EMERGING INFECTIOUS DISEASES®

September 2011

Disease Emergence the World Over

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Emerging Infectious Diseases is published monthly by the Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop D61, Atlanta, GA 30333, USA. Telephone 404-639-1960, fax 404-639-1954, email eideditor@cdc.gov.

∞ Emerging Infectious Diseases is printed on acid-free paper that meets the requirements of ANSI/NISO 239.48-1992 (Permanence of Paper)

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On the Cover

Frans Snyders (1579–1657)
Still Life with Fighting Monkeys (1635)
Oil on canvas (74.9 cm × 108 cm)
Bequest of John Ringling, 1936, The John and Mable Ringling Museum of Art, the State Art Museum of Florida, a division of Florida State University

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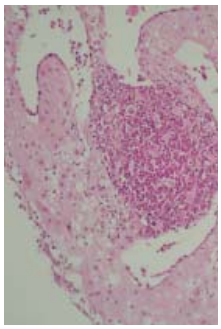

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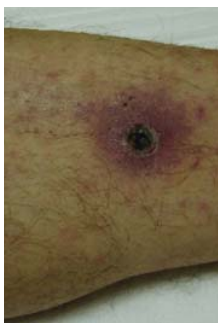

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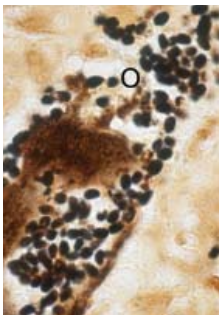

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Risk-based Estimate of Effect of Foodborne Diseases on Public Health, Greece

Elissavet Gkogka, Martine W. Reij, Arie H. Havelaar, Marcel H. Zwietering, and Leon G.M. Gorris

The public health effect of illness caused by foodborne pathogens in Greece during 1996–2006 was quantified by using publicly available surveillance data, hospital statistics, and literature. Results were expressed as the incidence of different disease outcomes and as disability-adjusted life years (DALY), a health indicator combining illness and death estimates into a single metric. It has been estimated that each year ≈370,000 illnesses/million inhabitants are likely caused because of eating contaminated food; 900 of these illnesses are severe and 3 fatal, corresponding to 896 DALY/million inhabitants. Ill-defined intestinal infections accounted for the greatest part of reported cases and 27% of the DALY. Brucellosis, echinococcosis, salmonellosis, and toxoplasmosis were found to be the most common known causes of foodborne illnesses, being responsible for 70% of the DALY. Overall, the DALY metric provided a quantitative perspective on the impact of foodborne illness that may be useful for prioritizing food safety management targets.

To initiate and sustain efforts for prevention and control of foodborne diseases, it is essential to determine the extent and dimensions of the problem (*1*). Accurate knowledge of disease incidence and severity is invaluable to competent national authorities for use in selecting appropriate management actions to reduce the overall public health impact. However, much of the information collated regarding foodborne illnesses by different systems cannot be directly translated into policy (*2*) for 3 main reasons. First, not all cases are reported to health authorities, and estimates of underreporting result in considerable uncertainty in burden of illness studies, which limits the interpretation and analysis of available information (*3*,*4*). Second, often only a fraction of illnesses caused by food-related pathogens are actually foodborne because transmission can also be through the environment, direct contact with animals, or from person to person (*5*). Third, foodborne illnesses may vary not only in their incidence but also in their severity, resulting in widely different clinical manifestations and potentially involving long-term sequelae, although for their accurate description and quantification a uniform health measure would be needed (*6*).

To circumvent the latter issue, the World Health Organization (WHO) recommends using disability-adjusted life years (DALY) as a metric to express the public health effects of foodborne diseases (*2*), and DALY is increasingly used for a wide variety of illnesses (*6*–*8*). The aim of this study was to test the feasibility of using publicly available relevant data sources combined with the DALY metric to quantify the annual impact of foodborne illnesses in a country in a format useful for policy decisions. The country selected was Greece. The study used available surveillance data, hospital statistics from 1996 through 2006, and literature. In an attempt to address the first 2 limitations of the types of study mentioned above, we account in our estimates for uncertainty caused by underreporting and food attribution by using probability distributions to describe a range of plausible values for these parameters. Results are also expressed as cases in the general population, reported or estimated severe cases, and deaths to enable comparisons with similar studies in other countries.

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DOI: http://dx.doi.org/10.3201/eid1709.101766

Methods

The various steps taken to estimate the incidence and impact of foodborne illness in Greece are shown in Figure 1. Reported cases of illnesses that may be transmitted through food were for the larger part collected from the Hellenic Statistical Authority (ELSTAT) (*9*) and the Hellenic Center for Infectious Diseases Control (HCIDC) (*10*). A limited number of data were obtained from WHO disease surveillance reports where HCIDC was mentioned to be the source (*11*,*12*) for better transparency and from other literature when no other information was available (*13*). The study included the period 1996–2006 for which data were available from both national sources. ELSTAT collects information regarding hospitalizations for patients who have a duration of stay ≥1 day based on the Basic Tabulation List (BTL) of the International Classification of Diseases, 9th Revision. ELSTAT data are based on sampling of hospitalized patients' bulletins.

This sampling includes bulletins of deceased patients, although these bulletins are not recorded separately. Hospitalizations recorded by the ELSTAT are likely to vary in their severity because the population in Greece had free access to hospital centers where it was possible to be treated even for minor health issues (*14*). HCIDC collects information on notified cases from hospital microbiologic laboratories and district health authorities (*11*) and also performs active surveillance on the general incidence of gastroenteritis through physicians' reports (*10*). HCIDC data can thus be representative of hospitalizations or visits to physicians and are a mixture of laboratory-confirmed and symptom-based notified cases. In the absence of a study validating these 2 systems of collecting information on disease incidence, we considered ELSTAT and HCIDC data to be representative of reported (severe) cases of illness. Corrections for undernotification or overnotification were not made because this would require a country-specific study that is not currently available. For the few illnesses for which data were available from both systems, ELSTAT data were preferred. For cryptosporidiosis, giardiasis, and toxoplasmosis, cases were estimated indirectly taking into account studies on prevalence of these parasites in the general population (*15*,*16*). The mean and standard deviation of reported and estimated cases for 1996–2006 were used to create normal distributions, which were considered representative of the annual incidence of these illnesses (*17*).

Deciding on a precise estimate of the proportion of cases that can be attributed to food is complicated (*5*). Because of differences in food production, consumption, and the ecology of pathogens, the percentage of foodborne transmission is expected to vary among countries and constitutes a major area of uncertainty. To make an adjustment for food attribution, PERT distributions were used as multipliers (*18*) (Table 1). Minimum and maximum parameters of PERT distributions were based on a literature search covering the range of potential values. Most likely values were based on data most relevant to Greece and Europe because endemicity of illnesses is often related to specific regions (*19*,*20*).

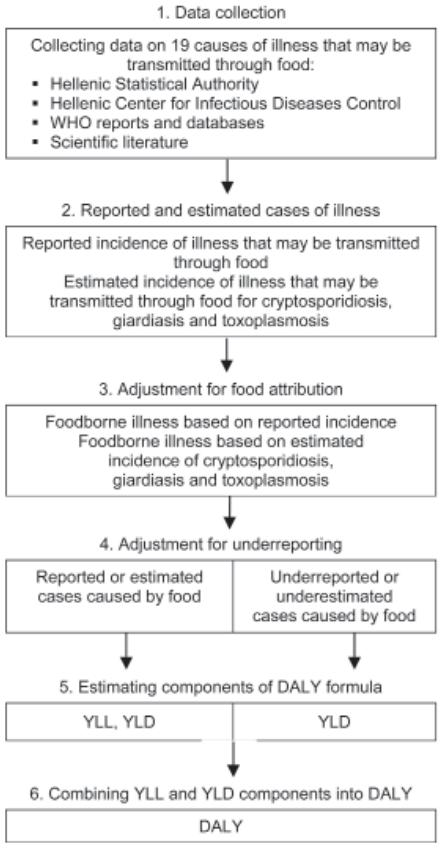

Figure 1. Working scheme for estimating the incidence and effects of foodborne illness in Greece. For cryptosporidiosis and giardiasis, because estimated cases are on the same level of the surveillance pyramid as reported cases, the cases occurring in the community (underestimated cases) were based on underreporting factors suitable for these pathogens. In the case of toxoplasmosis, disability-adjusted life years (DALY) are calculated only on the basis of estimated cases which cover the entire population. WHO, World Health Organization; YLL, years of life lost caused by premature death in the population; YLD, years lived with disability for incident cases of the health condition.

Not all cases of foodborne illness are reported to health authorities (*3*), and the degree of underreporting varies greatly among diseases between countries or within 1 country in different periods (*21*). To make an adjustment for underreporting, PERT distributions were used as multipliers (*18*) and extremes were selected to cover the full range of values found in literature. Most likely values were set at the middle of this range to give equal weight to extremes of each distribution (Table 1). We assumed that

Table 1. Parameters of the PERT distributions used to describe foodborne transmission, underreporting, and case-fatality rate for foodborne illnesses, Greece, 1996–2006*

Illness	Minimum, most likely, maximum†		
	Food attribution, %	Underreporting	Case-fatality rate, %
Bacterial			
Botulism	80, 100, 100	1.625, 1.8125, 2	3, 10.15, 17.3
Brucellosis	50, 84,100	2, 10.85, 19.7	0.9, 2, 5
Campylobacteriosis	30, 55, 80	7.6, 274.8, 542	0.1, 0.1265, 0.153
Enterohemorrhagic *Escherichia coli*	40, 51, 90	2, 14.05, 26.1	0.25, 0.54, 0.83
Leptospirosis	1, 5, 49	10, 15, 20	5, 10, 15
Listeriosis	69, 99, 100	1.1, 1.7, 2.3	10, 30, 44
Salmonellosis	55, 95, 95	3.2, 51.45, 99.7	0.5, 0.701, 0.902
Shigellosis	8.2, 10, 31	3.4, 18.35, 33.3	0.1, 0.13, 0.16
Typhoid and paratyphoid fever	55, 80, 95	2, 7.65, 13.3	0.4, 0.95, 1.5
Food poisoning	87, 100, 100	29.3, 185.65, 342	0, 0.025, 0.05
Parasitic			
Amebiasis	10, 50, 100	9.2, 9.6, 10	0.1, 0.2, 0.3
Cryptosporidiosis	5.6, 5.6, 8	7.4, 53, 98.6	0.07, 0.335, 0.6
Echinococcosis	30, 30, 100	2, 3, 4	1, 2.24, 3
Giardiasis	5, 10, 30	4.6, 25.45, 46.3	0, 0.05, 0.1
Toxoplasmosis	30, 50, 63	NA	3.3, 3.75, 4.8
Viral: acute hepatitis A	5, 8, 11	2, 5.55, 9.1	0.3, 1.35, 2.4
Mixed/ill-defined causes			
Other helminthiases	30, 90, 100	4.6, 51.6, 98.6	3.37‡
Intestinal infections due to other specified microorganism	1, 36, 70	2, 402, 1,562	0.25‡
Ill-defined intestinal infections	1, 36, 50	2, 402, 1,562	0.0045‡

*NA, not applicable.
†Minimum, most likely (mean), and maximum parameters of each PERT distribution. More information, including an expanded version of this table, can be found in the online Technical Appendix (www.cdc.gov/EID/content/17/9/101766-Techapp.pdf).
‡For these illnesses, an average fixed value was used for the case-fatality rates estimated by using data from the World Health Organization Mortality Database on the deaths and incidence data from the Hellenic Statistical Authority.

underreporting factors primarily represent underreported cases of serious illnesses that result in physician visits, and underreporting factors for gastrointestinal illnesses are primarily associated with cases not resulting in physician visits. Although in some studies an arbitrarily assigned factor is used to cover for misdiagnosed or undiagnosed hospitalizations and deaths (*3*,*18*), it was omitted in the absence of specific data for Greece and underreported cases caused by this phenomenon were considered to be included in the "ill-defined intestinal infections" BTL code as suggested by other authors (*17*). We also assumed that all reported cases were diagnosed and coded correctly.

DALY values were calculated as DALY = YLL + YLD, where YLL are the years of life lost because of premature death in the population and YLD are the years lived with disability for incident cases of the health condition (*22*). YLD was estimated for reported or estimated cases and underreported cases, and YLL was estimated based only on reported or estimated cases. The rationale for this was that fatal cases contributing to YLL occur at the top of the surveillance pyramid and, if diagnosed, most likely are notified, particularly for obligatory notifiable diseases such as most of the ones examined here. Moreover, for illnesses contributing to YLD such as gastrointestinal illnesses, underreported cases not resulting in hospitalization are not expected to have fatal outcomes. The sole exception was listeriosis, in DALY values mainly accounted for through YLL (*23*), because it has been under surveillance only since 2004. Thus, even serious cases of this infection were expected to be considerably undernotified in part of the period under study because physicians and laboratories might not immediately be aware of the new reporting requirements. Therefore, to avoid underestimation of deaths, YLD for listeriosis was estimated on the basis of reported and underreported cases.

The individual components of the DALY formula are estimated as follows: YLL = $d \times e$, where d is the number of deaths and e is the expected individual life span at the age of death in years; YLD = $n \times t \times w$, where n is the number of cases of a specific illness, t is its duration in years and w is a weight factor (disability weight) that reflects its severity on a scale from 0 (perfect health) to 1 (death) (*22*,*24*). In calculating YLL, the number of deaths (d) was estimated by multiplying reported or estimated cases caused by foodborne infection for each illness with a PERT distribution describing a plausible range of pathogen-specific case-fatality rates (*18*) on the basis of literature data from other industrialized countries (Table 1). Selected case-fatality rates were always from

the same level of the surveillance pyramid as reported for estimated cases. For some generic BTL codes (e.g., "Other helminthiases," "Intestinal infections due to other specified microorganism," and "Ill-defined intestinal infections"), the number of deaths was based on data from the WHO Mortality Database (*25*).

