates of *C. neoformans* from the United Kingdom and Uganda (3,4); the MICs of 5-flucytosine in our study also were similar to those previously reported for *C. neoformans* isolates from Uganda (4). The itraconazole MICs documented in our study were lower than those reported for isolates from the United Kingdom, Africa, and the United States (4,5). The differences between the susceptibility profiles of *C. neoformans* to itraconazole reported in

Table 1. *Cryptococcus neoformans* susceptibility results

Antifungal agent	MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	MIC geometric mean (μg/mL)
U.S. isolates (N = 20)				
Amphotericin B	1–2	1	2	1.4
Fluconazole	1–16	8	8	5.1
Itraconazole	0.016–0.125	0.06	0.125	0.06
5-Flucytosine	2–8	4	8	5.1
Ketoconazole	≤0.008–0.250	0.06	0.06	0.05
Thailand isolates (N = 29)				
Amphotericin B	0.5–2	1	2	1.2
Fluconazole	4–160	8	16	7.7
Itraconazole	0.030–0.125	0.06	0.06	0.06
5-Flucytosine	2–8	4	8	4.6
Ketoconazole	0.030–0.250	0.06	0.125	0.07
Malawi isolates (N = 16)				
Amphotericin B	1–2	2	2	1.6
Fluconazole	4–32	8	16	7.6
Itraconazole	0.030–0.125	0.03	0.125	0.05
5-Flucytosine	1–16	4	8	4.5
Ketoconazole	0.016–0.250	0.03	0.25	0.03

Table 2. Percentage of *Cryptococcus neoformans* isolates susceptible at each MIC dilution

	% Susceptible		
MICs (μg/mL)	U.S. isolates	Thailand isolates	Malawi isolates
Amphotericin B			
0.5		3	
1	50	72	31
2	100	100	100
Fluconazole			
1	5		
2	30		
4	40	21	25
8	90	83	87
16	100	100	94
32			100
Itraconazole			
0.016	5		
0.030	15	14	50
0.060	75	93	87
0.125	10	100	100
5-Flucytosine			
1			6
2	5	7	12
4	60	72	69
8	100	100	94
16			100
Ketoconazole			
≤0.008	5		
0.016	20		12
0.030	35	14	62
0.060	90	79	75
0.125	95	93	81
0.250	100	100	100

our study and those reported previously may be due in part to the poor solubility of this antimicrobial agent in an aqueous solution.

Using a standardized testing method, we found no significant or clinically meaningful differences between the antifungal susceptibility profiles of clinical isolates of *C. neoformans* from the United States, Thailand, and Malawi. Although rare strains of *C. neoformans* with elevated MICs to some antifungal agents may exist, they were not detected in this sampling of clinically significant *C. neoformans* isolates and, therefore, do not appear to be prominent in Cleveland, Ohio; Durham, North Carolina; Bangkok, Thailand; or Lilongwe, Malawi.

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Human Infection with M- Strain of *Brucella canis*

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The less mucoid strain of *Brucella canis* or M- strain is used for the serologic diagnosis of canine brucellosis. While this strain is avirulent in dogs, we report the case of clinical brucellosis that developed in a laboratory worker a few days after handling live M- cells for antigen production.

Brucella canis is the causative agent of canine brucellosis, which causes contagious abortion, orchiepididymitis, and uveitis. Transmission to human requires close contact with infected animals or bacterial cultures. Symptomatic human infections are rare, probably because of the low virulence of *B. canis*; 31 human cases have been reported (1).

In contrast to other *Brucella* species, which are pathogenic for humans (*B. abortus, B. melitensis, B. suis*) and yield smooth colonies, *B. canis* colonies are naturally rough. Therefore, serologic tests that use suspensions of smooth brucellae are not useful in diagnosing *B. canis* infections (2). Since suspensions of wild-type *B. canis* tend to aggregate even in the absence of specific antibodies, a less mucoid variant termed M-, which does not produce autoagglutination is used for serologic diagnosis (3). The M- strain has reduced virulence in dogs; even high doses of this strain do not induce the typical signs of brucellosis in dogs (4). The pathogenic potential of the M- strain in humans remains unknown, and to the best of our knowledge, human infection by this strain has not been reported. We report a clinical and immunologic study of a human infection by the *B. canis* M- strain that shows that this strain can produce human disease similar to that produced by wild-type *B. canis*.

Case Report

A 35-year-old male laboratory worker was referred to a physician with recurrent fever, headache, arthralgia, weakness, and constipation, which had begun 1 month before. The patient worked in a laboratory that produced antigens for diagnostic use. Three weeks before symptoms began, he had been handling a dense culture of live *B. canis* M- and was using no personal protection; the procedures were not performed in a biological safety cabinet. Moreover, the patient had attempted resuspension by repeated pipetting with his mouth. The clinical examination disclosed cervical adenomegaly, and laboratory tests indicated a mild increase of hepatic enzymes (Aspartate aminotransferase 46 U/L and alanine aminotransferase 65 U/L) and neutropenia. The patient reported not having close contact with dogs or other animals. Taking into account the unprotected exposure to *B. canis* M-, brucellosis was suspected, and blood samples were drawn for culture and serologic studies. Two weeks later blood cultures indicated a *Brucella* species that was later typed as *B. canis*. Conventional tests for antibodies to smooth brucellae (agglutination, complement fixation) yielded negative results. In contrast, slide agglutination for *B. canis* was strongly positive with undiluted serum and was also positive at 1:10 dilution. Serologic tests for hepatotropic viruses and *Toxoplasma gondii* were negative.

After diagnosis, a course with oral doxycycline, 100 mg twice a day for 42 days, plus parenteral gentamicin, 180 mg once a day for 10 days, was started. The patient clinically recovered, but on the last day gentamicin was administered, symptoms of VIII cranial nerve involvement occurred, which resolved with flunarizine and vitamin B12 administration.

Blood cultures performed 2 weeks after antimicrobial therapy ended were negative for *B. canis*. During follow-up, the patient remained asymptomatic, his cervical adenitis resolved, and serum levels of hepatic enzymes returned to normal. On his last visit, 4 years after infection, the patient was asymptomatic. While he continues to handle *B. canis* M-, cultures are now performed under strict biological safety measures (biological safety cabinet, personal protection including goggles, gloves and mask, and autoclaving of contaminated material).

Immunologic Studies

To assess the humoral immune response of the patient to *Brucella* antigens, the slide agglutination test with *B. canis* M- and 3 enzyme-linked immunosorbent assays (ELISA) were used. ELISA used a hot-saline extract of *B. canis* M- (HS, mainly composed of rough lipopolysaccharide [LPS] and outer membrane proteins), a preparation of cytoplasmic proteins of *B. abortus* depleted of LPS (CP [cytoplasmic proteins]) or recombinant *Brucella* lumazine synthase (RBLS), which were obtained as described previously (5–7). As shown in the Figure, antibodies to the three antigens were detected at the time of diagnosis, but antibodies to proteinaceous antigens (CP and RBLS) were negative earlier than those against HS. The slide agglutination test that used *B. canis* M- and undiluted serum was strongly positive at diagnosis and 48 days later (beginning

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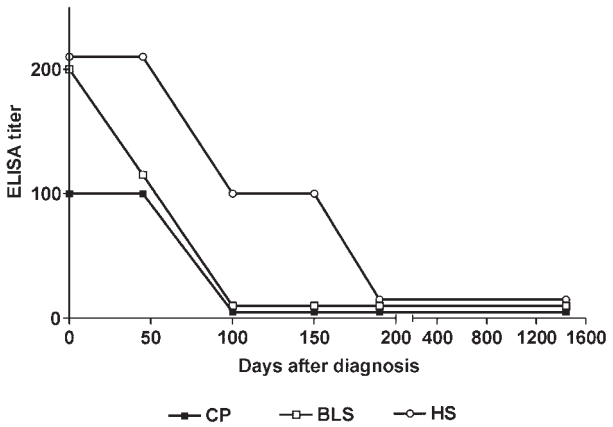

Figure. Serological follow-up of a human infection by *Brucella canis* M-. CP, cytoplasmic proteins; BLS, *Brucella* lumazine synthase; HS, *B. canis* hot-saline extract. The enzyme-linked immunosorbent assay (ELISA) titer was calculated as the inverse of the last serum dilution that yielded an optical density higher than the cut-off of the assay.

and end of therapy, respectively), weakly positive at 103 and 150 days, and negative at 190 days and 4 years after diagnosis. Positive samples were assayed in serial dilutions starting at 1:10; only the initial sample was positive at 1:10 dilution (negative at 1:20).

The cellular immune response against proteinaceous activity from *Brucella* in peripheral blood mononuclear cells (PBMCS) was also evaluated. In vitro proliferation and cytokine gene expression were investigated as previously described (8). For blastogenesis assays, PBMCS were cultured with CP (10 μg/mL), RBLS (5 μg/mL), or phytohemagglutinin (10 μg/mL). Results were expressed as stimulation index (counts per minute of stimulated cultures divided by counts per minute of unstimulated cultures). Stimulation indices (SI) >2 were considered positive. For reverse transcription-polymerase chain reaction (RT-PCR), RNA was extracted from PBMCS cultured in the presence of CP, RBLS, or phytohemagglutinin for 24 hours. Results were expressed as fold increase over the messenger ribonucleic acid levels of cells cultured in the absence of antigen; increases >2 were considered specific.

CP and RBLS induced T-cell proliferation (SI >2) in PBMCS obtained from the patient before antimicrobial therapy (Table). PBMCS from a healthy person, which were run in parallel, showed no response to CP and RBLS (SI <2) (not shown). In addition, CP and RBLS induced a significant ($p<0.001$, nonparametric Mann-Whitney *U* test) upregulation of interferon-gamma (IFN-γ), interleukin (IL)-2, and IL-10 transcripts only in PBMCS from the patient. No IL-4 induction was observed with PBMCS from the patient or the healthy control (not shown). The cellular immune response declined with antimicrobial treatment (Table), but CP-specific IFN-γ remained increased 55 days after therapy ended. In the last sample (obtained 250 days after therapy ended), all the parameters of the cellular immune response were normal (not shown).

Conclusions

The main finding of our study is that the M- strain of *B. canis* can produce human disease, which was unexpected in view of the reported avirulent phenotype of this strain in dogs. To the best of our knowledge, this case is the first of human *B. canis* M- infection ever reported. The M- strain has been widely used for the diagnosis of canine brucellosis because it is less prone to autoagglutination than its wild-type counterpart (called M+). Based on the low virulence of the M- strain in dogs (4), the production protocol of the laboratory where this case occurred did not include bacterial inactivation or personnel protection during initial handling of cultures, which led to a prolonged exposure to a high number of viable bacteria. As the patient was the only person involved in the production of this strain, his co-workers were not tested for *B. canis* M-. Similar illness in the production plant was not reported. Because *Brucella* spp. is not usually transmitted from patients to healthy persons, the patient's family members were not tested for *B. canis* M- infection.

The clinical manifestations in our patient were similar to those reported for human, wild-type *B. canis* infections (e.g., fever, headache, anorexia, asthenia, and adenitis). Previous studies in dogs experimentally infected with the M- strain showed that this strain does not revert to the M+ phenotype in vivo (4). Our case may be analogous to cases of human illness by attenuated strains of *Brucella* species used for animal vaccination, mainly *B. melitensis* Rev-1 (9) and *B. abortus* S19 (10). Altogether, these human infections indicate that attenuation for animals does not

Table. Cellular immune response in vitro to *Brucella* cytoplasmic proteins[a]

	Antigen	Lymphocyte proliferation (SI)	IL-2[b] (fold increase)	IFN-γ[b] (fold increase)	IL-10[b] (fold increase)
Before therapy	BLS	4	8	3	3
	CP	3	10	7	4.5
End of therapy	BLS	1	1	2	1
	CP	2.5	6	4	2
55 days after end	BLS	1	1	1	1
of therapy	CP	1	1	3	1

[a]BLS, *Brucella* lumazine synthase; CP, cytoplasmic proteins; SI, stimulation indices; IL, interleukin; IFN, interferon.
[b]By reverse transcription-polymerase chain reaction.

necessarily mean innocuity for humans and that biological safety measures must be followed in each case.

To assess the humoral immune response to the infection with the M- strain, antibodies against outer membrane antigens (HS) and to internal antigens (CP and *Brucella* lumazine synthase) were measured. Overall, low titers of antibodies were found by all tests, which is similar to those found for M- infections in dogs (4). Low antibody titers also could be related to early administration of antimicrobial therapy, as has been shown in patients infected with smooth brucellae (11). Antibodies to both external and internal antigens declined after antimicrobial therapy was begun and were undetectable 6 months after diagnosis (Figure). This decline, with longer persistence of antibodies to external antigens, is in agreement with our previous findings in human infections by smooth *Brucella* species (12).

An early and strong cellular Th1-type response to *Brucella* internal antigens developed in this patient, in agreement with our previous observations in acute human brucellosis (8). The reasons for the decline of this response during follow-up are unknown, but conceivably, bacteria levels were substantially diminished by the early antimicrobial therapy, thus eliminating the internal antigens needed to develop a long-lasting cellular immune response.

In summary, this case shows that, in spite of its reduced virulence in dogs, *B. canis* M- can produce human disease with a clinical picture similar to that produced by the infection with wild-type strains of *B. canis*. Therapeutic and immunologic parameters seem to be very similar to those observed in infections by smooth brucellae.

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Adenovirus Type 7 Genomic-Type Variant, New York City, 1999

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An outbreak of respiratory illness occurred in a long-term care facility in New York City. Investigation of the outbreak identified confirmed or suspected adenoviral infection in 84% of the residents from October 19 to December 18, 1999. Further identification by type-specific neutralization and restriction analysis identified a new genomic variant of adenovirus type 7.

Human adenoviruses are known to cause a variety of illnesses, including cystitis, diarrhea, intussusception, meningoencephalitis, epidemic keratoconjunctivitis, and encephalitis (1). Communitywide outbreaks of respiratory illness attributable to adenovirus, particularly serotypes 3, 4, 7, and 21, have also been described in civilian (2,3) and military (4) populations. Institutional outbreaks involving pediatric populations or persons with underlying pulmonary disease can be particularly severe, with high illness and death rates (5,6).

On November 27, 1999, three residents in the specialty hospital of a large long-term care facility in New York City were hospitalized with respiratory failure. When additional illnesses were identified, control measures and an outbreak investigation were initiated. We present the results of that investigation and document a novel genome type of adenovirus serotype 7 (Ad7) as the etiologic agent of the outbreak.

The Study

Chart reviews were conducted for all residents of the specialty hospital and retrospectively for persons who had died or were discharged in the previous 2 months. A confirmed case-patient was defined as a resident of the specialty hospital in whom new respiratory symptoms developed from October 19, 1999, to January 10, 2000, and who had a positive laboratory test for adenovirus or a histopathologic finding that was indicative of adenovirus infection. A suspected case-patient was defined as a resident of the specialty hospital in whom new respiratory symptoms developed from October 19, 1999, to January 10, 2000, and who tested negative for adenovirus or was not tested. A noncase-patient was defined as anyone without respiratory symptoms, regardless of test results, or anyone with respiratory symptoms and with an organism other than adenovirus identified through laboratory testing in the same period.

Specimens initially were requested only from symptomatic residents. As the outbreak continued, we attempted to obtain specimens from all residents in an effort to identify carriers. Nasal washings or tracheal secretions were tested for respiratory syncytial virus (RSV), influenza A and B, parainfluenza 1, 2, and 3, and adenovirus antigens by a commercial immunofluorescence assay (VRK Bartels Viral Respiratory Screening and Identification Kit, Bartels Inc., Issaquah, WA) and by enzyme immunoassay (Directigen RSV, Becton Dickinson, Le Pont de Claix, France) for RSV. Specimens were also added to MRC5, RMK, and A549 cells, and the cells were monitored for cytopathic effect. Selected specimens were tested for adenovirus by polymerase chain reaction (PCR) assay, using adenovirus group- and Ad7-specific primer sets (7,8). Autopsied lung tissue specimens also were sent to the Centers for Disease Control and Prevention (CDC) for routine histopathologic examination.