Regarding the expected individual life span at the age of death in years (*e*), the age of death was estimated on the basis of data collected by the HCIDC and ELSTAT on patients' age in reported cases. When no explicit information was available in these sources, which was the case for 5 illnesses, age at time of death was assumed to

Table 2. Disability weights related to the diseases included in study of the effects of foodborne infections, Greece, 1996–2006

Illness	Disability weights	
	Reported or estimated cases	Underreported cases
Bacterial		
Botulism		
Moderate cases	0.600	0.600
Severe cases	0.906	0.906
Brucellosis	0.200	0.200
Campylobacteriosis		0.067
Gastroenteritis	0.393	
Reactive arthritis	0.140	
Guillain-Barré syndrome, first year*	0.250	
Guillain-Barré syndrome, long-term sequelae	0.160	
Inflammatory bowel disease	0.260	
Irritable bowel syndrome	0.042	
Enterohemorrhagic *Escherichia coli*		0.067
Watery diarrhea and hemorrhagic colitis	0.393	
Hemolytic uremic syndrome and end-stage renal disease	†	
Leptospirosis	0.920	0.096
Listeriosis	‡	‡
Salmonellosis		0.067
Gastroenteritis	0.393	
Inflammatory bowel disease	0.260	
Irritable bowel syndrome	0.042	
Reactive arthritis	0.150	
Shigellosis	0.220	0.096
Irritable bowel syndrome	0.042	
Typhoid and paratyphoid fever	0.600	0.096
Food poisoning	0.220	0.067
Parasitic		
Amebiasis	0.400	0.067
Cryptosporidiosis	0.393	0.067
Echinococcosis		
Cured	0.200	0.200
Postsurgical conditions	0.239	0.239
Relapse	0.809	0.809
Undiagnosed	0.200	0.200
Giardiasis	0.393	0.067
Toxoplasmosis		
Clinical symptoms in the first year of life§	0.140	¶
Asymptomatic at birth, chorioretinitis later in life	0.080	¶
Viral: acute hepatitis A	0.500	0.500
Mixed/ill-defined causes		
Other helminthiases	0.463	0.067
Intestinal infections caused by other specified microorganism	0.400	0.067
Ill-defined intestinal infections	0.400	0.067

*For an explanation of this selection, see the online Technical Appendix (www.cdc.gov/EID/content/17/9/101766-Techapp.pdf).
†For hemolytic uremic syndrome (including end-stage renal disease as a sequela), it is estimated that every case corresponds to 1.05 years lived with disability (*24*).
‡Not applicable for listeriosis because the high case-fatality rate (>95%) of the disability-adjusted life year estimates is composed of years of life lost (*24*) that mainly determine the burden of the disease. Therefore, no years lived with disability were estimated.
§Clinical symptoms in the first year of life include chorioretinitis, intracranial calcifications, hydrocephalus, and central nervous system abnormalities that lead to neurologic deficiencies such as mental retardation.
¶Toxoplasmosis cases are estimates for the entire population. Consequently, underreporting does not apply.

be 40 years. To check the impact of this assumption on the ranking of foodborne risks, we tested both extremes by assuming 0 years as the age at death and by assuming YLL to be 0. For "Other helminthiases," data from the WHO Mortality Database were used. General life expectancy was based on the life table for Greece for 2000 (*22*). For comparison, estimates were also made by using the WHO standard West Level 26 life table (*22*).

In calculating YLD, duration of illness (*t*) was based on data collected by ELSTAT and on literature regarding serious and mild forms of each cause of illness. Different disability weights (*w*) were used for each disease based on the severity of its sequelae and whether estimated cases likely reach the health system or not (Table 2). All underreported cases were assumed to be mild or self-limiting for gastroenteritis-related illnesses. For serious, non–self-limiting diseases such as brucellosis or echinococcosis that are not related to gastroenteritis, nonreported cases were considered to be as severe as reported or estimated cases.

All estimations were performed by using the @RISK 5.7 software (Palisade Corporation, Ithaca, NY, USA) as an add-in in Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Full details regarding estimations of DALY, selection of input distributions and simulation settings can be found in the online Technical Appendix (www.cdc.gov/EID/content/17/9/101766-Techapp.pdf).

Results

Annual Incidence of Foodborne Illnesses

For 1996–2006, we estimated 369,305 (95% credible interval [CrI] 68,283–910,608) illnesses per million inhabitants per year attributable to eating contaminated food, at least 905 of which (95% CrI 499–1,340) are reported or estimated to be severe and 3 fatal (95% CrI 2.0–4.8) (Table 3). Ill-defined intestinal infections accounted for most (94%) cases (sum of reported/estimated and underreported cases). Regarding reported/estimated cases, ill-defined intestinal infections were responsible again for the greatest part (72%), followed by salmonellosis (8.2%), brucellosis (7.1%), food poisoning (4.0%), and echinococcosis (2.7%). Most deaths (48%) were estimated to be caused by brucellosis, although salmonellosis, echinococcosis, listeriosis, and toxoplasmosis also contributed substantially to deaths.

Table 3. Mean estimated incidence of total foodborne illnesses, reported/estimated illnesses, and deaths attributed to food in Greece per 1 million inhabitants, 1996–2006*

	Incidence per million inhabitants					
	Total illnesses		Reported/estimated illnesses		Deaths	
Illnesses	Mean†	95% CrI‡	Mean†	95% CrI‡	Mean†	95% CrI‡
Bacterial						
Botulism	0.13	0.011–0.28	0.066	0.056–0.15	0.0067	0.00052–0.017
Brucellosis	699	225–1,378	**64**	30–102	**1.5**	0.52–3.0
Campylobacteriosis	**3,571**	851–7,733	13	5.6–22	0.016	0.0069–0.029
EHEC	1.0	0.069–2.8	0.072	0.0058–0.17	0.00039	0.000030–0.00098
Leptospirosis	4.0	0.34–13	0.27	0.023–0.84	0.027	0.0022–0.087
Listeriosis	0.89	0.11–1.9	0.41	0.049–0.85	**0.19**	0.021–0.45
Salmonellosis	**3,793**	750–8,350	**74**	22–128	**0.52**	0.15–0.93
Shigellosis	25	1.1–77	1.4	0.068–3.8	0.0018	0.000088–0.0050
Typhoid and paratyphoid fever	37	3.3–92	4.8	0.47–10	0.046	0.0043–0.11
Food poisoning	**6,636**	450–17,569	**36**	2.8–80	0.0089	0.00055–0.025
Parasitic						
Amebiasis	13	1.9–29	1.3	0.19–3.0	0.0026	0.00037–0.0064
Cryptosporidiosis	197	71–360	3.7	2.4–5.3	0.013	0.0050–0.022
Echinococcosis	72	29–140	**24**	10–45	**0.52**	0.19–1.0
Giardiasis	159	47–358	6.3	2.7–12	0.0031	0.00069–0.0074
Toxoplasmosis	3.4	2.5–4.1	3.2	2.4–4.0	**0.12**	0.090–0.16
Other helminthiases	137	22–322	2.7	0.56–5.1	0.089	0.019–0.17
Viral: hepatitis A	6.9	1.4–15	1.2	0.27–2.4	0.017	0.0031–0.038
Mixed/ill-defined causes						
Intestinal infections caused by other specified microorganism	**7,394**	354–25,558	14	1.2–36	0.035	0.031–0.091
Ill-defined intestinal infections	**346,558**	45,985–886,276	**655**	256–1,082	0.030	0.012–0.049
Total of gastroenteritis	368,520	67,536–909,457	812	408–1,245	0.95	0.52–1.4
Total	369,305	68,283–910,608	905	499–1,340	3.1	2.0–4.8

*Values have been rounded to include significant digits and thus not all summations necessarily tally. **Boldface** indicates the top 5 contributors to each estimate category. EHEC, enterohemorrhagic *Escherichia coli*; CrI, credible interval.
†These estimates correspond to the mean of the output distributions.
‡95% CrI representative of the 2.5 and 97.5 percentiles.

Public Health Impact of Foodborne Illnesses Expressed as DALY

Foodborne illnesses accounted for ≈896 DALY per 1 million inhabitants annually (95% CrI 470–1,461), of which 14% were attributable to YLL and 86% to YLD (Table 4). As much as 34% of the estimated effects of foodborne disease in Greece could be attributed to gastroenteritis-related illnesses, and the remaining 66% was unevenly split among 6 non–gastroenteritis-related illnesses (brucellosis, echinococcosis, toxoplasmosis, leptospirosis, hepatitis A, and botulism). Notwithstanding attendant uncertainty (Figure 2), the most serious foodborne illness in Greece was brucellosis, representing ≈55% of the estimated DALY and contributing greatly to illness (>88%). Ill-defined intestinal infections were the second most serious contributor to disease burden (≈27% of DALY), followed by echinococcosis (7.8%) and salmonellosis (4.6%) as known causes of illness.

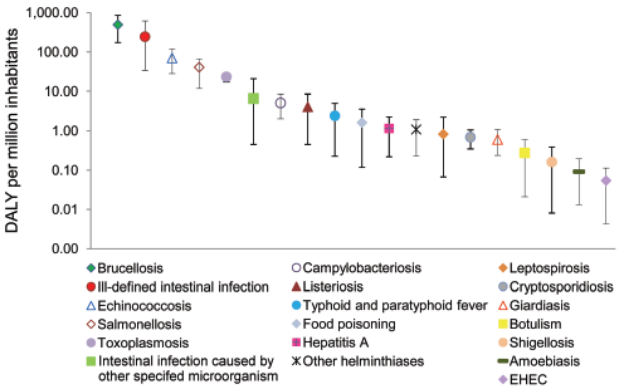

Figure 2. Disability-adjusted life years (DALY) caused by different foodborne diseases per million inhabitants in the course of an average year in Greece, including uncertainty. Estimates are presented on a logarithmic scale on the y-axis. Whiskers represent 95% credible intervals. EHEC, enterohemorrhagic *Escherichia coli*.

Discussion

The DALY metric provided a different view on the effects of foodborne illnesses on public health in comparison to incidence estimates (Table 5). Although salmonellosis was captured as a major contributor by all 4 rankings, there was variation regarding other causes of illness. Interestingly, diseases that have the highest effect on public health either in terms of illness (ill-

Table 4. Estimates of YLL, YLD, and DALY caused by foodborne illnesses in an average year in Greece per 1 million inhabitants, including plausible range attributable to uncertainty*

Illnesses	Estimated YLL (95% CrI)†	Estimated YLD (95% CrI)†	Estimated DALY (95% CrI)†
Bacterial			
Botulism	0.27 (0.021–0.67)	0.0066 (0.00056–0.015)	0.28 (0.021–0.69)
Brucellosis	**59 (21–121)**	**434 (140–856)**	**493 (174–943)**
Campylobacteriosis	1.2 (0.51–2.1)	3.9 (1.5–7.5)	5.14 (2.0–9.4)
Enterohemorrhagic *Escherichia coli*	0.016 (0.0012–0.039)	0.039 (0.0031–0.091)	0.054 (0.0043–0.13)
Leptospirosis	0.81 (0.066–2.7)	0.015 (0.0013–0.046)	0.83 (0.067–2.7)
Listeriosis	**4.1 (0.45–9.7)**	‡	4.1 (0.45–9.7)
Salmonellosis	**31 (8.7–55)**	**10 (2.9–19)**	**41 (12–72)**
Shigellosis	0.12 (0.060–0.34)	4.1 (0.0021–0.12)	0.16 (0.0081–0.46)
Typhoid and paratyphoid fever	2.3 (0.21–5.4)	0.17 (0.016–0.38)	2.4 (0.23–5.7)
Food poisoning	0.36 (0.022–0.98)	1.3 (0.088–3.3)	1.6 (0.12–4.1)
Parasitic			
Amebiasis	0.079 (0.011–0.20)	0.013 (0.0019–0.030)	0.092 (0.013–0.22)
Cryptosporidiosis	0.50 (0.20–0.88)	0.20 (0.10–0.32)	0.69 (0.35–1.2)
Echinococcosis	**16 (5.9–31)**	**54 (22–106)**	**70 (28–135)**
Giardiasis	0.12 (0.028–0.29)	0.48 (0.18–0.99)	0.61 (0.24–1.2)
Toxoplasmosis	**9.7 (7.0 –13)**	**14 (10–17)**	**23 (17–29)**
Other helminthiases	0.92 0.19–1.8)	0.17 (0.029–0.38)	1.1 (0.23–2.1)
Viral: hepatitis A	1.1 (0.20–2.4)	0.089 (0.018–0.19)	1.2 (0.22–2.6)
Mixed/ill-defined causes			
Intestinal infections caused by other specified microorganism	1.4 (0.12–3.6)	5.2 (0.26–18.0)	6.6 (0.45–21)
Ill-defined intestinal infections	1.2 (0.5–2.0)	**243 (33–621)**	**245 (34–622)**
Total of gastroenteritis§	43 (20–68)	265 (55–643)	308 (94–687)
Total	130 (81–196)	767 (361–1,308)	896 (470–1,461)

*Values have been rounded to include significant digits and thus not all summations necessarily tally. **Boldface** indicates the top 5 contributors to each estimate category. YLL, years of life lost; YLD, years lived with disability; DALY, disability-adjusted life years; CrI, credible interval.
†95% CrI representative of the 2.5 and 97.5 percentiles.
‡DALY due to listeriosis are mainly determined by the YLL (*23*); therefore, no YLD were estimated.
§Gastroenteritis-related illnesses are considered to be all of the above except: botulism, brucellosis, leptospirosis, echinococcosis, hepatitis A, and toxoplasmosis.