Adenovirus isolates were typed by use of a microneutralization assay (9). Genome typing by restriction analysis was performed by using 12 restriction enzymes, *Bam*HI, *Bcl*I, *Bg*I, *Bgl*II, *BstE*II, *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I , and *Xho*I, as previously described (5). Restriction patterns were interpreted by using the genome type classification scheme of Li and Wadell (10) and Li et al. (11). An additional 27 unrelated Ad7 community isolates collected between 1995 and 2000 were provided by several healthcare centers in the New York City area for comparison with the outbreak strains. Data were analyzed by using EpiInfo version 6.04b (CDC) for descriptive statistics.

Of the 50 residents in the specialty hospital from October 19, 1999, to January 10, 2000, 23 (46.0%) were females and 27 (54.0%) were males. Their ages ranged from 1 to 46 years (mean 11; median 10). The residents were located in two units: A with 21 (42.0%) and B with 29 (58.0%) residents. On average, the residents in B were younger than those in A (mean age 7 years [median 5;

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range 1-32] versus mean age 16 years [median 14; range 3-46]).

Of the 50 residents, 30 (60.0%) had confirmed cases of adenovirus infection, 12 (24.0%) had suspected cases, and 8 (16.0%) did not have adenovirus. Of the 30 patients with confirmed cases, 19 (63.3%) were hospitalized, and 5 died (confirmed case-fatality rate, 16.7%). Of the 12 patients with suspected cases, 7 (58.3%) were hospitalized and 2 died (suspected case-fatality rate, 16.7%). Of the eight noncase-patients, one person (12.5%) exhibited respiratory symptoms and was confirmed to be infected with herpes simplex virus; the other seven (87.5%) exhibited no respiratory symptoms, and three of six tested positive for adenovirus.

Confirmed case-patients were on average younger than suspected and noncase-patients (mean age 9 years [median 6; range 1-32] versus 12 years [median 12; range 1-25] and 13 years [median 12; range 1-6], respectively. More than 80% of confirmed and suspected case-patients had a tracheostomy, versus 37% of noncase-patients.

Symptom onset dates for residents with respiratory illness ranged from October 19, 1999, to December 18, 1999 (Figure 1). The Table describes the clinical characteristics of confirmed and suspected case-patients. The mean duration of illness for confirmed patients was 21 days (median 20; range 2-62) versus 28 days (median 22; range 6-93) for suspected case-patients.

Specimens from 48 (96.0%) of the 50 residents were submitted for testing. Of them, 26 (60.5%) of 43 were adenovirus positive by culture, 13 (54.2%) of 24 were positive by adenovirus group–specific PCR, 6 (22.2%) of 27 were positive by antigen testing, and 3 (100%) of 3 were positive by histopathology. Overall, 33 (68.8%) persons tested positive for adenovirus. Of 15 outbreak isolates submitted to CDC for further characterization, all were identified as serotype 7 by type-specific neutralization. Restriction analysis with 12 previously described restriction enzymes further identified all isolates as *Sma*I variants of genome type 7b (Figure 2). Restriction patterns obtained with 11 other enzymes were identical with those predicted for genome type 7b (12,13). Of the 28 additional Ad7 field isolates from the New York City area that were epidemiologically unrelated to the outbreak, 24 were identified as Ad7b and 4 as Ad7d2; none possessed the unique *Sma*I restriction pattern of the outbreak strain (data not shown).

Conclusions

This outbreak illustrates the difficulty inherent in controlling the spread of adenovirus in a closed community and the potential for severe disease and death in persons with underlying respiratory disease. When both confirmed and suspected cases are included, a remarkably high attack rate of 84% was documented among the 50 residents of the specialty hospital, with 26 hospitalizations and 7 deaths. The epidemiologic association and similar hospitalization and fatality rates for confirmed and suspected cases support our conclusion that most suspected cases likely had adenovirus infections.

A possible explanation for the 1-month lapse between the initial case in October and the other cases may be that the virus was first introduced into the facility in October but was transmitted more efficiently during the colder months, when there were more indoor activities and crowding on the units. A previously published report of an outbreak in a chronic-care facility (5) identified younger age and tracheostomy as risk factors for adenovirus infection. The high attack rate in this outbreak prevented calculation of risk factors. However, we observed a higher percentage of confirmed cases among the younger residents and persons with a tracheostomy. Therefore, we conclude that the underlying chronic respiratory disease in this population facilitated the transmission of this pathogen and that Ad7b variant may represent an emerging risk to similar persons.

The Ad7b genome type is widely distributed geographically and has been the predominant strain circulating in the United States since the late 1960s (12,13) (D. Erdman, pers. observation). Although genetic variants of Ad7b have been described using other restriction enzymes (12), to our knowledge, this is the first description of the *Sma*I variant of Ad7b. The novel *Sma*I pattern was most likely derived from the loss of the single restriction site that forms frag-

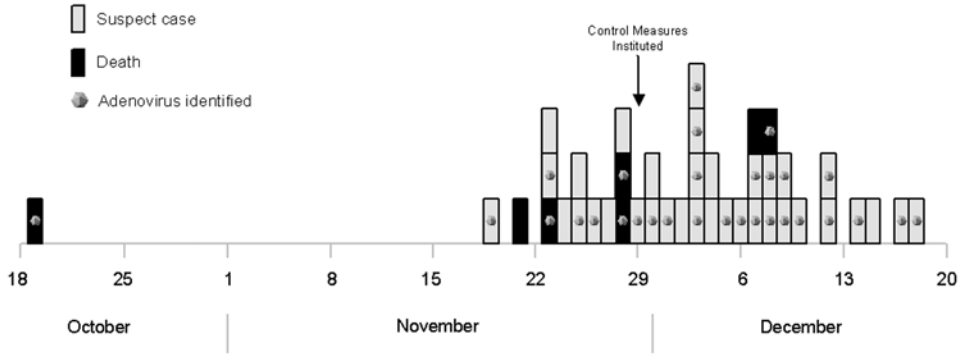

Figure 1. Epidemic curve showing onset date of illness for confirmed and suspected cases of adenovirus infection, New York City, 1999.

Table. Clinical characteristics of the confirmed and suspected adenovirus case-patients in a specialty hospital unit of a long-term care facility, New York City, October 19, 1999, to January 10, 2000[a]

Clinical characteristic	Confirmed (%) (N = 30)	Suspected (%) (N = 12)
Female	13 (43.3)	5 (41.7)
Male	17 (56.7)	7 (58.3)
Mean age, in y	9	12
Median (range)	6 (1–32)	12 (1–25)
Fever ≥38.3°C	30 (100.0)	11 (91.7)
Increased tracheal secretions	24 (80.0)	10 (83.3)
Chest congestion	23 (76.7)	11 (91.7)
Tracheitis	23 (76.7)	9 (75.0)
Pneumonia	18 (60.0)	7 (58.3)
Wheezing	16 (53.3)	5 (41.2)
Cough	9 (30)	4 (33.3)
Nasal congestion	2 (6.7)	0
Runny nose	1 (3.3)	1 (8.3)

[a]Except for age, data are number of patients with the characteristic (%).

ments 12,700 bp and 2,500 bp; loss of these two fragments corresponded with the appearance of a new 15,200-bp fragment. Failure to identify this variant in past studies or among recent Ad7s isolates circulating in the New York community suggests that it arose recently and supports the hypothesis that this outbreak resulted from nosocomial spread of the virus within the chronic care unit. Whether the genetic mutation(s) in the outbreak strain contributed to the severity of illness documented in these patients could not be determined.