Table 5. Ranking of the top 5 causes contributing to the effects of foodborne illness in Greece as estimated on the basis of individual incidence parameters and disability-adjusted life years, 1996–2006

Rank	Incidence estimates			Disability-adjusted life years
	All foodborne illnesses	Reported/estimated illnesses	Deaths	
1	Ill-defined intestinal infections	Ill-defined intestinal infections	Brucellosis	Brucellosis
2	Intestinal infections due to other specified causes	Salmonellosis	Salmonellosis	Ill-defined intestinal infections
3	Food poisoning	Brucellosis	Echinococcosis	Echinococcosis
4	Salmonellosis	Food poisoning	Listeriosis	Salmonellosis
5	Campylobacteriosis	Echinococcosis	Toxoplasmosis	Toxoplasmosis

defined intestinal infections), death (toxoplasmosis) or both (brucellosis) are not identified in the ranking based on a single individual incidence parameter, but they are captured by DALY, which has the advantage of enabling comparisons between different disease endpoints. For instance, although toxoplasmosis is not among the 5 major contributors on the basis of the total incidence or on reported/estimated cases, it is given more prominence through using the DALY metric because this also accounts for severe outcomes and sequelae of this disease. Although self-limiting diseases may appear to be essential in terms of incidence, on the basis of DALY they do not greatly contribute to either illness or death. Therefore, use of the DALY metric gives a different and risk-based perspective of the influence of foodborne illnesses on the health of a country's population because it is estimated on the basis of the diseases' frequency (incidence) and severity (health effect).

Most of the foodborne illness cases in Greece were caused by ill-defined intestinal infections (Table 3). This finding is consistent with results from similar studies in other countries (*3*,*17*). Using the current Greek surveillance system, we cannot attribute this burden to known causes of gastroenteritis other than the ones included in this study. Noroviruses could be the etiologic agents in a large proportion of these ill-defined intestinal infections because they have been considered the most likely agent of foodborne illness caused by unknown agents (*26*) and have been found in other studies to be a most common cause of foodborne illness due to known agents (*17*,*18*). Outbreak data found for these pathogens were scarce (*27*) and therefore not included in this study. A considerable part of this category might also have been caused by other unknown agents of illness or known agents that have been misdiagnosed. For instance, campylobacteriosis is expected to be undiagnosed to a great extent in Greece because few laboratories in the country have the ability to identify the pathogen (*10*). This finding could partially explain the high underreporting factor estimated for this illness for Greece, based on the approach of Ekdahl and Giesecke (*28*) compared with results for other Western countries (*3*,*29*).

Brucellosis was found to be the leading cause of illness and death in Greece. Although its incidence showed a reasonably consistent decline during the period of this study, it still constitutes a serious public health problem (Figure 3). The disease is most common in rural areas of the country, and risk factors for its contraction are occupational contact with animals and the consumption of unpasteurized milk and milk products (*30*,*31*).

Echinococcosis was the second most notable foodborne illness. This disease has been recognized as a serious health problem in the country (*32*) and linked with contaminated food (*10*,*33*). Echinococcosis caused by *Echinococcus granulosus* (cystic echinococcosis) is the dominant form in Greece (*32*), where the infection is hyperendemic (*19*). Although its incidence has gradually decreased since 1984 as a result of a long anti-echinococcosis campaign and general improvements in living and hygiene standards (*32*), it still is a serious health risk for the population (Figure 3).

Salmonellosis was the third most serious foodborne illness of known etiology in terms of public health impact, and it also was the most prominent gastroenteritis-related illness of identified cause (Table 3). This finding is consistent with it being a noteworthy zoonosis, which contributes to a high prevalence of gastrointestinal illness in the European Union (*34*), and the most often reported causative agent of outbreaks of an identified etiologic agent worldwide (*35*).

After salmonellosis, congenital toxoplasmosis was also a major contributor to the disease burden, although in terms of incidence it is an uncommon illness with <4 cases per million inhabitants. The disease has not been

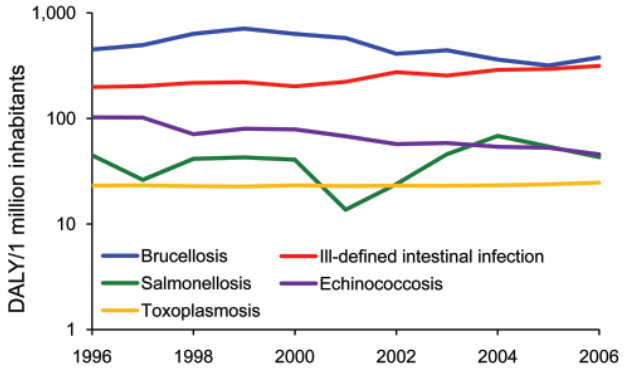

Figure 3. Trends for the top 5 contributors to the burden of foodborne diseases in Greece, 1996–2006. DALY, disability-adjusted life years.

recognized as a major foodborne illness in the country, although its serious health consequences have been well documented (*36*).

There are 4 major factors that add to the uncertainty in our estimates that are not independent: 1) underreporting, 2) food attribution, 3) the quality of incidence data, and 4) value choices in the DALY formula. Given the limited data available for Greece, data from other countries have been used to create multipliers for underreporting and foodborne transmission (online Technical Appendix); these data were of variable quality and representativeness. For instance, in the case of campylobacteriosis and salmonellosis, underreporting factors based on tourist studies (*28*,*37*) have been included in the multipliers, which were higher than underreporting factors from other Western countries for the same pathogens (*4*,*18*). Such underreporting factors might not be completely representative of the difference between reported cases resulting in physician visits and cases in the general population because these studies can be subject to several biases (e.g., tourists differ from natives in exposure) (*28*), although at the same time they cover for phenomena such as undernotification and misdiagnosis of illnesses that were beyond our intention. As a consequence of including data derived by using different method approaches, the plausible range of these multipliers was wide, which resulted in DALY estimates with similarly wide credible intervals (Figure 2). However, despite this limitation, our estimates can still be used for risk ranking purposes.

Uncertainty is also an inherent property of incidence data. Specifically, data for reported cases in Greece (and elsewhere) rely on insufficiently detailed codes, there is incomplete or lacking separate surveillance for many foodborne pathogens, and a specific diagnosis is not given for most episodes of enteric illness requiring hospitalization. These factors result in the greater part of reported cases of gastroenteritis being attributed to ill-defined causes. As with other studies of this kind, assumptions had to be made, notably considering the age of death. Although this assumption did not change the 5 major foodborne risks, it had considerable impact on the individual estimates. We also had to assume that serious cases of illness that have been reported because of a specific agent have been diagnosed and coded correctly or notified to the appropriate authorities. This assumption might not always be the case because at least some of these illnesses are expected to be part of the ill-defined illnesses. A correction for misdiagnosis and undernotification cannot be included for the reported illnesses until country specific data are available. Assigning an arbitrary factor as in other studies (*3*) introduces new uncertainties and, unlike incidence data in the case of DALY, can affect the ranking of foodborne risks. Thus, our estimates are based only on the illnesses that the surveillance system in Greece currently exposes, and the estimates' robustness can only be further improved through improved surveillance.

As for uncertainty resulting from value choices in the DALY formula itself, in the present study no age-weighting or discounting were used because their combined use has been criticized as attributing considerably fewer disease impacts and effects to younger age groups (*38*), and disability weights were carefully selected. For policy-making purposes, ideally, disability weights should be based on the opinion of the general public because they should reflect preferences of the society being studied (*21*). Conceivably, use of the DALY metric could help reduce a considerable part of overall uncertainty by accounting for sequelae, which are not normally taken into consideration in studies focusing solely on incidence of foodborne illness yet do constitute a substantial part of the overall effects on a population. In our study, all well-defined sequelae for which information existed in literature were used for DALY calculations, but our findings could be subject to change when new insights become publicly available. For instance, rates of posthospitalization morbidity related to gastrointestinal illnesses have not been taken into account in the absence of a specific study, although the duration of illness can be longer than the actual hospital stay.

Finally, selection of life tables is another factor that can influence the DALY estimates. When our estimates could be based on West Level 26 life tables, total burden of illness expressed as DALY increased by only 0.0042%, although individual estimates for illnesses could differ by up to 5.0% (results not shown).

Regarding the total incidence of foodborne illnesses, our estimates were in the same range as the estimates for Australia (Table 6), although somewhat higher because the study by Hall et al. was restricted to gastroenteritis-related foodborne illnesses (*17*). Our estimates of severe reported or estimated cases are between the range of hospitalization rates mentioned for different countries, and the same is the case for our case-fatality rates. Our DALY estimates were higher than estimates for the Netherlands (*7*) or New Zealand (*39*), although our estimated overall impact for gastrointestinal illnesses is still comparable to the one from the Netherlands where brucellosis is not a major foodborne risk.

Our finding that brucellosis, salmonellosis, echinococcosis, and toxoplasmosis together accounted for ≈70% of annual DALY means that these diseases might be major targets for policy making regarding appropriate food safety management actions, especially because their causative agents and likely transmission routes are generally known. Overall, the approach may be of interest to competent authorities in other countries requiring risk-based estimates ranking the impact of foodborne pathogens on public health to prioritize risk management actions.

Table 6. Comparison of foodborne illness effects on public health in Greece with estimates from other countries*

Country (reference)	Target	Disease estimates per 1 million inhabitants			
		All illnesses†	Hospitalizations	Deaths	DALY
United States (*3*)	All causes	270,057	1,155	18	NA
United States (*18*)	Known agents	31,438 (90% CrI 22,074–42,475)	187 (90% CrI 132–253)	5 (90% CrI 2–8)	NA
United States (*40*)	Unspecified agents	128,404 (90% CrI 66,318–204,670)	240 (90% CrI 33–526)	6 (90% CrI 1–11)	NA
England and Wales (*29*)	All causes	26,161	406	9	NA
Australia (*17*)	Gastro	281,250 (95% CrI 208,333–359,375)	766 (95% CrI 594–922)	4 (95% CrI 2–6)	NA
The Netherlands (*7*)	All causes	79,725–104,256	NA	1–12	184–613
New Zealand (*39*)	6 agents‡	128,421 (95% CrI 34,801–330,075)	NA	NA	632 (95% CrI 344–1,066)
Greece (this study)	All causes	369,305 (95% CrI 68,283–910,608)	905 (95% CrI 499–1,340)§	3.1 (95% CrI 2.0–4.8)	896 (95% CrI 470–1,461)
Greece (this study)	Gastro only	368,520 (95% CrI 67,536–909,457)	812 (95% CrI 408–1,245)§	0.95 (95% CrI 0.52–1.4)	308 (95% CrI 94–687)

*Data have been normalized for population differences and are expressed per million inhabitants. DALY, disability-adjusted life years; NA, not available; CrI, credible interval; gastro, gastroenteritis.
†Credible interval not available for all studies.
‡The study was limited to campylobacteriosis, salmonellosis, listeriosis, infection with Shiga toxin–producing *Escherichia coli*, yersiniosis, and infection with norovirus.
§The reported/estimated cases of severe illness in this study can be considered to be approximately the same as hospitalizations.

Acknowledgments

We thank Unilever for sponsoring this research, and the Hellenic Statistical Authority and the Hellenic Center of Infectious Diseases Control for providing us with data necessary for the completion of this study.

Ms Gkogka is a PhD student in the Laboratory of Food Microbiology of Wageningen University. She is interested in food safety management, risks caused by foodborne illnesses, and approaches for assessing risk-based decision making.

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Estimating Effect of Antiviral Drug Use during Pandemic (H1N1) 2009 Outbreak, United States

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From April 2009 through March 2010, during the pandemic (H1N1) 2009 outbreak, ≈8.2 million prescriptions for influenza neuraminidase-inhibiting antiviral drugs were filled in the United States. We estimated the number of hospitalizations likely averted due to use of these antiviral medications. After adjusting for prescriptions that were used for prophylaxis and personal stockpiles, as well as for patients who did not complete their drug regimen, we estimated the filled prescriptions prevented ≈8,400–12,600 hospitalizations (on the basis of median values). Approximately 60% of these prevented hospitalizations were among adults 18–64 years of age, with the remainder almost equally divided between children 0–17 years of age and adults ≥65 years of age. Public health officials should consider these estimates an indication of success of treating patients during the 2009 pandemic and a warning of the need for renewed planning to cope with the next pandemic.

From April 23, 2009, through April 10, 2010, it is estimated that pandemic (H1N1) 2009 virus caused ≈61 million cases of influenza (range 43–89 million cases), ≈270,000 related hospitalizations (range 195,000–403,000 hospitalizations), and ≈12,500 deaths (range 8,900–18,300 deaths) in the United States (*1*). Even before the impact was fully known, the Centers for Disease Control and Prevention (CDC) recommended prompt empiric treatment with influenza antiviral drugs, principally the neuraminidase-inhibiting influenza antiviral drugs oseltamivir and zanamivir, of persons with suspected or confirmed influenza and who also met ≥1 of the following conditions: 1) illness that required hospitalization; 2) progressive, severe, or complicated illness, regardless of previous health status; and 3) risk for severe disease (e.g., patients with asthma, neurologic and neurodevelopmental conditions; chronic lung or heart disease; blood, endocrine, kidney, liver, and metabolic disorders; pregnancy; and those who were old or young) (*2*). The primary goal of these recommendations was to reduce the number and severity of pandemic (H1N1) 2009 cases, especially hospitalizations.