Adenoviruses cause severe and often fatal respiratory disease in immunocompromised patients (1). A large outbreak of acute respiratory disease and pneumonia at a chronic mental health facility recently was attributed to

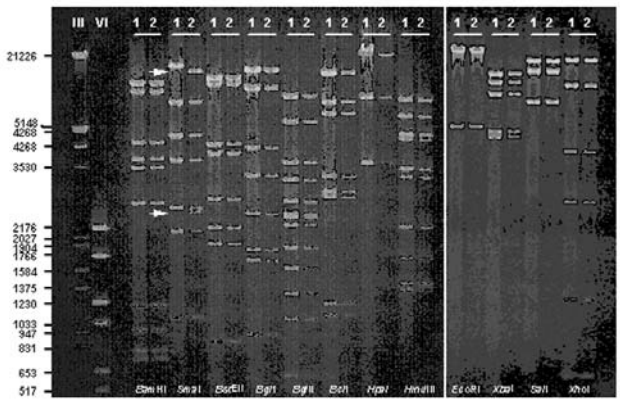

Figure 2. DNA fragment patterns obtained with selected restriction enzymes of representative outbreak (1) and community (2) Ad7 isolates resolved by gel electrophoresis with ethidium bromide staining. DNA markers III (λ*Hin*dIII/*Eco*RI) and VI (pBR328 *Bgl*I/*Hin*fI) were run simultaneously to facilitate fragment size estimates. Arrows highlight loss of 2,500- and 12,700-bp fragments and corresponding appearance of a new 15,200-bp fragment for the outbreak strain (1) as compared with the expected pattern for Ad7b (2).

adenovirus 35, a pathogen that has typically affected immunocompromised patients (14). Investigators speculated that immunity to Ad35 may have waned in the long-term residents, who may have been less likely to have been exposed to adenoviruses circulating in the community.

Recent outbreak investigations and surveillance studies (15) suggest that certain U.S. populations at risk may be experiencing an increase in acute respiratory disease caused by adenoviruses. In settings such as long-term care facilities that house patients with susceptible underlying conditions, infection with virulent adenoviral strains should be considered when patients are seen with sudden respiratory disease, and appropriate control measures should be implemented quickly, pending pathogen identification.

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Virus Taxonomy: One Step Forward, Two Steps Back

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Taxonomy, the science of identifying and naming entities, has long been an integral component of biologic sciences, both in botany and zoology. Not all biologists are actively engaged in taxonomy (only a few truly enjoy working in this field), yet everyone, even lay persons, recognizes the value of consistency and standardization in the naming of animate and inanimate objects. The naming of biologic entities is an exact tool that conveys a precise meaning and ensures maximal continuity and universality for present and future generations. It further confirms that when reference is given to a scientific name, such as *Quercus albus* or *Gorilla gorilla beringei*, everyone recognizes we are speaking about white oak trees or mountain gorillas, respectively, and not northern red oak trees (*Quercus rubra*) or lowland gorillas (*Gorilla gorilla gorilla*). However, taxonomy has not been, and still is not, without difficulties. Very early on, it was recognized that universal "codes" needed to be developed to guide the naming of biologic entities so that each name would be as unique and distinct as the object being named.

Virologists seem to be struggling with taxonomy more than scientists in other disciplines. Much of this struggle seems self-induced, and the article by van Regenmortel and Mahy, "Emerging Issues in Virus Taxonomy"(1), continues to provide highly controversial reading for those who might have an interest in how to approach taxonomic issues. For taxonomists and other scientists with a strong sense of historical perspective, that article may be difficult reading. The article illustrates the inconsistencies between viral taxonomy and taxonomy of other biologic disciplines.

The authors do continue to chip away at several fundamental issues, and they are to be commended for that. For instance, the authors acknowledge that viruses are biologic entities, they advocate applying species names in virus taxonomy, and they recognize that use of a binomial naming system is preferred. That virologists also recognize the value of using a combination of characters to define a species is not novel but reflects that elements other than morphologic features, i.e., host and geographic distribution, vector requirements, and molecular sequences, contribute to defining a species. Although seemingly at the very core of taxonomy, agreement on such basic principles is a major step, when one considers that not all virologists subscribe to standard biologic principles.

As the authors note, virus taxonomy is an emerging discipline that allows working virologists to communicate without misunderstanding. However, the issue is larger than that, and virologists need to know that they are not only working to solve a problem in virology, but they are also accountable to the larger biologic community so that we can communicate clearly and effectively across disciplines. Only with consistency and uniformity will this communication occur. The overall goal should be to provide consistency not only within the field of virology, but also and more importantly, across the broader field of biology. In a recent article by Ashford, the need for consistency in defining terms was noted, and the author stated, "When we all agree on what we are talking about, we will understand each other better" (2). Whether dealing with definition of terms or taxonomic categories, consistency across fields is paramount.

This is where this article (and seemingly most efforts to date on virus taxonomy) falls short. Inconsistencies in virus taxonomy—some perpetuated in this article, some introduced in it—indicate that, unless more attention is paid to what has gone before, virus taxonomy will never achieve the respect it deserves. For instance, insistence on italicizing names above genus level is out of character with most other zoologic disciplines. Similarly, the idea that viruses are unique and need their own set of rules appears presumptuous. All biologic entities are unique: humans are unique, a particular bacterium is unique, as are specific parasites, plants, algae, and the like. Viruses are different and distinct but occupy a spot along a continuum in the bigger biologic spectrum. Prions may be even more problematic than viruses to characterize and name.

The most perplexing proposal offered in this article is the placement of the genus name after the species name.

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Taxonomy, and the more specific aspect of nomenclature, is based on a traditional system of naming organisms beginning with the highest order (kingdom) and descending to lowest order (species). Why virologists would wish to be discordant with the rest of biologic science is unclear, and no immediate value to such a system is evident. Virologists also need not worry about having a universally acceptable definition of a species before applying rigorous rules of taxonomy. Every discipline, botanical and zoologic, is wrestling with a working definition of what constitutes a species. Virologists should also not fret over whether a species concept only applies to sexually reproducing organisms. Other biologists are not so encumbered, as the International Code for Zoological Nomenclature (3) and the International Code of Botanical Nomenclature (4) very nicely handle plants, fungi, bacteria, and protozoa that reproduce asexually. This point is further supported by the fact that even newly discovered fossil plants and animals are routinely named according to rules found in these codes, and it can be stated with a fair degree of certainty that most fossils have not engaged in sexual activity for centuries, if not millennia.

Other troubling areas of current taxonomy of viruses include the concept that viruses are abstract entities. How comforting will it be to patients with serious diarrhea caused by Norwalk virus to learn that their illness is caused by an abstract entity? Viruses cause disease just as parasites and bacteria cause disease, and we do not consider them abstract. Because parasites or bacteria are different from viruses in composition, life cycle, and the like does not make a virus an abstract entity. Developing scientific names for viruses has also provided some humorous fodder for other taxonomists. Using English rather than latinized words for names appears capricious and is dismissive of centuries of distinguished scientists and pioneers in the field, including virology. Lastly, virologists' concern about having to demarcate and coin new names for an estimated 1,550 virus species is puzzling. One wonders what their response would be to naming and cataloguing in other disciplines, such as entomology, where there are >1 million recognized species, some 10,000 new species described each year, and an estimated 4–6 million species yet to be discovered and named (5,6).

From the perspective of a nonviral taxonomist, virologists might do well not to reinvent the wheel (7) but rather to adopt and use existing, conventional taxonomic structure, as in the International Code of Zoological Nomenclature (3). The rules, concepts, and framework have all been worked out, have been tested over time, and, best of all, are immediately available for use.

Drs. van Regenmortel and Mahy are to be commended for trying to bring virus taxonomy to a higher order of consistency. However, given the controversies, virus taxonomy may not get it right (8) for some time. This situation is unfortunate as there is an increasing need, as recently evidenced by the severe acute respiratory syndrome outbreak, to detect, study, develop effective treatments for, and ultimately control and prevent viral infections. Virus taxonomy should become a stabilizing force, rather than a distraction, during these challenging times.

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Haemophilus influenzae Type b Meningitis in Children, Eritrea

To the Editor: Bacterial meningitis is a major cause of death and disability in children worldwide: >1,000,000 cases and 200,000 deaths are estimated to occur each year. *Neisseria meningitidis*, *Haemophilus influenzae* type b (Hib), and *Streptococcus pneumoniae* are major causative agents of bacterial meningitis in children. A region in sub-Saharan Africa, extending from Ethiopia in the east to the Gambia in the west and containing 15 countries with >260 million people, is known as the "meningitis belt" because of its high prevalence of endemic disease with periodic epidemics caused by *N. meningitidis.*

Eritrea, a small country with an estimated population of 3.5 million in northeast Africa, is part of the meningitis belt. Eritrea gained independence in 1993 and borders the Red Sea on the east, Djibouti on the southeast, Ethiopia on the south, and Sudan on the north. Asmara, with a population of about 500,000, is the capital city. The estimated infant mortality rate is 73 deaths/1,000 live births.

In 2002, a prospective laboratory-based study was carried out in Asmara to gain insight regarding the distribution of bacterial agents causing bacterial meningitis in children. Starting in January 2002, cerebrospinal fluid (CSF) specimens were collected from every child who had a spinal tap administered at Mekane Hiwet Pediatric Hospital, Asmara. This facility serves as the national reference hospital for pediatric care. Within 1 hour of collection, all CSF specimens were processed at the Department of Microbiology, Central Health Laboratory. This laboratory, located <200 m from the Mekane Hiwet Pediatric Hospital, serves as the national reference health laboratory and is the only facility in Eritrea with the capabilities to perform cultures. Standard methods were used to process all specimens and to isolate and identify bacterial agents from the CSF specimens (1–3). All CSF specimens were cultured on chocolate agar plates with IsoVitalex supplement (BBL Microbiology Systems, Cockeysville, MD) and tested for bacterial antigens by using Wellcogen latex agglutination kits (Remel, Inc., Dartford, UK). Hib strain ATCC 49247 was used as the control strain. All Hib strains were tested for susceptibility to ampicillin, penicillin, chloramphenicol, gentamicin, and cefotaxime by using the disk diffusion method (BBL); isolates determined to be penicillin-resistant were also tested for β-lactamase by using the Nitrocefin touch sticks (Oxoid, Basingstoke, UK).

From January 1 to December 31, 2002, a total of 81 CSF specimens were collected: 38 (47%) were from patients <1 year of age, 28 (35%) from patients 1 to 2 years of age, and 15 (18%) from patients 2 to 14 years of age. Twelve (15%) of the 81 specimens tested positive; 10 were positive by both culture and latex agglutination test (5 Hib, 2 *S. pneumoniae*, 1 *N. meningitidis*, and 2 *Enterobacteriaceae*), and 2 were positive only by the latex agglutination test (1 Hib and 1 *N. meningitidis*). The patients' age and sex and the results of microbiologic tests are presented in the Table.

This study preceded the implementation of the Integrated Disease Surveillance (IDS) in Eritrea (last quarter of 2002) and does not allow for calculation of incidence of Hib disease at the national level. However, implementation of IDS will enable microbiologists to prospectively monitor the incidence of infectious diseases, including meningitis caused by Hib.

In many countries, Hib is still reported as a major cause of bacterial meningitis (4–9), and while Hib meningitis has a relatively low case-fatality rate in developed countries (3% to 5%), high case-fatality rates (20% to 30%) are common in tropical Africa. Rapid laboratory diagnosis and treatment with appropriate antimicrobial drugs, such as third-generation cephalosporins, are crucial in reducing the risk for severe complications. The decrease in Hib meningitis cases after the introduction of Hib vaccination and the use of vaccine to control Hib meningitis are well documented (10–12). Additionally, the findings of this study suggest that Hib remains the leading cause of childhood meningitis in this region and lead us to advocate for the introduction of vaccination in Eritrea.

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Table. Results of microbiologic tests of specimens from five case-patients with *Haemophilus influenzae* type b[a]

Age/sex	Latex agglutination test	Antimicrobial susceptibility					β-lactamase
		Pen	Amp	Cefot	Chl	Genta	
3 months/female	Positive	R	R	S	S	S	Positive
5 months/male	Positive	R	R	S	R	R	Positive
6 months/male	Positive	R	R	S	S	S	Positive
1 year/male	Positive	R	R	S	S	S	Positive
3 years/male	Positive	R	S	S	S	S	Positive

[a]Pen, penicillin; Amp, ampicillin; Cefot, cefotaxime; Chl, chloramphenicol; Genta, gentamicin; S, sensitive; R, resistant.

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Quinolone Safety and Efficacy More Important than Potency

To the Editor: In a recent article, Scheld defines two principles for appropriate quinolone use based on the goal of maintaining class efficacy, namely avoiding unnecessary antimicrobial drug therapy and "using the agents with optimal activity against the expected pathogens" (1). He presents a large body of evidence supporting an inverse correlation between quinolone activity and the selection of antimicrobial drug resistance. On the basis of this concept, Scheld favors ciprofloxacin for known or suspected *Pseudomonas aeruginosa* infection and moxifloxacin for infections in which *Streptococcus pneumoniae* is likely, including community-acquired pneumonia (CAP). Preventing the emergence of antimicrobial drug resistance is certainly an important goal in drug therapy decision-making. However, this goal should be balanced by the clinical criteria of safety and efficacy.

Serious adverse drug effects in patients led to the withdrawal or restriction of four quinolones in the past decade (temafloxacin, grepafloxacin, trovafloxacin, and sparfloxacin). Safety may differ substantially among the quinolones discussed in Scheld's review (ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin). On the basis of spontaneous reports to the U.S. Food and Drug Administration (FDA), gatifloxacin is associated with a higher rate of torsades de pointes than ciprofloxacin or levofloxacin ($p = 0.001$) (2). Torsades cases have been reported in association with moxifloxacin, but their rate cannot be estimated with any precision by using FDA spontaneous reporting data because of the relatively small number of U.S. prescriptions (2). In a crossover study, a single oral dose of moxifloxacin 800 mg was associated with greater QT interval prolongation (16–18 milliseconds) than ciprofloxacin 1,500 mg (2–5 milliseconds) or levofloxacin 1,000 mg (4–5 milliseconds) (3). Gatifloxacin has been associated with alterations in glucose metabolism, both in prospective trials and in postmarketing surveillance. Gatifloxacin underwent two "safety-related drug labeling changes" in 2001 and is the only quinolone that carries a "warning" about disturbances in glucose metabolism. Gemifloxacin was approved after Scheld's review and has pharmacodynamic potency similar to moxifloxacin against *S. pneumoniae*. Gemifloxacin is associated with a high rate of rashes, especially in women <40 years of age.

Serious but uncommon adverse side effects may not be recognized until drugs are used in large populations (4). More than 100 million prescriptions were written for terfenadine and astemizole before they were withdrawn from the market because of torsades and sudden death. On the basis of the number of U.S. prescriptions in the past decade (January 1993–December 2002), patient experience with ciprofloxacin (119 million prescriptions) and levofloxacin (44 million) is larger than with gatifloxacin (8 million) or moxifloxacin (5 million) (5–7).

The clinical efficacy of ciprofloxacin and levofloxacin is better established for a broad range of indications in comparison to the newer agents. A full discussion of the literature is beyond the scope of this letter. A simple MEDLINE (U.S. National Library of Medicine, Bethesda, MD) search in April 2003 provided the following raw numbers of peer-reviewed, randomized, controlled trials reporting clinical outcomes: >200 trials using ciprofloxacin, 28 using levofloxacin, 13 using moxifloxacin, 7 using gatifloxacin, and 6 using gemifloxacin (search terms, inclusion

criteria, and exclusion criteria available from the author). The quality of these studies is quite variable, and quality is certainly more important than quantity. Most trials of the newer agents were designed and funded by industry. In general, ciprofloxacin and levofloxacin have been studied in patient populations with more severe illnesses, including nosocomial infections, than the newer quinolones. With the exception of a single moxifloxacin trial (8), the trials of the newer quinolones have enrolled patients with predominantly mild or moderate community-acquired infections and low overall mortality rates.