We present estimates of the number of pandemic (H1N1) 2009–related hospitalizations, by age group, averted because of use of antiviral drugs given to treat clinical cases of influenza. These results can be used by public health policy makers to plan and prepare for the next pandemic. For example, these estimates can be used to help evaluate the policy option of replenishing state and federal influenza antiviral drug stockpiles

Methods

We developed a spreadsheet-based model to calculate the number of pandemic (H1N1) 2009–related hospitalizations averted because of treatment with the neuraminidase-inhibiting influenza antiviral drugs oseltamivir and zanamivir (online Technical Appendix, www.cdc.gov/EID/content/17/9/110295-Techapp.htm). The risk for hospitalization (and thus potential benefit from antiviral drugs) differed by age groups (*1*). Therefore, we estimated the reduced number of hospitalizations separately for 3 groups: persons 0–17 years of age, 18–64 years of age, and ≥65 years of age. We calculated the hospitalizations averted by using the following general equation: no. hospitalizations averted (by age group) = [no. prescriptions written – estimated no. written for prophylaxis, stockpiling, or incomplete adherence to drug regimen] × age group–specific risk for hospitalizations caused by pandemic

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DOI: http://dx.doi.org/10.3201/eid1709.110295

(H1N1) 2009 × age group–specific effectiveness of drugs in preventing hospitalizations.

Prescriptions Filled

We used the number of prescriptions filled for these drugs for weeks ending April 24, 2009, through March 26, 2010 (Table 1), collected from the IMS Health (IMS) Xponent proprietary prescription database (IMS Health, Norwalk, CT, USA) (*3*). This database contains all retail prescriptions filled from a representative sample of 35,000 (73%) of ≈50,000 US-based retail pharmacies, including independent pharmacies, chain pharmacies, pharmacies in discount outlets, pharmacies in food stores, mail order pharmacies, and pharmacy benefit management companies. IMS then proportionately extrapolates their data on the basis of populations served by the included pharmacies to provide weekly estimates of all prescriptions filled in the United States for these drugs. The Xponent database does not track prescriptions filled by in-hospital pharmacies. Therefore, in-hospital prescriptions are not part of our calculations.

The IMS Xponent database captures all filled prescriptions related to influenza antiviral drugs within its sample pharmacies. However, it does not identify the source of the drugs. During 2009, there were 2 main potential supplies for the antiviral drugs—the regular commercial supply system and state and federal government-maintained drug stockpiles. The IMS database does not track medications dispensed from public domains, such as public health departments. Furthermore, the federal and state stockpiles of antiviral drugs were meant to supplement the commercial supply

Table 1. Number of pandemic (H1N1) 2009 cases versus number of influenza antiviral prescriptions filled during pandemic (H1N1) 2009 outbreak, United States, April 24, 2009–March 26, 2010*

		Filled influenza antiviral prescriptions		
Week†	Mid-level estimate of cases‡	Oseltamivir	Zanamivir	Total
2009 Apr–Jul	3,052,768	1,243,827	69,513	1,313,340
2009 Aug	1,605,760	342,386	11,645	354,031
35	626,256	146,282	5,097	151,379
36	1,675,630	234,211	7,171	241,382
37	1,302,846	265,626	7,892	273,518
38	1,508,514	331,060	8,735	339,795
39	2,319,691	383,759	9,981	393,740
40	4,461,542	435,546	11,625	447,171
41	6,549,205	471,323	11,226	482,549
42	7,120,298	527,362	11,218	538,580
43	6,297,210	671,741	12,046	683,787
44	5,899,647	640,887	9,306	650,193
45	5,013,181	537,781	6,338	544,119
46	3,350,286	386,569	4,863	391,432
47	1,767,166	273,092	3,039	276,131
48	1,020,606	152,482	1,857	154,339
49	804,901	133,998	1,782	135,780
50	646,358	99,565	1,348	100,913
51	612,204	88,718	1,338	90,056
52	619,080	64,807	1,010	65,817
1	418,803	56,569	1,009	57,578
2	520,390	50,642	981	51,651
3	516,958	50,326	1,057	51,307
4	356,400	44,770	1,048	45,827
5	493,448	43,757	1,211	44,805
6	322,623	42,474	1,251	43,685
7	312,327	43,809	1,228	45,060
8	281,986	47,146	1,487	48,374
9	245,707	48,671	1,494	50,158
10	288,215	47,261	1,587	48,755
11	225,448	33,867	1,043	34,910
12	312,575	26,072	730	26,802
Total	60,548,030	7,966,386	211,156	8,177,542

*IMS Health Xponent database (3) includes 57,544 oseltamivir prescriptions and 877 zanamivir prescriptions for week 53. Because the Centers for Disease Control and Prevention only reports 52 weeks for 2009, we removed week 53 from the IMS data set (IMS Health, Norwalk, CT, USA).
†Estimates of cases for April–August 2009 are not available on a weekly basis.
‡Mid-level weekly cases estimated from (*1*) and www.cdc.gov/h1n1flu/estimates_2009_h1n1.htm.

chain in times of drug shortages anticipated to occur during a pandemic emergency.

As of August 2010, the estimated total amount of antiviral drugs managed by states throughout the pandemic was 38 million treatment regimens. This estimate includes antiviral drugs purchased by states (26 million treatment regimens) plus ≈12 million treatment regimens distributed early in the pandemic to states from the CDC Strategic National Stockpile (SNS). Preliminary reports from state public health departments to the CDC show that most SNS product was either retained by the health departments or deployed at the local level (to dispensing sites such as drug stores and health departments). Sites received directions that the SNS-provided supplies were to be dispensed if commercial supplies could not keep up with demand or used to treat uninsured or underinsured persons who could otherwise not afford treatment. Preliminary data reported to CDC through SNS show that minimum quantities of stockpiled antiviral drugs were actually dispensed to patients. Because the commercial supply chain for antiviral drugs remained relatively robust, most states did not need to use stockpiled antiviral drugs. Therefore, we did not include any estimates of impact on antiviral drugs dispensed from these government stockpiles.

Prescriptions by Age Group

IMS collects for filled prescriptions deidentified data regarding age of patient from the pharmacy systems. We thus divided the total number of prescriptions given into 3 age groups (0–17 years, 18–64 years, ≥65 years) by using age-specific data from IMS that covered prescriptions written for oseltamivir from October 9, 2009, through March 26, 2010. The percentages were as follows: 0–17 years, 38.6%; 18–64 years, 53.4%; ≥65 years, 5.3% (Table 2). Note that ≈3% of prescriptions filled during this period did not have the age of the patient recorded. Therefore, we did not include those prescriptions in our analysis.

Prescriptions over Time

We plotted the total number of prescriptions filled per week, from the IMS database, against the weekly number of estimated pandemic cases for April 24, 2009, through March 26, 2010. Estimates of cases for April through the end of July 2009 are not available on a weekly basis. Thus, all cases were combined into a single estimate for that period (*1*). We combined for the same period all filled prescriptions and directly compared cases and prescriptions. A notable divergence in the correlation between plots of cases and prescriptions over time would indicate the possibility of prescriptions being filled for reasons other than the immediate treatment of influenza-related illness (e.g., stockpiling or use for prophylaxis).

Percentage of Prescriptions Written for Prophylaxis

We assumed in the absence of any data that 10% of all prescriptions for these antiviral drugs were written for

Table 2. Input values used to estimate influenza antiviral drug–related reduction in hospitalizations during pandemic (H1N1) 2009 outbreak in the United States, April 24, 2009–March 26, 2010

Input	Initial value	Sources
Distribution of prescriptions by patient age group, y*		IMS Health Xponent database (*3*)
0–17	38.6%	
18–64	53.4%	
≥65	5.3%	
Prescriptions filled for prophylaxis†	10%	Assumption: Some prescriptions were written to prevent infection and disease without presentation of symptoms.
Prescriptions for patients who failed to adhere to drug regimen or used for personal stockpiles	20%	Assumption: Not all patients will adhere with the drug regimen as prescribed. Also, some prescriptions were for personal stockpiles
Antiviral drug effectiveness against hospitalization, by age group, y‡		Literature review (see Table 3)
0–17	22%–32%	
18–64	34%–50%	
≥65	30%–50%	
Median (range) risk for hospitalization, given pandemic (H1N1) 2009–related illness, by age group, y§		Reed et al. (*4*)
0–17	0.0038 (0.00314–0.00428)	
18–64	0.00496 (0.0041–0.00558)	
≥65	0.0155 (0.0128–0.0174)	

*Age group–based distribution of prescriptions based on IMS (IMS Health, Norwalk, CT, USA) that covered prescriptions written for oseltamivir (only) from October 9, 2009, through March 26, 2010.
†These inputs were subjected to sensitivity analyses (see Table 4).
‡Effectiveness estimate assumes that the patient follows the drug regimen, i.e., these estimates do not allow for those who do not take the complete course. Failure to follow prescribed drug regimen was assumed to have 0% effect on reducing risk of hospitalization. This assumption was accounted for in a separate input.
§Risk of per-person hospitalization, given symptomatic illness caused by pandemic (H1N1) 2009 virus.

prophylaxis. This assumption was subject to sensitivity analyses (described below). We further assumed that such prescriptions essentially had no impact on reduction of hospitalizations (Table 2).

Adherence to Drug Regimen and Stockpiling

We also assumed that a total of 20% of all prescriptions were for either personal stockpiles (i.e., not written for a clinically ill patient at time of prescription) or patients who did not sufficiently follow the recommended drug regimen so that the prescription had no impact on risk of hospitalization caused by nonadherence (Table 2). A study conducted in the United Kingdom during the (H1N1) 2009 pandemic found that 76%–80% of the patients did complete the full course of prescribed antiviral drugs (*5*). Another study among schoolchildren in London, UK, that examined adherence among those offered oseltamivir for prophylaxis found that 89% actually took ≥1 dose and 66%

Table 3. Literature review of effectiveness of neuraminidase inhibitors in preventing influenza-related hospitalizations*

Drug	Study type	Population	Reduction in hospitalization point estimate (95% CI)	Reference
Zanamivir	Randomized, double-blind, placebo-controlled trial	455 patients residing in Australia, New Zealand, and South Africa age ≥12 y with influenza-like symptoms of ≤36 hours' duration	NA	(*14*)
Oseltamivir	Open-label, multicenter international study	1,426 patients (age range 12–70 y) seeking treatment ≤48 h after onset of influenza symptoms	NA	(*15*)
Oseltamivir	Retrospective cohort analysis	The oseltamivir and untreated control groups each included 36,751 eligible patients	22%; HR 0.78 (0.67–0.91)	(*8*); claims data
Oseltamivir	Retrospective cohort study	Oseltamivir and untreated propensity matched control groups each included 45,751 eligible patients	30% any cause; OR 0.71 (0.62–0.83)	(*9*); insurance claims data
Zanamivir	Randomized, double-blind studies in 38 centers in North America and 32 centers in Europe during the 1994–95 influenza season	417 adults with influenza-like illness of ≤48 hours' duration were randomly assigned to 1 of 3 treatments	NA	(*16*)
Amantadine/ rimantadine	Two randomized, double-blind, placebo-controlled trials	≈80 patients with laboratory-documented influenza A virus (H3N2) illness ≤2 days' duration	NA	(*13*)
Oseltamivir	Combined analysis of 10 prospective, placebo controlled, double-blind trials	3,564 persons (age range 13–97 y) with influenza-like illness enrolled in 10 placebo-controlled, double-blind trials of oseltamivir treatment	59% any cause reduction; 50% influenza, at risk patients	(*7*)
Zanamivir	Retrospective pooled analysis of data; all studies were randomized, double-blind, and placebo-controlled with 21–28 day follow-up	2,751 patients were recruited; of these, 321 (12%) were considered high risk and 154 were randomized to receive zanamivir	NA	(*17*)
Zanamivir	Randomized, double-blind, placebo-controlled trial in primary care and hospital clinics	356 patients age ≥12 y were recruited within 2 d of onset of typical influenza symptoms	NA	(*12*)
Zanamivir	Pooled analyses of secondary endpoints		NA	(*18*)
Oseltamivir	Randomized controlled trial	726 healthy nonimmunized adults with febrile influenza-like illness of ≤36 hours' duration	NA	(*19*)
Oseltamivir	Retrospective cohort study	9,090 patients with diabetes and influenza	30% any cause; RR 0.70 (0.52–0.94)	(*10*); insurance claims data
Oseltamivir	Retrospective cohort study	The oseltamivir and untreated control groups each included 36,751 eligible patients, 50% with a claim for oseltamivir, 50% without	38%; RR 0.62 (0.52–0.74)	(*11*); insurance claims data
Oseltamivir	Double-blind, stratified, randomized, placebo-controlled, multicenter trial	Healthy adults (age range 18–65 y) who sought treatment ≤36 h after onset of influenza symptoms	NA	(*20*)
Oseltamivir	Randomized, double blind, placebo-controlled study	Children age 1–12 y with fever (≥100°F [≥38°C]) and a history of cough or coryza <48 hours' duration	NA	(*21*)

*CI, confidence interval; NA, not applicable; HR, hazard ratio; OR, odds ratio; RR, relative risk.

of this group completed (or said they would complete) a full 10-day prophylaxis course (*6*). One of the drug effectiveness studies that we reviewed (discussed below) and used for model input values asked patients to self-record adherence; it found that ≈90% of enrolled patients were fully compliant (*7*). Our assumption that 20% of prescriptions were for either stockpiling or nonadherence was subject to sensitivity analyses (described below).