Scheld provides a table that lists case reports of clinical failures of levofloxacin for the treatment of pneumococcal infections. Some cases were associated with primary or secondary levofloxacin resistance. These case reports should not be surprising, since CAP trials regularly identify clinical failures regardless of the therapy chosen. The rate of clinical failure is best determined by data from prospective trials rather than case reports. Both levofloxacin and moxifloxacin have performed well in patients with severe pneumococcal infections, on the basis of the rates of therapeutic success and death (8–10).

Scheld's choice of ciprofloxacin as a component of combination therapy for suspected *P. aeruginosa* infections can be affirmed. Ciprofloxacin has pharmacodynamic potency against *P. aeruginosa*, a track record of safety in large populations, and a large published literature. Ciprofloxacin has demonstrated efficacy in patient populations with severe illnesses, including nosocomial infections.

Antimicrobial drug therapy decision-making for patients with CAP and other respiratory tract infections is much more complex. Individual patient factors should be considered, including the severity of illness, coexisting illnesses, risk factors for drug-resistant *S. pneumoniae*, and risk factors for specific adverse effects. A respiratory quinolone will be an appropriate choice for some patients with CAP. Among the respiratory quinolones, a wholesale switch from levofloxacin to moxifloxacin, on the basis of pneumococcal potency alone, would be premature. Clinicians should use newer quinolones cautiously until their safety has been established in large patient populations.

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Vancomycin-resistant *Enterococcus faecalis* in Serbia

To the Editor: First isolated in France (1), vancomycin-resistant enterococci (VRE) have become pathogens of major importance, particularly in the United States (2). Infections due to VRE are still uncommon in most European countries (3). We report the first isolation of high-level vancomycin-resistant *Enterococcus faecalis* in Serbia.

A 55-year-old woman was admitted to the Clinic for Cardiovascular Diseases, Belgrade, on April 1, 2002, for aortobifemoral bypass surgery. Three weeks after she was admitted to the hospital, an infection developed in the surgical wound and treatment with trimethoprim-sulfamethoxazole (160/800 mg q 12 h) was empirically introduced. Bacteriologic analysis of the wound swab sample showed a methicillin-resistant strain of *Staphylococcus aureus*, a multiresistant strain of *Acinetobacter* sp., a commonly susceptible strain of *Enterococcus* sp., and a VRE strain.

According to the results of susceptibility testing, imipenem (1 g q 6 h) was added to the patient's treatment protocol. VRE were not isolated from subsequent wound samples or any other sample submitted for microbiologic analysis. The patient was discharged at the end of the 14-day treatment period.

The isolate was identified as *E. faecalis* by biochemical characterization, as recommended by Facklam and Collins (4) and confirmed by API 20 Strep (bioMérieux, Marcy-l'Etoile, France). Susceptibility testing, performed by the disk diffusion method, showed that the isolate was resistant to vancomycin, teicoplanin, gentamicin, streptomycin, tetracycline, and ciprofloxacin, while susceptible to ampicillin, amoxicillin, amoxicillin and clavulanic acid, and imipenem. Resistance to vancomycin, teicoplanin, gentamicin, and streptomycin was confirmed by the broth dilution method, according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations (5). The obtained MICs were 256 μg/mL for vancomycin, 64 μg/mL for teicoplanin, >4,000 μg/mL for gentamicin, and >2,000 μg/mL for streptomycin. This phenotype, with high-level resistance to vancomycin and teicoplanin, is typical for the *vanA* genotype (2). The strain was subsequently genotyped by pulsed-field gel electrophoresis, using previously described methods (6). The presence of the *vanA* gene was confirmed by polymerase chain reaction assay, according to a previously described procedure (7). *E. faecium* EF228 was used as the positive control.

The enterococci are among the most frequent causes of nosocomial infections, particularly in intensive care units, and present a major therapeutic challenge (2). While the emergence of VRE strains in the United States is probably associated with extensive use of vancomycin, the occurrence of VRE in Europe is possibly due to application of avoparcin (glycopeptide analog) as a growth promoter in animal husbandry (3). However, avoparcin has not been used in Serbia, and vancomycin application has been restricted to hospitalized patients and quite limited due to its high cost. Thus, emergence of VRE strains in Serbia has not been likely.

The origin of this VRE isolate is unknown: the strain may have been imported or may have originated from the hospital environment. The first prospective pan-European VRE surveillance study (January–April 1997) showed VanA-VRE strains in only eight European countries, with isolates numbering from one to four per country (3). No epidemiologic relations were established among the VanA isolates, and only 2 out of 18 isolates (11%) were identified as *E. faecalis* (3). Since our patient-case had no history of travel outside Serbia, we assumed that the VRE isolate originated from the hospital environment. However, a study investigating the occurrence of VRE strains in Belgrade, the capital of Serbia, detected no such isolates in five different hospitals (8). Although the study did not analyze samples from the Clinic for Cardiovascular Diseases, it did include samples from the Clinic for General Surgery, which is located within the same building. The susceptibility of 191 isolates of enterococci to vancomycin was tested by agar dilution method according to NCCLS recommendations. Of the 191 isolates, 159 were classified as susceptible and 32 as intermediately susceptible.

This report of the first isolation of VRE in Serbia, as well as the previously shown presence of enterococci displaying intermediary susceptibility to vancomycin, provides the rationale for future active screening for VRE in hospital environments in the region.

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Q Fever in Como, Northern Italy

To the Editor: Q fever is a widespread zoonosis caused by the intracellular gram-negative bacterium *Coxiella burnetii.* Infection in humans usually occurs through inhalation of contaminated aerosols from parturient fluids of infected animals or contaminated wool (1). *C. burnetii* also forms spores that may survive for months in the environment in an area where animals have been present, representing a source of infection for persons without any evident contact with animals (2). A self-limited febrile illness, Q fever has the major signs and symptoms of atypical pneumonia and hepatitis. Diagnosis is based on serologic test results. Recently, outbreaks of Q fever have been described in urban areas (3,4), affecting people without any evident risk factor. We describe an outbreak of Q fever in Como, northern Italy, which affected 133 persons.

From January 2003 to February 2003, over a 5-week period, 16 men and 1 woman from the prison in Como were admitted to the local hospital with acute pneumonia. At the same time, a 26-year-old man and a 79-year-old woman living in the same area were admitted with the same diagnosis. On admission, all the patients had a high-grade fever and reported a dry cough; 80.0% of patients had a headache, and 70.0% of patients complained of fatigue and weakness. In a few patients, nausea and abdominal pain developed after they were hospitalized. Physical examination of the lungs showed minimal auscultatory abnormalities. Hemoptysis was observed in four patients. Routine blood examinations were performed. In all patients, the leukocyte count was normal, with an increase in the erythrocyte sedimentation rate and elevated C-reactive protein levels. The transaminase levels were elevated in eight (47.0%) patients, usually two to three times normal values. Radiographic findings were nonspecific and differed greatly among the patients, who exhibited single and multiple opacities, diffuse interstitial pneumonia, and pleural effusion (in one patient). Bilateral involvement was seen in five (29.4%) patients. All patients received empirical antimicrobial therapy with a β-lactam antimicrobial drug in association with a macrolide or an advanced fluoroquinolone, following the guidelines for community-acquired pneumonia. Patients improved clinically in 48 to 72 hours after antimicrobial drug therapy was started: the fevers resolved and the inflammatory indexes and liver enzyme levels returned to normal.

An epidemiologic investigation was started by the Department of Prevention, Azienda Sanitaria Locale, of Como, after it was notified that six patients with acute pneumonia of unknown cause had been admitted to St. Anna Hospital during a 6-day period (January 11–17, 2003); all six patients were men from the Como prison. Investigation showed that the case-patients resided in different sections of the prison in different rooms; thus, human-to-human transmission was excluded. After exclusion of *Streptococcus pneumoniae, Legionella* spp., *Mycoplasma pneumoniae,* and *Chlamydia pneumoniae* as etiologic agents, the diagnosis of Q fever was made on the basis of positive serologic results for *C. burnetii* from immunofluorescence assay. Further investigation was directed to determine the source of the epidemic, with a focus on animals in the area surrounding the prison. We identified two flocks of sheep and other animals that grazed in the meadows next to the prison from December 2002 to January 2003, in an annual migration pattern, and a colony of pigeons nesting in the prison. The first flock of animals was composed of 950 sheep; 50 goats and 4 dogs accompanied them. The Laboratory of the Zooprofilattico Institute, Brescia, tested 80 animals from this flock, including both males and females, chosen randomly. All four dogs were tested. All the animals tested (sheep, goats, and dogs) were negative for *C. burnetii*. The second flock included 748 animals (sheep and goats) and 4 dogs. All these animals were tested because the biological specimens, collected for another purpose, were available. In this flock, 255 of 748 animals were positive for *C. burnetii,* and 2 of the 4 dogs were positive, for a prevalence rate of 34.2%. We know that a parturient sheep died on December 31, 2002. The source of the epidemic was declared extinct on March 5, 2003, on the basis of a negative result of DNA amplification by polymerase chain reaction assay conducted on animals' milk, urine, and feces after antimicrobial drug treatment of the infected animals.

At the same time, in collaboration with physicians of general medicine in the area, we actively sought patients with pneumonia of unknown etiology living in the urban area close to the meadows where the flock grazed. Since the beginning of the epidemic, 133 cases of acute Q fever, defined as clinical symptoms (high grade fever, dry cough, auscultatory abnormalities, arthromyalgia, fatigue) plus positive serologic results (immunoglobulin [Ig] G phase II >1:64; values were included between 1:64 and 1:1024), were reported to the Department of Prevention, ASL, in Como. Of these, 59 were prisoners, 37 were prison officers, 33 were persons living in the area in which the flock traveled**,** and 4 were personnel of the Veterinarian Service, who participated in performing the autopsy on the sheep. Analysis of the data showed a prevalence of disease in the prisoners of 10.8% (59/547), comparable to the prevalence obtained in the guards, 16.5% (37/224) and significantly lower than that for the residents, 3.2%

(33/1,025). These differences could be ascribed to the prison's being situated in a natural setting, with the flock grazing for 1 month in the meadows nearby.

C. burnetii is an infrequent cause of community-acquired pneumonia in our region. These data suggest that the infected sheep were the source of this large outbreak. None of the patients had any contact with animals, except for the Veterinarian Service personnel, which suggests airborne transmission of infected dust particles from contaminated soil, favored by the dry weather recorded in that period. To our knowledge, this is the second outbreak of Q fever reported in northern Italy (5). Since 1999, neither the Department of Prevention nor the Veterinary Service had received any reports of Q fever in Como. In Italy, the total number of cases of rickettsial diseases, which includes Q fever, reported to the Ministry of Health was 769 in 2002 and 739 in 2001, with 5 and 13 cases, respectively, from Lombardia, which includes Como. This Q fever epidemic in Como is thus an exceptional event in our area.

The collaboration between epidemiologists and veterinarians of the Department of Prevention and staff from Saint Anna Hospital allowed us to share epidemiologic and medical information, which proved useful in diagnosing the outbreak and treating patients. Our experience emphasizes the necessity of a greater awareness of this occupational zoonosis in areas with a high rate of urbanization.

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Correction Vol. 9, No. 6

In "Clinical Implications of Varying Degrees of Vancomycin Susceptibility in Methicillin-Resistant Staphylococcus aureus Bactermia, by Mitchell J. Schwaber et al., errors occurred in some reference numbers. In the Discussion, fourth paragraph, p. 661, the last sentence should read as follows: 'It is possible that some or all of the isolates from our cases are potential precursors of truly heteroresistant isolates (hetero-VISA), which may in turn be forerunners of VISA (6,18,32)." In the final paragraph, pp. 662-663, the next to last sentence should read as follows: "These results add weight to assertions that clinical microbiology laboratories need not routinely screen for vancomycin heteroresistance in S. aureus isolates with vancomycin MICs in the susceptible range (1,7).

Correction Vol. 9, No. 11

In "Genetic Variation among Temporally and Geographically Distinct West Nile Virus Isolates, United States, 2001, 2002, by C. Todd Davis, et al., errors occurred in Tables 1 and 2. The correct title for Table 1 is "Nucleotide mutations in sequences of the prM gene of 22 West Nile virus isolates obtained during 2001 and 2002 compared to WN-NY99." In Table 1, line 6, Nueces Co., Tx-1, under "prM 491" should read "G (Arg)"; line 19, Galveston Co, TX-2, under "prM 679" should read "A (Thr)." The correct title for Table 2 is "Nucleotide mutations in sequences of the E gene of 22 West Nile virus isolates obtained during 2001 and 2001 compared to WN-NY99." In Table 2, line 3, Harris Co., TX, under "Envelope 2,392," should read "A (Thr)"; line 8, Nueces Co., TX-2, under "Envelope 1,118," should read "U (Val)"; line 12, Randall Co., TX, under "1,192" should read "C (Asn)"; line 24, Galveston Co., TX-3, under "Envelope 1,192" should read "G (Ala)." Corrected versions of Tables 1 and 2 are available at: URL: http://www.cdc.gov/ncidod/EID/vol9no11/03-0301.htm

We regret any confusion these errors may have caused.

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Office International des Epizooties
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International Conference on Women and Infectious Diseases: From Science to Action
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Atlanta, GA
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Johannes [Jan] Vermeer (1632–1675). The Astronomer (1668)
Oil on canvas (51 cm x 45 cm). Musée du Louvre, Paris, France. Photo: Erich Lessing/Art Resource, New York

"What is it about Johannes Vermeer?" contemporary art lovers and historians ask. The enigmatic painter, apparently well known in his day, lapsed into obscurity after his death only to surface again in the 19th century and capture the imagination and esthetic taste of modern times. Even though he produced no more than 40 paintings, their originality and refinement place him among the greatest 17th-century Dutch artists (1).

Vermeer's life story can only be patched together from public records, and no portrait is available of his physical appearance. He was sufficiently trained in his trade to belong to an artists' guild and was esteemed enough by his colleagues to be twice appointed guild leader. Nonetheless, financial hardship, aggravated by his inability to support a brood of 15 children, 11 of whom survived to adulthood, limited his artistic output. He made little money from his paintings and died poor at age 43 (2). "Because of…the large sums of money we had to spend on the children, sums he was no longer able to pay," his wife lamented after his death, "he fell into such a depression and lethargy that he lost his health in the space of one and a half days and died" (3).

Though untraveled, Vermeer was well connected with other artists, including Gerald Ter Borch and Dirk van Baburen, and might have been influenced by Caravaggio and Carel Fabritius, the brilliant student of Rembrandt. Vermeer's work is also reminiscent of 15th-century Flemish art, especially in the use of color and meticulous detail; however, he brought to these elements unique sensitivity and novelty (4).

During the 17th century, the arts broke away from classical style into genre (scenes of everyday life). Vermeer's work assumed the domestic intimacy of the Dutch school yet moved beyond its monochromatic, evenly lit, raucous gatherings. Although more than any of his contemporaries Vermeer saw poetry in everyday activities (The Lacemaker, The Procuress, The Milkmaid), his characters had an air of introspection and seemed to be engaged in more than the activities themselves. In scenes of extraordinary simplicity and clarity, he placed solitary figures, whose dimensions were sometimes enlarged in relation to the surroundings, within the confines of carefully constructed spaces (1). Then, elaborating on textures and details, he used light to peer through the external trappings into the soul.