This allowance for nonadherence also acts as a proxy for those who may have started the treatment too late. To maximize drug effectiveness in alleviating the duration of symptoms, it is recommended that antiviral drug treatment start <48 hours after onset of clinical symptoms (*2*).

Risk for Hospitalization Given Clinical Case of Pandemic (H1N1) 2009

We used the risk for hospitalization by age group, given clinical illness caused by pandemic (H1N1) 2009, from Reed et al. (*4*) (Table 2). We identified 17 published studies that evaluated the effectiveness of neuraminidase inhibitors given influenza-induced clinical illness (*7*,*8*–*21*; Table 3). Although many studies were random placebo-controlled trials, the studies did not use hospitalizations averted as a measured endpoint (*13*,*15*–*17*). We identified only 4 studies that specifically evaluated the impact of the antiviral drugs on risk for hospitalization, given clinical illness. One study provided an estimate of 50% reduction in the probability of influenza-specific hospitalizations (no confidence interval was published) (*7*). Three retrospective studies, using health insurance claims data, reported effectiveness in reducing hospitalizations (any cause) that ranged from 22% to 59%, with some variation by age (*8*–*10*). For each age group, we used lower and upper estimates of effectiveness, from a lower estimate of 22% reduction for children 0–17 years to an upper estimate of 50% for adults (Table 2).

Calculating Ranges and Sensitivity Analyses

For each level of antiviral effectiveness (lower, upper), and for each age group, we calculated the median and lower and upper estimates of hospitalizations averted. We also conducted sensitivity analyses by altering from 0% to 30% the assumed percentages of prescriptions written for prophylaxis, personal stockpiles, and patients who did not adhere to the drug regimen.

Results

Pandemic influenza vaccine became available in week 40 of 2009 (near the peak of cases). We hypothesized that before this date is when doctors would have been most likely to try to protect patients by prescribing prophylactic courses of antiviral drugs. However, the plot of the prescription data against estimated cases over time shows a close correlation between the occurrence of pandemic (H1N1) 2009 clinical cases and filled prescriptions (Table 1; Figure). This comparison suggests that antiviral drugs were mostly prescribed to treat the occurrence of clinical cases of pandemic (H1N1) 2009.

The total number of prescriptions filled before adjustments was 8.2 million (Table 1). After removing the prescriptions presumed filled for prophylaxis and for patients who failed to adhere to the drug regimen or had prescriptions filled for personal stockpiles, 5.7 million prescriptions were filled that may have reduced hospitalizations (Table 4). Most (97%) were filled for oseltamivir, and ≈55% of all prescriptions filled were for persons 18–64 years of age, and ≈40% were filled for children 0–17 years of age.

We estimated that the median number of hospitalizations averted ranged from 8,427 (lower 6,961; upper 9,479) to 12,641 (lower 10,442; upper 14,219) (Table 5). Approximately 60% of averted hospitalizations were among persons 18–64 years old. The estimated hospitalizations averted in children and adults ≥65 years of age (Table 5) were similar. Although adults ≥65 years of age received only ≈5% of filled prescriptions (Table 4), these prescriptions had a relatively substantial impact in averting hospitalizations because the risk for hospitalization is higher in this age group than the other risk groups (Table 2).

Doubling the assumed percentages of filled prescriptions for prophylaxis and personal stockpiles/

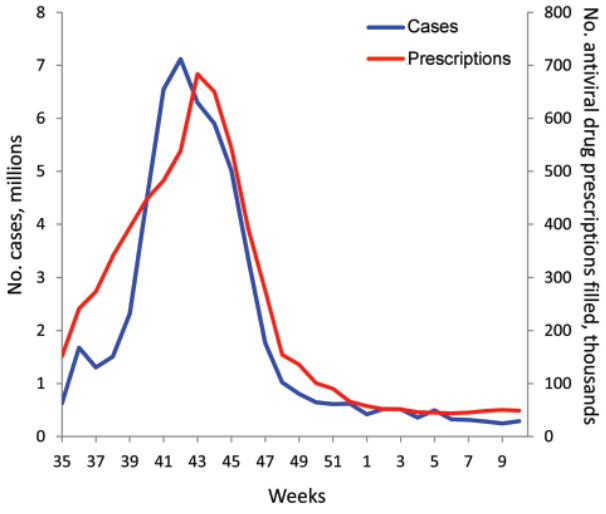

Figure. Number of estimated influenza cases and filled prescriptions for influenza antiviral drugs during pandemic (H1N1) 2009 in the United States, September 2009–March 2010. The estimates of cases for April–August 2009 are not available on a weekly basis. During April 12–July 23, 2009, there were 3.1 million cases and 1.3 million prescriptions filled for influenza antiviral drugs. For the month of August 2009, there were 1.6 million cases and 354,000 prescriptions filled for influenza antiviral drugs. Estimates of cases from Shrestha et al. (*1*); number of prescriptions filled from the IMS Health Xponent database (*3*).

Table 4. Estimated number of influenza antiviral drugs prescribed for treatment, after adjusting for prescriptions for prophylaxis, nonadherence, and personal stockpiling, pandemic (H1N1) 2009 outbreak, United States

Influenza antiviral drug*	No. prescriptions, by patient age group†			Total
	0–17 y	18–64 y	≥65 y	
Oseltamivir	2,152,915	2,979,711	297,700	5,430,326
Zanamivir	57,065	78,980	7,891	143,936
Subtotal‡	2,209,980	3,058,690	305,591	5,574,262

*These antiviral drugs were prescribed in a variety of forms (e.g., capsules, tablets, syrup, and inhaled powder). The estimated numbers came from the IMS database (*3*), which records ≈73% of all prescriptions filled by >50,000 US-based retail pharmacies. IMS then proportionately extrapolates their data, based on populations served by pharmacies, to provide weekly estimates of all prescriptions filled in the U.S. for these drugs. The IMS Health Xponent database does not cover in-hospital prescriptions.
†These subtotals, by age group, are the estimates of prescriptions filled to treat pandemic (H1N1) 2009–related clinical illness, after removing the prescriptions filled for prophylaxis and for patients who failed to adhere to drug regimen or prescriptions filled for personal stockpiles (see Table 1). The total number of prescriptions filled, before adjustments, was 8,177,542 (Table 1). Note that ≈3% of prescriptions filled during this period did not have age of patient recorded, and we omitted those prescriptions from our calculations.
‡These subtotals, by age group, were the estimates used to calculate the hospitalizations averted as shown in Table 5.

nonadherence from 30% to 60% (i.e., a 100% increase) produced only a 40% reduction in median hospitalizations averted, from ≈12,600 to 7,200 (Table 6). Thus, the major factors influencing hospitalizations averted were total prescriptions filled and (assumed) effectiveness of the drugs in preventing hospitalizations.

Discussion

The close correlation between estimated pandemic influenza cases and filled prescriptions (Figure) can be used as evidence that antiviral drugs were mostly used to treat those who were clinically ill (i.e., recommendations regarding use were essentially followed). Restricting the use of antiviral drugs to treating the clinically ill meant that preventing clinical cases from deteriorating into severe cases requiring hospitalizations was likely to have been among the major effects of antiviral drug use. By our estimates, this strategy worked; ≈8,000–13,000 hospitalizations were averted (Table 5). This reduction is equivalent to ≈4–5% of the total estimated pandemic (H1N1) 2009–related hospitalizations (*1*).

We found no other studies with which to compare our methods and results. We compared the accuracy of the IMS database using unpublished data from the Behavioral Risk Factor Surveillance System (BRFSS), conducted in 49 states (excluding Vermont, the District of Columbia, and Puerto Rico). From September 1, 2009, through March 31, 2010, adults (≥18 years old) responding to the BRFSS telephone survey were asked whether they had influenza-like illness (ILI) (defined as having had a fever with cough or sore throat) in the month preceding the interview. They were also asked if they sought medical care for their ILI condition and if they were prescribed antiviral drugs to treat their illnesses. Extrapolating the results to the national level in the period covered by the survey, we found that ≈54 million adults reported having ILI symptoms. Of those who reported having ILI and sought medical care, 4.1 million adults reported they were prescribed influenza antiviral drugs (oseltamivir or zanamivir) during August 2009–March 2010. The IMS database recorded 6.86 million prescriptions in the same period (Table 1); ≈40% for those 0–17 years of age (Table 2), leaving ≈4.1 million filled prescriptions for adults. This estimate is close to the number recorded by the BRFSS survey and further supports the idea that few prescriptions were for prophylaxis or personal stockpiles.

There are many limitations to this study; the biggest is the uncertainty regarding the effectiveness of the drugs in preventing hospitalizations. The effectiveness of the drugs in reducing risk for hospitalization caused by pandemic (H1N1) 2009 may vary considerably from estimates reported for nonpandemic strains of influenza virus. The data are also limited in that we cannot verify if those persons who filled a prescription were actually clinically ill from pandemic (H1N1) 2009 or to what extent they adhered to the drug regimen. We addressed this issue by allowing a wide range in drug effectiveness and a relatively large percentage of prescriptions filled for conditions other than direct treatment of pandemic (H1N1) 2009.

We were unable, because the available literature did not contain sufficiently reliable estimates of effectiveness of antiviral drugs against death, to estimate the number of deaths averted by treatment with antiviral drugs. Shrestha et al. (*1*) estimated that deaths caused by pandemic (H1N1)

Table 5. Estimates of hospitalizations averted, by age group, assuming lower and upper estimates of influenza antiviral drug effectiveness, United States, 2009–2010*

Drug effectiveness estimate	No. hospitalizations averted, by patient age group, y, median (range)			
	0–17	18–64	≥65	Total
Lower	1,848 (1,527–2,081)	5,158 (4,264–5,803)	1,421 (1,171–1,595)	8,427 (6,961–9,479)
Upper	2,687 (2,221–3,027)	7,586 (6,270–8,534)	2,368 (1,951–2,659)	12,641 (10,442–14,219)

*Estimates of antiviral drug effectiveness are shown Table 2 (source, Table 1). Lower, median, and upper estimates are generated by using the range of age-specific probabilities of hospitalization, given influenza-related clinical illness (Table 2).

Table 6. Sensitivity analysis, altering the assumed percentage of prescriptions written for prophylaxis, nonadherence to drug regimen, and stockpiling, United States 2009–2010*

% Prescriptions written for prophylaxis	% Prescriptions resulting in nonadherence + stockpiling	Net no. prescriptions used to treat clinically diagnosed influenza	Median no. hospitalizations averted, by patient age group, y†			
			0–17	18–64	≥65	Total
0	0	8,177,542	3,839	10,837	3,383	18,059
10	10	6,542,034	3,071	8,669	2,707	14,447
>10	>20	5,724,279	2,687	7,586	2,368	12,641
20	20	4,906,525	2,303	6,502	2,030	10,835
20	30	4,088,771	1,920	5,418	1,692	9,030
30	30	3,271,017	1,536	4,335	1,353	7,224

*Baseline data used displays 10% for prophylaxis and 20% for personal stockpiling and non-adherence. This baseline assumption was used to generate results in Table 5.
†Results of sensitivity analysis were calculated by using the upper median estimates of antiviral effectiveness in preventing hospitalization among the clinically ill (Tables 1, 2).

2009 were equivalent to 1.5% of children's hospitalizations and 6% of hospitalizations for persons of all other ages. Assuming that hospitalizations averted generate similar percentages of deaths averted, then the use of antiviral drugs prevented 27–40 deaths in children 0–17 years of age and 395–597 deaths in adults of all ages (using median values of hospitalizations averted; Table 4).

If during the next pandemic there is a desire to produce better quality estimates (perhaps even produce estimates at regular intervals during the event), then additional data collection systems must be developed to overcome some of these limitations. For example, measuring the number of prescriptions filled for prophylaxis or personal stockpiles or degree of adherence can only reliably be conducted by interviewing patients and physicians. Improving estimates of impact of filled prescriptions in reducing adverse health outcomes during an event will require a large case–control study. Policy makers will have to determine if the value of such information warrants the investment in such data collection systems.

Our results also highlight how the use of influenza antiviral drugs during a pandemic is likely to be beneficial, notably through a presumed reduction in the demand for hospital-based resources. Reduced demand will also reduce costs of hospitalizations. Assuming a cost per influenza-related hospitalization of US$5,000–$7,000 per patient admitted (adjusted to 2009 dollars) (*22*–*26*), averted hospitalizations saved ≈$42 million to $88 million (based on median values of hospitalizations averted; Table 4). A detailed cost-effectiveness analysis, including an in-depth consideration of the costs of hospitalizing pandemic (H1N1) 2009 patients, is the subject of a separate analysis.

If the next influenza pandemic causes greater numbers of severe cases and hospitalizations than in 2009, there may be an increased demand for antiviral drugs for treatment and prophylaxis. Such increased demand could overwhelm the existing commercial distribution chains. Therefore, public health officials should consider these estimates as an indication of success of treating patients during the 2009 pandemic and a warning for the need for renewed planning to cope with the next pandemic.

Acknowledgments

We thank Alicia Fry for her thoughtful and helpful contributions.

Ms Atkins is a research assistant at the Centers for Disease Control and Prevention. She is assisting with the development and revisions of several tools that will assist state and local public health organizations with pandemic influenza planning needs.