While the arts were abandoning classical themes for scenes of everyday life, the sciences were inventing new methods of inquiry, dispelling the shadows of antiquity to view the world through a lens. Newton was making the first reflecting telescopes, Louis XIV was building an observatory in Paris, and Huygens had detected the first moon of Saturn (5,6). An innovator himself, Vermeer was likely acquainted with the science of his time and is often linked with Anton van Leeuwenhoek, Dutch inventor of the microscope. Van Leeuwenhoek, a merchant who sold cloth not far from Vermeer's abode in Delft and who later became the court-appointed executor of the artist's will, discovered through his microscope the cellular nature of spermatozoa and bacteria and was skilled in navigation, astronomy, and mathematics. Van Leeuwenhoek, who was born the same year as Vermeer, may have inspired both The Astronomer (on this month's cover of Emerging Infectious Diseases) and its companion, The Geographer (c.1668–1669) (3).

The Astronomer harmonizes space, color, and light to convey a single human activity, a unified moment in time. Perfectly staged, the scene is a subtle composite of interlocking diagonal, rectangular, or elliptical fields and has no empty or undefined surface. The composition is not narrative but rather forms the context of a sole figure, frozen in a pose of profound preoccupation.

Like many Vermeer characters, the astronomer is placed near a window on the onlooker's left, which casts a glow on the man of science, revealing youthful freshness, sudden insight, and nervous anticipation. Expressive hands define the geometric space between the sympathetic figure and the celestial globe (drafted by Dutch cartographer Jodocus Hondius in 1600) and drive the forward move-

ment of the body. The desk, framed by a thick tapestry, holds an astrolabe (precursor of the sextant) and a book. On the wall is a circular figure with radial lines.

The moment of discovery reflected on the astronomer's face captures centuries of human fascination with the universe. The generic physiognomy, unremarkable features, untended hair, and drab attire draw the eyes to the illuminated face of the thinker, in a room where the only light is that of knowledge.

A model to all stargazers, old and new, Vermeer's scientist reaches beyond the globe at hand into the mysterious continuum of time and space, charting, measuring, counting, categorizing, naming, recording. His contemporary counterpart, whether an astronomer exploring the cosmos or a biologist investigating the microcosm, is still guided by the light of discovery. Uncharted in Vermeer's days, the spatial distribution of disease follows the evolution over time of agent, host, and environment and is the domain of those today who trace the time-space continuum of emerging pathogens, from Ebola to influenza and SARS.

Polyxeni Potter

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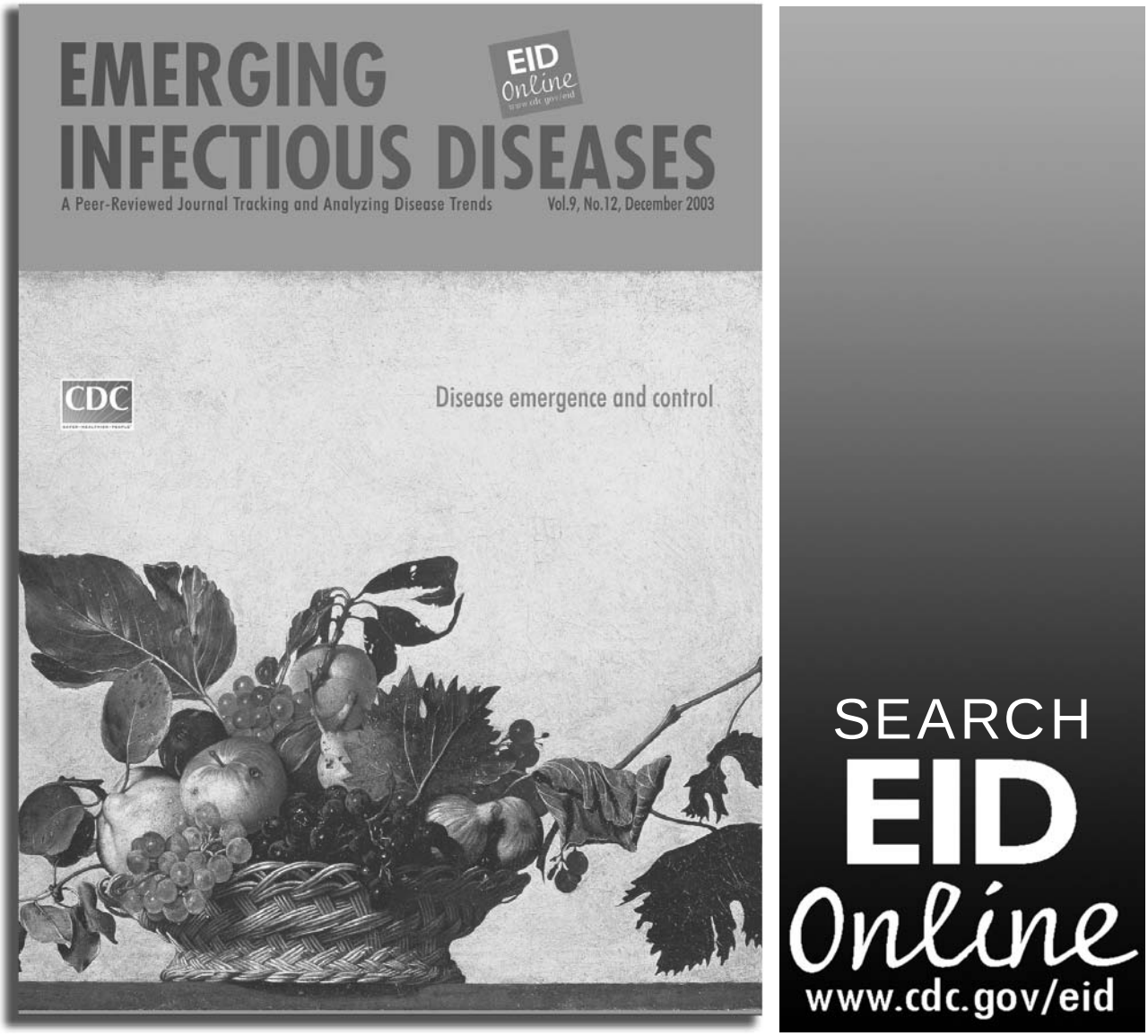

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EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.10, No.2, February, 2004

Upcoming Issue on Severe Acute Respiratory Syndrome (SARS)

For a complete list of articles included in the February issue, and for articles published online ahead of print publication, see http://www.cdc.gov/ncidod/eid/upcoming.htm

The February issue includes more than 40 SARS articles on SARS origins, epidemiology, transmission, infection control, and laboratory and clinical studies:

Wresting SARS from Uncertainty

Global Surveillance, National Surveillance, and SARS

SARS-related Virus Predating SARS Outbreak, Hong Kong

SARS in France, March-April 2003

Lack of SARS Transmission among Healthcare Workers

SARS among Critical Care Nurses, Toronto

Surgical Helmets and SARS Infection

Serologic and Molecular Biologic Methods for SARS-associated Coronavirus

Combining Clinical and Epidemiologic Features for Early Recognition of SARS

Crisis Prevention and Management during SARS Outbreak, Singapore

Body Temperature Monitoring and SARS Fever Hotline, Taiwan

SARS Epidemic in the Press

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 and Historical Reviews, Dispatches, Commentaries, Another Dimension, Letters, Book Reviews, and News and Notes. 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.

Chinese, French, and Spanish translations of some articles can be accessed through the journal's home page at http://www.cdc.gov/eid.

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Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

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Tables and Figures. Create tables within MS Word's table tool. Do not format tables as columns or tabs. Send graphics in native, high-resolution (200 dpi minimum) .TIF (Tagged Image File), or .EPS (Encapsulated Postscript) format. Graphics should be in a separate electronic file from the text file. For graphic files, use Arial font. Convert Macintosh files into the suggested PC format. Figures, symbols, letters, and numbers should be large enough to remain legible when reduced. Place figure keys within the figure. For more information see EID Style Guide (http://www.cdc.gov/ncidod/EID/style_guide.htm).

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Manuscript Types

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. 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 (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be 1,000–1,500 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed two); and a brief biographical sketch of first author—both authors if only two. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but should not include figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

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

Book Reviews. Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted on the journal Web page only, depending on the event date.)

Conference Summaries. (500–1,000 words) of emerging infectious disease conferences may provide references to a full report of conference activities and should focus on the meeting's content rather than on individual conference participants.