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Intrahousehold Transmission of Pandemic (H1N1) 2009 Virus, Victoria, Australia

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To examine intrahousehold secondary transmission of pandemic (H1N1) 2009 virus in households in Victoria, Australia, we conducted a retrospective cross-sectional study in late 2009. We randomly selected case-patients reported during May–June 2009 and their household contacts. Information collected included household characteristics, use of prevention and control measures, and signs and symptoms. Secondary cases were defined as influenza-like illness in household contacts within the specified period. Secondary transmission was identified for 18 of 122 susceptible household contacts. To identify independent predictors of secondary transmission, we developed a model. Risk factors were concurrent quarantine with the household index case-patient, and a protective factor was antiviral prophylaxis. These findings show that timely provision of antiviral prophylaxis to household contacts, particularly when household members are concurrently quarantined during implementation of pandemic management strategies, delays or contains community transmission of pandemic (H1N1) 2009 virus.

Households play a major role in secondary transmission of pandemic influenza. Modeling estimates that household transmission has accounted for 25%–40% of all pandemic (H1N1) 2009 cases (*1*,*2*). Although understanding the effect of individual-level and household-level factors on secondary transmission of pandemic (H1N1) 2009 is paramount to informing population-level prevention strategies, few studies have evaluated household-level risk factors (*3*–*8*).

The Australian Health Management Plan for Pandemic Influenza (AHMPPI), revised in 2008, provides a framework for preparedness and response to pandemic influenza (*9*). The emergence and magnitude of pandemic (H1N1) 2009 in Melbourne, Australia (*10*–*15*), coupled with intensive follow-up and case identification data collected during the delay and contain phases of the AHMPPI (*16*), presented a unique opportunity to characterize intrahousehold transmission during a period of community transmission. Introduction of a suite of prevention and control measures in accordance with AHMPPI also provided an opportunity to measure the effects of these interventions on pandemic (H1N1) 2009 virus transmission.

We therefore conducted a retrospective cross-sectional study of index case-patients and their household contacts in Melbourne (population >3.5 million), Australia (*17*). We examined transmission of pandemic (H1N1) 2009 in households, identified possible risk factors for intrahousehold secondary transmission, and assessed the effects of prevention and control measures introduced to limit transmission.

Methods

Participants

The sample population consisted of all persons with confirmed cases of pandemic (H1N1) 2009 reported to the

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DOI: http://dx.doi.org/10.3201/eid1709.101948

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Victorian Department of Health (VDOH) during the delay and contain phases of AHMPPI (May 18–June 3, 2009) from 2 neighboring municipal regions in Melbourne with high numbers of pandemic (H1N1) 2009 notifications. To ensure that only the first reported case in a household could be randomly selected, we flagged households with >1 confirmed case. The index case-patient and household contacts were then recruited by mail and telephone (up to 5 calls were attempted). Of those who could be contacted, we assessed the household's eligibility according to the Australian Bureau of Statistics definition of a family (households of ≥2 persons residing together, including at least 1 person <18 years of age, related by blood, marriage, de facto, adoption, or fostering) (*18*).

Data Collection

During November 18–December 21, 2009, interviewers administered questionnaires to index case-patients and their household contacts. Data collected included demographics, case details, and prevention and control measures used. Participants indicated dates of symptom onset and prevention and control measures used in a retrospective diary of the period of interest (May 11–June 14, 2009). Interpreters were used as requested or needed. A parent or guardian was also interviewed when a participant was <18 years of age. If a household member was not available, a parent, guardian, or partner provided information. Written informed consent was obtained for all participants; parents or legal guardians provided written informed consent for participants <18 years of age.

Definitions

Index case-patients were defined as patients with the first laboratory-confirmed case of pandemic (H1N1) 2009 in a household reported to the VDOH. Household contacts were defined as persons residing in the same household at the time of the index case-patient's symptom onset.

Cultural and linguistic diversity was defined as speaking English only or speaking languages other than English in the home. The latter category included those households in which English was a second language.

A secondary case-patient was defined as a household contact who met the case definition for having an influenza-like illness (ILI), defined as self-described fever plus chills and/or respiratory tract signs or symptoms such as cough, sore throat, or shortness of breath with onset 1–9 days after onset for the index case-patient. This interval was based on a serial interval (the number of days between symptom onset in the index case-patient and household contacts) of up to 9 days to identify secondary cases, given that shedding of seasonal influenza virus rarely lasts >8 days (*7*,*19*) and a median incubation period for seasonal influenza of ≈1.4 days (*7*,*20*). Secondary cases were not required to be laboratory confirmed. Household contacts who met our definition for having ILI but who reported symptom onset on the same day as or before that of the index case-patient were not considered to be at risk for secondary transmission and were not included in analysis for exposures associated with secondary transmission.

Use of antiviral drugs (treatment or prophylaxis) was self-reported. VDOH provided antiviral treatment to those who met the case definition (confirmed or suspected case) and whose symptom onset was within 48 hours and provided antiviral prophylaxis to household contacts. Quarantine was self-reported and defined as separation and restriction of movement of case-patients and contacts in their homes (*21*). During the contain phase, patients with confirmed cases were advised to quarantine themselves for 7 days after symptom onset, and contacts were advised to quarantine themselves at home for 7 days after the most recent exposure to an infectious case-patient. A case-patient was considered infectious for 7 days after symptom onset or until acute respiratory symptoms resolved, whichever was longer (*21*).

Analysis

Chi-square tests were used to determine differences in clinical signs and use of prevention and control measures between index case-patients and household contacts. The Fisher exact test statistic, used to determine nonrandom associations between 2 categorical variables, was used when the expected value was <6. Secondary attack rates (SARs) were calculated by dividing the number of secondary cases by the total number of susceptible household contacts. We stratified SARs for several potential predictors, including individual-level factors, prevention and control measures, and household-level factors. Potential predictors included gender, age group (0–4, 5–19, 20–49, ≥50 years), relationship to index case-patient (parent/child, sibling, partner, other family member, or other), use of antiviral drugs (treatment or prophylaxis), number of days quarantined with index case-patient, household size (2–3, 4–5, ≥6 persons), number of children living in the household (1, 2, ≥3 children), and cultural and linguistic diversity (English only spoken at home and English and/or other languages spoken at home).

Unadjusted logistic regression was used to identify significant candidate predictors ($p<0.05$) for inclusion in the final adjusted model. The final model used reverse stepwise selection procedures in which all significant predictors of secondary transmission were included in the initial model and removed sequentially until only significant predictors ($p<0.05$) remained. We accounted for household clustering in the unadjusted and adjusted logistic regression models; that is, we adjusted for dependency of all potential predictors based on

membership in the same household by using a generalized estimated equation with robust error estimates, assuming conditional independence within each family (i.e., within the family, each member had independent probability of becoming a case-patient). Goodness of fit for both models was assessed by using the Hosmer–Lemeshow test to 0.05 significance. Statistical analyses were conducted by using Stata version 10 (StataCorp LP, College Station, TX, USA). To indicate precision of the measurement, we have reported 3 significant (i.e., nonzero) figures.

Ethical Considerations

Participants were reimbursed with $A30. Ethical approval was obtained from the Alfred Hospital Ethics Committee and Australian National University Ethics Committee.

Results

Participation and Response Rates

Data extracted on October 20, 2009, contained records for 857 confirmed cases of pandemic (H1N1) 2009, representing 772 households, reported on or before June 3, 2009, including a total of 181 cases for persons residing in the selected municipalities. We then randomly selected 72 case-patients to participate in this study, of which 12 refused, 21 could not be contacted, and 3 did not meet eligibility requirements; the remaining 36 index case-patients and their 131 household contacts participated. Participating and nonparticipating index case-patients were similar in age and student status; however, more nonparticipating (n = 4) than participating (n = 2) index case-patients required an interpreter. Among the 36 households that participated in the study, 32 (88.9%) persons were interviewed face to face and 4 (11.1%) were interviewed by telephone. Interpreters were used for interviews in 2 households.

Participant Characteristics

The analysis included 36 index case-patients and 131 household contacts (Table 1). The age range of index case-patients was 6–47 years; that of household contacts was 1–74 years. The number of persons living in each household was 2–14, median 4.5 persons. The number of children living in each household was 1–7; most (75.0%) households had 1–2 children. In half of the households (n = 18), a language other than English was spoken at home.

Prevention and Control Measures

Antiviral treatment was taken by 30.6% of index case-patients and 4.58% of all household contacts (Table 2). Just under half (45.8%) of all household contacts reported taking antiviral prophylaxis; and among those who did, 1 person reported subsequent symptoms consistent with ILI. The proportion of index case-patients and household contacts who reported being quarantined differed significantly (88.9% and 69.5%, respectively, p = 0.013).

The median number of days to initiate quarantine was 3 days for index case-patients and 4 days for household contacts. Greater than half (61.1%) of household contacts reported concurrent quarantine with the index case-patient for at least 1 day; the range of concurrent quarantine was 1–15 days, median 4 days.

The median number of days before antiviral treatment was initiated for index case-patients and household contacts was 2 days (Figure 1). The median number of days before antiviral prophylaxis was initiated among household contacts was 6 days.

Clinical Features

Among 131 household contacts, 122 (93.1%) were considered to be at risk for secondary transmission. Among these, 18 reported symptoms consistent with ILI within 1–9 days of symptom onset for the index case-patient and were thus considered secondary case-patients (Figure 2). Household contacts who reported symptom onset before the index case-patient (n = 5), on the same day as the index case-patient (n = 4), or >9 days after onset of symptoms in the index case-patient (n = 3) were not considered to be secondary case-patients and were not included in analyses. The serial interval for secondary cases included in the analysis was 1–9 days, median 2 days.

With the exception of vomiting, clinical features reported by index and secondary case-patients did not differ significantly (range p = 0.275–0.667, Table 3). The most frequent duration of symptoms for index and secondary case-patients was 4–6 days; 31.3% and 37.0% of index and secondary case-patients, respectively, reported symptom duration within this range. Approximately three fourths (77.8%) of secondary case-patients sought medical care (p = 0.01). Prevention or control measures used by index case-patients and secondary case-patients did not differ significantly (quarantine p = 0.429, antiviral prophylaxis p = 0.429, antiviral treatment p = 0.095)

Secondary Transmission

The overall SAR in this study was 14.8% (95% confidence interval [CI] 8.90%–22.3%, Table 4). The SAR varied when stratified for different individual-level and household-level factors. In unadjusted analysis, predictors of intrahousehold secondary transmission were being female, concurrent quarantine with the index case-patient, and use of antiviral prophylaxis (Table 5). We did not find a significant association between secondary case-patients and age group, relationship to the index case, household size, number of children living in the household, or cultural

Table 1. Characteristics of pandemic (H1N1) 2009 case-patients and household contacts, Victoria, Australia, May 18–June 3, 2009*

Characteristic	No. (%) index case-patients, n = 36	No. (%) household contacts, n = 131	p value
Individual level			
Sex			
M	25 (69.4)	69 (52.7)	0.07
F	11 (30.6)	62 (47.3)	
Age, y			
0–4	0	13 (9.92)	<0.001
5–19	31 (86.1)	40 (30.5)	
20–49	5 (13.9)	68 (51.9)	
≥50	0	10 (7.63)	
Household level		NA	NA
No. persons			
2–3	5 (13.9)		
4–5	22 (61.1)		
≥6	9 (25.0)		
No. children		NA	NA
1	12 (33.3)		
2	15 (41.7)		
≥3	9 (25.0)		
Cultural and linguistic diversity		NA	NA
English only spoken at home	18 (50.0)		
English and/or other language(s) spoken at home	18 (50.0)		

*NA, not applicable.

and linguistic diversity. In the adjusted analysis, p value for gender decreased from 0.037 to 0.83 and was thus removed from the final model. In the final model, the odds of a household contact who was concurrently quarantined with the index case-patient becoming a secondary case-patient increased for each additional day (adjusted odds ratio 1.25, 95% CI 1.06–1.47), and the odds of secondary transmission among household contacts who reported use of antiviral prophylaxis decreased (adjusted odds ratio 0.042, 95% CI 0.004–0.434). We did not identify a significant interaction term to include in the multivariate model.

Discussion

This study fully characterizes transmission of pandemic (H1N1) 2009 in households in Australia during implementation of pandemic management strategies to delay or contain community transmission. The findings are relevant for prevention and control strategies used at the household level indicated in the AHMPPI and for international pandemic influenza planning. Overall, 14.8% of susceptible household contacts became secondary case-patients, assumed to have been infected by the index case-patient. The SAR for ILI observed in this study is within the range of reported SARs for ILI used as a proxy for pandemic (H1N1) 2009 in similar international studies, which were 3.7%– 45% (*4–8*,*22–27*).

The odds of seeking medical care were lower for secondary than for index case-patients. Although this finding was expected because of the case ascertainment methods used, other factors involved with health care–seeking behavior should be considered. For example, household contacts may have not sought care because VDOH provided antiviral treatment and prophylaxis to household contacts without requiring evidence of laboratory-confirmed disease. Furthermore, symptomatic household contacts may have reasonably assumed that they were infected with pandemic (H1N1) 2009 given their proximity to a confirmed case-patient and may not

Table 2. Prevention and control measures used by pandemic (H1N1) 2009 case-patients and household contacts, Victoria, Australia, May 18–June 3, 2009*

Reported measure	No. (%) index case-patients, n = 36	No. (%) household contacts, n = 131	p value†
Antiviral			
Treatment	11 (30.6)	6 (4.58)	<0.001
Prophylaxis	0	60 (45.8)	<0.001
Quarantine duration, d			
≥1	32 (88.9)	91 (69.5)	0.013
≥1 with index case-patient	NA	80 (61.1)	

*NA, not applicable.
†Fisher exact test statistic used when expected value <6.

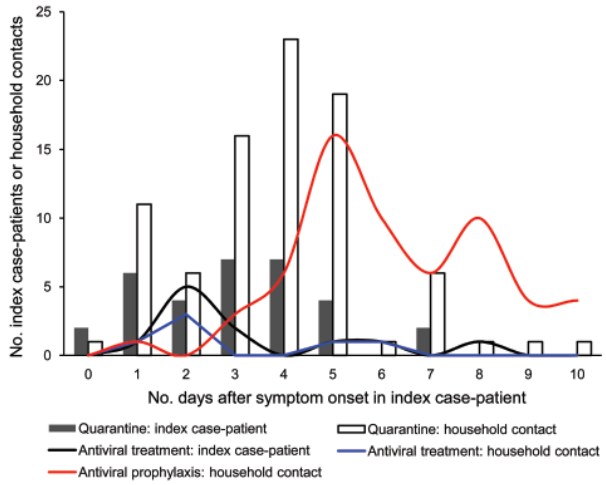

Figure 1. Timeliness of quarantine initiation and administration of antiviral (treatment and prophylaxis) by pandemic (H1N1) 2009 index case-patients and household contacts after onset of symptoms in the index case-patients, Melbourne, Victoria, Australia, May 18–June 3, 2009.

have considered confirmation necessary. The differences in health care–seeking behavior have implications for the pandemic influenza response, particularly during the phases of the AHMPPI when emphasis is on active case finding and slowing community transmission. This finding highlights the need for timely household-level, rather than individual-level, provision of treatment and prevention strategies by health care professionals, at the point of care of the index case-patient.

Several individual-level and household-level factors influenced the SAR and the odds of secondary transmission within households. The odds of becoming a secondary case-patient were almost 3× greater for female than male contacts, possibly because more women assume caregiver roles and therefore having a greater likelihood of exposure. This explanation is supported by France et al. (*4*), who reported that providing care to a case-patient was associated with a higher risk for ILI among parents. A study with greater power may be able to demonstrate this association in adjusted analyses. Other studies have also reported findings that older age was protective against secondary transmission of pandemic (H1N1) 2009, possibly as a result of prior immunity in older age groups (*4*,*5*). Although a decreasing trend of secondary transmission was observed for participants 5–19 years to 20–49 years of age, the size of this study was insufficiently powered to demonstrate a significant association between age group and rate of secondary transmission.

Our finding that antiviral prophylaxis reduced the odds of secondary transmission by 95% among at-risk household contacts was greater than that reported by France et al., who reported a 68% reduction in risk (*4*). Although this finding highlights the potential for antiviral prophylaxis to prevent secondary transmission, it should be considered along with the finding that initiation of antiviral treatment and prophylaxis for index case-patients and household contacts was considerably delayed. Current evidence highlights that rapid implementation of prevention measures such as antiviral prophylaxis is critical for control of pandemic influenza as soon as community transmission is identified; our findings identify an area for improvement in the implementation of pandemic influenza management plans. For example, the need for timely use of antiviral prophylaxis was demonstrated by Donnelly et al., who found that only 18% of pandemic influenza transmission events take place >2 days after onset of symptoms in case-patients (*28*). Ghani et al. also demonstrated this need when they reported a 3-fold increase in odds of intrahousehold secondary transmission in households that did not receive antiviral prophylaxis within 3 days of index case-patient symptom onset (*2*). Similarly, Goldstein et al. report that early antiviral treatment (on the day of or day after symptom onset) reduced the odds of household secondary transmission by 42% (*29*).

The issue of timeliness was also identified with regard to initiation of quarantine. We identified a considerable delay between onset of symptoms in the index case-patient and initiation of quarantine for index case-patients and household contacts, thus prolonging community exposure to pandemic (H1N1) 2009. Quarantine of case-patients and close contacts is considered an essential strategy for mitigating community transmission of pandemic influenza (*9*); however, to reduce the rate of community transmission, case-patients need to be quarantined as early as possible during their infectious period.

Although quarantine has been demonstrated to be effective at reducing community attack rates in pandemic influenza modeling studies, it has been hypothesized

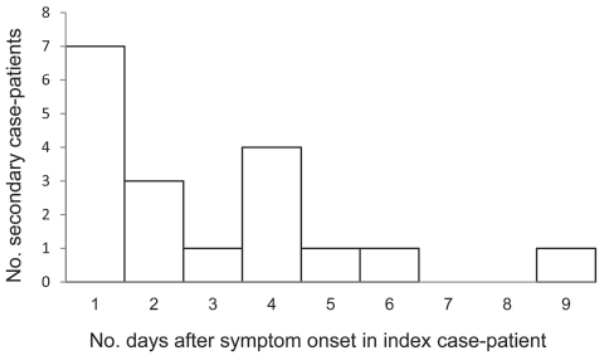

Figure 2. Serial interval for symptom onset in pandemic (H1N1) 2009 index case-patient to symptom onset in secondary case-patients, Melbourne, Victoria, Australia, May 18–June 3, 2009.

Table 3. Clinical features for pandemic (H1N1) 2009 case-patients and household contacts, Victoria, Australia, May 18–June 3, 2009

Feature	No. (%) index case-patients, n = 36	No. (%) secondary case-patients, n = 18	p value*
Sign or symptom			
Fever	35 (97.2)	18 (100)	0.67
Chills	17 (47.2)	8 (44.4)	0.54
Headache	25 (69.4)	13 (72.2)	0.55
Muscle pain	20 (55.6)	8 (44.4)	0.32
Joint pain	15 (41.7)	7 (38.9)	0.54
Fatigue	30 (83.3)	16 (88.9)	0.46
Diarrhea	8 (22.2)	2 (11.1)	0.28
Vomiting	16 (44.4)	2 (11.1)	0.01
Upper respiratory tract symptoms	32 (88.9)	17 (94.4)	0.45
Sign or symptom duration, d			
1–3	9 (25.0)	2 (11.2)	0.49
4–6	13 (36.1)	9 (50.0)	
7–9	9 (25.1)	3 (16.7)	
≥10	5 (13.8)	4 (22.2)	
Any medical care received	36 (100)	14 (77.8)	0.01
Reported prevention and control measures taken			
Quarantine	32 (88.9)	15 (83.3)	0.43
Antiviral prophylaxis	0	1 (5.56)	0.43
Antiviral treatment	11 (33.3)	2 (11.1)	0.10

*Fisher exact test statistic used when expected value was <6.

that the subsequent increase in contact rates between household members during quarantine may increase intrahousehold transmission (*30*). We found evidence supporting this hypothesis, demonstrating that the odds of secondary transmission increased >20% for each additional day of quarantine with the index case-patient. Similar effects of quarantine on intrahousehold secondary attack rates have not been reported for pandemic (H1N1) 2009; however, a study of university students in the People's Republic of China found an increased attack rate among contacts who shared a room or bathroom with confirmed pandemic (H1N1) 2009 case-patients (*31*), and a study in New York reported increased risk between siblings who interacted closely with the index case-patient (*4*). Thus, to prevent community transmission, effective communication to confirmed case-patients as well as their household contacts to ensure timely implementation of quarantine measures is needed. This finding should be considered along with previously discussed public health implications, including the recommendation for implementation of prevention and control measures at the household level rather than the individual level to ensure that messages reach household contacts. Furthermore, to counter the increased risk associated with quarantine with the index case-patient, quarantine should be implemented concurrently with distribution of antiviral prophylaxis to household contacts.

The influence of cultural and linguistic diversity on secondary transmission served as a proxy for a range of social and environmental determinants of intrahousehold transmission of pandemic (H1N1) 2009 transmission, including recognition and understanding of health promotion messages and access to antiviral treatment and prophylaxis during the containment stages of the AHMPPI. A key finding was a higher SAR among persons who spoke languages other than English at home. This finding suggests that control and prevention measures were not effectively communicated, comprehended, and adhered to by a major community subset in Victoria. Although a higher SAR was observed among persons who spoke languages other than English at home, the study had insufficient power to provide evidence for the relative contribution of cultural and linguistic diversity on secondary transmission. Nonetheless, the potential issues associated with effective communication, comprehension, and adherence to prevention and control measures by cultural and linguistically diverse communities suggest that further work should explore the social and cultural determinants of pandemic (H1N1) 2009.

This study has some limitations. First, it was subject to recall bias, which we attempted to reduce by using tools to improve accurate recall of illness (such as case notification information from VDOH and calendars of major events that occurred during the period of interest). Second, information bias may have been introduced by household members who provided information for household contacts not available at the time of interview. This bias occurred during a few interviews; however, any information bias is likely to underestimate the true association between exposures and pandemic (H1N1) 2009. Third, ILI was used as an indicator for pandemic (H1N1) 2009, and thus some misclassification may have occurred. However, because sentinel surveillance

indicated that most respiratory infections during the same period were pandemic (H1N1) 2009, misclassification was probably minimal (*32*). Fourth, recruitment of households on the basis of the confirmed status of 1 household member may introduce selection bias; however, during the study period, rates of testing of persons with mild to severe illness were high, and thus household contacts should be representative of influenza infections in the community. Fifth, the sample size was small; nonetheless, we identified several factors significantly associated with secondary transmission of pandemic (H1N1) 2009. Sixth, some ILI might be community acquired and therefore overestimate the rate of secondary transmission; we attempted to mitigate any overestimation by excluding concurrent primary cases and household contacts who reported symptom onset before that of the index case-patient.

Our study findings can aid the continued development of future pandemic influenza preparedness plans in Australia and internationally. In particular, the provision of treatment and prevention strategies at the household level, rather than at the individual level alone at the point of care of the index case-patient, should be considered. The need for engagement at the household rather than the individual level is further emphasized by the benefit of timely provision of antiviral prophylaxis to household contacts, particularly when household contacts are quarantined concurrently with the index case-patient. The integration of these practical findings in the development of pandemic influenza preparedness plans in Australia and internationally can help reduce the potential for intrahousehold transmission of influenza during future pandemics.

Acknowledgments

We gratefully acknowledge the participation of the household members for participating in this study; data collectors and research assistants Anna Bowring, Katie Hall, Megan Lewis, Phuong Nguyen, Fabian Kong, Anita Fegan, Johanna Mithen and Cecil Madsen for working on this project; and the Victorian Department of Health for providing data on confirmed cases of pandemic (H1N1) 2009 in Victoria.

This work was funded by an Australian Government National Health and Medical Research Council grant (application ID 603753) for research on pandemic (H1N1) 2009 to inform public policy. This work was conducted as part of a field epidemiology

Table 4. Secondary attack rates for susceptible household contacts of index case-patients with pandemic (H1N1) 2009, Victoria, Australia, May 18–June 3, 2009*

Variable	Total no. household contacts	No. with influenza-like illness	Secondary attack rate, % (95% CI)
Individual-level associations			
Sex			
M	58	5	8.62 (1.08–14.4)
F	64	13	20.3 (11.3–32.2)
Age, y			
0–4	11	1	9.09 (0.230–41.3)
5–19	35	6	17.1 (6.50–33.6)
20–49	66	10	15.2 (7.51–26.1)
≥50	10	1	10.0 (0.25–44.5)
Relationship to index case-patient			
Parent/child/partner	65	10	15.4 (7.63–26.5)
Sibling	44	8	18.2 (8.19–32.7)
Other family member	13	0	0 (0–24.7)
Prevention and control measures reported			
Antiviral prophylaxis	57	1	1.8 (0.04–9.39)
Quarantined ≥1 d with index case-patient	73	15	20.5 (12.0–31.6)
Household-level associations			
No. persons			
2–3	7	2	28.6 (3.67–71.0)
4–5	75	10	13.3 (6.58–23.2)
≥6	40	6	15.0 (5.71–29.8)
No. children			
1	31	6	19.4 (7.45–37.5)
2	47	7	14.9 (6.20–28.3)
≥3	44	5	11.4 (3.79–24.6)
Cultural and linguistic diversity			
Only English spoken at home	53	5	9.4 (3.13–20.7)
English and/or other language(s) spoken at home	69	13	18.8 (10.4–30.1)

*CI, confidence interval.

Table 5. Unadjusted associations with secondary transmission for pandemic (H1N1) 2009, Victoria, Australia, May 18–June 3, 2009*

Variable	OR (95% CI)	p value
Individual level		
Sex		
M	1.00	
F	2.70 (1.060–6.860)	0.037
Age, y		
0–4	1.00	
5–19	2.06 (0.179–23.90)	0.560
20–49	1.79 (0.228–14.00)	0.581
≥50	1.11 (0.529–23.30)	0.946
Relationship to index case-patient		
Parent/child/partner	1.00	
Sibling	1.22 (0.562–2.660)	0.613
Other family member	†	
Reported prevention and control measures		
Antiviral prophylaxis‡	0.05 (0.006–0.429)	0.006
Quarantined for ≥1 d with index case-patient§	1.22 (1.03–1.44)	0.019
Household level		
No. persons		
2–3	1.00	
4–5	0.385 (0.035–4.280)	0.437
≥6	0.441 (0.024–8.070)	0.581
No. children		
1	1.00	
2	0.729 (0.163–3.260)	0.679
≥3	0.534 (0.05–5.74)	0.605
Cultural and linguistic diversity		
Only English spoken at home	1.00	
English and/or other language(s) spoken at home	2.23 (0.448–11.100)	0.328

*Backwards stepwise selection procedures were used to develop the final adjusted model whereby predictors (p>0.05) were removed sequentially until only significant predictors (p<0.05) remained. Gender was not significant in the adjusted model (p = 0.83) and was thus removed. Goodness of fit for both models was assessed by using the Hosmer and Lemeshow test to 0.05 significance. Goodness of fit for the final model was 0.2. OR, odds ratio; CI, confidence interval.
†No secondary cases occurred in this group, and this level is not included in the unadjusted model.
‡Adjusted OR 0.042 (95% CI 0.004–0.434); p = 0.008.
§Logistic regression using number of days quarantined with index case-patient as continuous exposure. Adjusted OR 1.25 (95% CI 1.06–1.47); p = 0.008.

training program at the Australian National University (Master of Applied Epidemiology, which was funded by the Australian Department of Health and Ageing). M.H. received funding from the National Health and Medical Research Council for a senior research fellowship.

Ms van Gemert was a Masters of Applied Epidemiology Scholar at the Australian National University at the time of the study. She now works as a researcher in the Centre for Population Health, Burnet Institute, Melbourne. Her primary research interest is the link between behavior and transmission of infectious diseases.

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Epidemiologic Modeling with FluSurge for Pandemic (H1N1) 2009 Outbreak, Queensland, Australia

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At the beginning of the pandemic (H1N1) 2009 outbreak, we estimated the potential surge in demand for hospital-based services in 4 Health Service Districts of Queensland, Australia, using the FluSurge model. Modifications to the model were made on the basis of emergent evidence and results provided to local hospitals to inform resource planning for the forthcoming pandemic. To evaluate the fit of the model, a comparison between the model's predictions and actual hospitalizations was made. In early 2010, a Web-based survey was undertaken to evaluate the model's usefulness. Predictions based on modified assumptions arising from the new pandemic gained better fit than results from the default model. The survey identified that the modeling support was helpful and useful to service planning for local hospitals. Our research illustrates an integrated framework involving post hoc comparison and evaluation for implementing epidemiologic modeling in response to a public health emergency.

Influenza pandemics can result in substantial excess illness and death (*1*,*2*). In the past century, 3 influenza pandemics have occurred, commonly referred to as the 1918 Spanish flu, the 1957 Asian flu, and the 1968 Hong Kong flu. It is estimated that 40–50 million persons died during the 1918 pandemic, which is considered to be one of the most severe disease events in history (*1*,*2*). The following 2 relatively mild pandemics caused approximately 2 million (1957) and 1 million (1968) deaths, respectively (*1*,*2*).

It is widely foreseen that excess illness and deaths in a future pandemic may place serious demands on and even exhaust the available hospital resources in a community. For example, modeling studies consistently predict that current intensive care unit (ICU) services in several industrialized countries could be overwhelmed during a future event of pandemic influenza (*3*–*5*). The prediction of the expected impact of an emerging pandemic would enable appropriate preparation to be made without diversion of excess resources and thus have the potential to reduce pandemic- and nonpandemic-related illness and death.

Since April 2009, a new variant of influenza virus A (H1N1) initially discovered in Mexico and the United States has caused a wave of pandemic influenza. On May 8, 2009, the first case of pandemic (H1N1) 2009 influenza in Australia was confirmed in Queensland (*6*). During the initial "Delay" and "Contain" phases of the Australian Health Management Plan for Pandemic Influenza (*7*), during April 26–June 22, 2009, a total of 593 laboratory-confirmed cases were notified in Queensland. Among the patients these cases represent, 16 hospitalizations and no deaths were reported (*8*). However, the reported number of cases, hospitalizations, and deaths may only represent a small fraction of the true numbers because not all persons who are infected seek medical care and have a specimen collected. Further, not all specimens will have positive results and be reported (*9*).

To assist hospital planners in their preparation for pandemic (H1N1) 2009, at the emerging stage of the pandemic in 2009, regional epidemiologists made predictions of the potential need for general hospital resources and ICU services for 4 Health Service Districts in Queensland, Australia, by using the FluSurge model developed by the Centers for Disease Control and Prevention in the United States (*10*,*11*). A follow-up survey was conducted in early 2010 to evaluate the application of

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DOI: http://dx.doi.org/10.3201/eid1709.102012

these predictions. The aims of the studies were to describe the modeling work, to explore the fitness of the predictions to the actual hospital data, and to evaluate the application and usefulness of the modeling to hospital planners.

Methods

Design

This article describes results from 2 studies. The first study compared the model's predictions to the actual data for the largest district, Metro North. The modeling techniques used to calculate these predictions are described. The second study was a Web-based cross-sectional survey among the hospital staff who had access to and used these predictions in the response to the pandemic. The aim of this survey was to examine how the projections were applied and whether they were perceived as useful in planning.

Modeling was undertaken during May 29–June 29, 2009, when cases in Australia emerged. The Web-based survey was conducted in May 2010.

Service Provision Area

Central Regional Services is 1 of 3 regional services of Queensland Health that provide public health services to 4 Health Service Districts, including Metro North (the northern side of Brisbane, population 770,000), Sunshine Coast-Wide Bay (SCWB, population 501,000), Central Queensland (CQ, population 189,000), and Central West (CW, population 12,000). Together these districts account for 38% of Queensland's population and 32% of Queensland's area. According to the remoteness structure defined by the Australian Bureau of Statistics (*12*), most of Metro North and SCWB are classified as major city or inner regional (urban), and CQ and CW are mainly outer regional or remote areas.

Modeling Tool

FluSurge (*11*) predicts the surge in demand for hospital-based services during an influenza pandemic, yielding estimates of the number of hospitalizations (including ICU admissions) and deaths caused by a pandemic in comparison to the existing hospital capacity. Major assumptions of the FluSurge model include that hospital admissions for pandemic influenza pose an extra inconvenience to the current resources and that the admissions are normally distributed over a given time period of the pandemic. Prior to 2009, the model was used to predict the demand for hospital-based services of a future pandemic in the United States (*3*,*10*), England (*4*), Mexico (*13*), and the Netherlands (*14*,*15*). More recently, this tool was also used in the Australian state of Victoria for the pandemic (H1N1) 2009 (*16*).

Data Sources

District and age-specific population data (estimated resident population 2007 data from Australian Bureau of Statistics, released August 19, 2008; cat. no. 3235.0) and district-specific hospital resource data (for both public and private hospitals) were used as model inputs. Resource data for public hospitals were obtained from the Monthly Activity Collection produced by the Health Statistics Centre and verified by hospital managers. Private hospital data were based upon licensed capacity of operational status by designations, obtained from the Private Health Unit. As Queensland data on ventilators were unavailable, we used the proportion (53.3%) of available ventilators among available ICU beds in New South Wales (Health New South Wales, unpub. data) to estimate the number of available ventilators in Queensland hospitals. To compare modeling predictions with actual data, weekly numbers of hospital admissions because of pandemic (H1N1) 2009 by care type (general bed care, ICU admission and/or ventilation) for each district were extracted from EpiLog, a Web-based application on which hospital-admitted patients who were suspected of having pandemic (H1N1) 2009 were registered.

Postservice Survey

We conducted a postservice survey using a Web-based survey tool, SurveyMonkey (*17*), seeking information on 1) users of the predictions (including position, role, and district); 2) use of the predictions; 3) perceived usefulness of the predictions; and 4) recommendations and suggestions. Both closed and open-ended questions were used.

Thirty-one hospital planning staff from the 4 districts who were involved in the pandemic response for their district in June 2009 were approached; 16 (52%) responded to the survey. Data analysis was conducted with 15 respondents (48%) as 1 questionnaire was incomplete. Among the respondents, 6 (40%) were from Metro North, 4 (27%) from SCWB, 3 (20%) from CW, and 2 (13%) from CQ. A large percentage of respondents (40%) held nursing positions. Many other respondents were pandemic planning directors and coordinators (13%), or infection control officers (13%). All played a role in the district pandemic (H1N1) 2009 planning and response.

Data Analysis

Modeling efforts were summarized, and the main inputs and results of an example district (Metro North) were presented in a descriptive manner. The rationale and procedure of modifications to the default FluSurge assumptions were also reported. Modeling predictions and the actual data from the EpiLog were compared visually by plotting.

Responses to survey questions were presented in count and percentages. Content analysis was used to examine the open-ended questions and examples were provided.

Ethics Approval

Ethical approval was obtained from the Royal Brisbane and Women's Hospital Human Research Committee for the survey. Participation in the survey was voluntary. Anonymity, confidentially, and privacy of participant responses and any personal details were assured. All participants completed the consent section before responding to the questionnaire.

Results

Modeling for Planning

In the initial modeling stage (May 20–29, 2009) predictions regarding the potential hospital load caused by pandemic (H1N1) 2009 in the 4 districts, were made by using FluSurge 2.0 (www.cdc.gov/flu/tools/flusurge). Given that data regarding the current pandemic were unavailable, we used assumptions derived from previous pandemics in the United States, as recommended by the authors of FluSurge (*11*) and other studies (*4*; Health New South Wales, unpub. data). Assumptions included a 25% attack rate and 12-week outbreak duration (Table).

Using the largest district, Metro North, as an example, we found by initial modeling that a moderate pandemic would result in a total of 2,840 hospital admissions (Table). Peak hospital admission would likely occur at week 6 and week 7 with 426 (range 166–571) admissions per week (Figure 1). During the peak week of the pandemic, 9% of available general beds and 83% of available ICU beds, in both public and private hospitals, would be occupied by patients with pandemic (H1N1) 2009 (Table). The model also predicted that the need for ventilator capacity would exceed the current availability by 17% (Table).

In early June, these results were then presented to those involved in pandemic response and/or hospital planning. However, it was evident through discussion that some major assumptions of the initial model, such as the attack rate, hospitalization rate, and proportions that require ICU or ventilation care, needed revision in line with emerging evidence. For example, although at that time Australia was still in the early stage of responding, data emerging from North America suggested that the new pandemic was much less severe than originally expected. Specifically, it appeared that the present attack rate and incidence of both hospitalization and related death was lower than that used in the model (*18*,*19*; Australian Government Department of Health and Ageing, unpub. data). In addition, historic seasonal influenza data for Queensland suggested that the duration would be longer than 12 weeks (*20*). However, a limitation of the initial model was that the FluSurge only provided 3 options for the attack rate (15%, 25%, and 35%), 3 options for the duration (6, 8, and 12 weeks), and an unadjustable hospitalization risk (*11*).

A modified model with more flexible inputs was then developed to address these limitations. In this modified model, the number of cases related to pandemic (H1N1) 2009 was estimated by total population multiplied by the gross attack rate (same as the original FluSurge

Table. Modeling attempts for pandemic (H1N1) 2009, Metro North Health Service District, Queensland, Australia, 2009*

Variable	FluSurge 2.0	Modified model
Parameter and assumption/source		
Population	812,941	812,941
Gross attack rate	25%	15%
Hospitalization rate	Default	0.5%
Duration of hospitalization	12 wk	14 wk
Proportion of patients needing ICU care	20%	10%
Proportion of patients needing ventilation	15%	7.5%
Average length of non-ICU hospitalization	5 d	5 d
Average length of ICU stay	10 d	10 d
Average length of ventilator usage	10 d	10 d
Available hospital resources	Private and public hospitals	Public hospitals only
Main output		
Hospital admissions	2,840 (range 1,104–3,810)	610
Patients needing general beds only	–	549
Patients needing ICU care	–	61
ICU patients needing ventilator care	–	46
Bed demands/availability during peak week		
General	9%	2%
ICU	83%	10%
Ventilator	117%	13%

*ICU, intensive care unit; –, not applicable.

model). The number of hospital admissions was then calculated by multiplying the number of cases by the gross hospitalization rate. The modified model also assumed that the total number of admissions would be allocated to each day within the pandemic period according to a symmetric distribution. The number of admissions and bed demands by care type and time were estimated according to the original FluSurge model methods. The main advantage of the modified model was that any value of attack rate (0%–100%), hospitalization rate, and duration could be accepted. However, because a single hospitalization rate was used instead of the default range in the original FluSurge model, the modified model could no longer generate a confidence interval. Nevertheless, the modifications were expedient and addressed the planners' concerns of unreasonable default assumptions.

On the basis of the modified model, repeated analyses were conducted in June 2009. We hypothesized a 15% attack rate, 0.5% hospitalization rate (among infected persons), and 14-week duration (Table). A series of sensitivity analyses were also made by increasing or decreasing the value of major assumptions within the model. The main results under the base-case assumptions are presented in the Table. At the peak week of hospitalization, the required numbers of 3 types of beds were predicted to account for 2% general, 10% ICU, and 13% ventilator of the current availabilities of public hospital resources (Table). The modified assumptions and results, along with sensitivity analyses were endorsed by Metro North planners and used to inform resource planning.

Comparisons with Actual Data

We determined that, according to the number of hospital admissions, the 2009 outbreak of pandemic (H1N1) 2009 in Metro North started with week 25. During a 14-week period (weeks 25–38), a total of 308 patients with confirmed or probable pandemic (H1N1) 2009 influenza were hospitalized in Metro North hospitals. The majority (92.3%) were admitted into public hospitals. Seventy-four (21.1%) patients were cared for in an ICU, and 42 (13.6%) were treated with mechanical ventilation.

Compared with actual data above, the original FluSurge model (Figure 1; Table) largely overpredicted the effects on hospital-based services. This supported the appropriateness of modifying the assumptions as proposed. The comparison between predictions of the total and ICU admissions based on the modified model and the actual data are shown in Figure 2. This figure shows that the predicted and actual hospitalizations mapped fairly closely for the first 6 weeks of the pandemic, after which there was a rapid drop. Of particular interest to hospital managers was that the actual number of ICU admissions for each week fitted reasonably well to the modeling line (Figure 2).

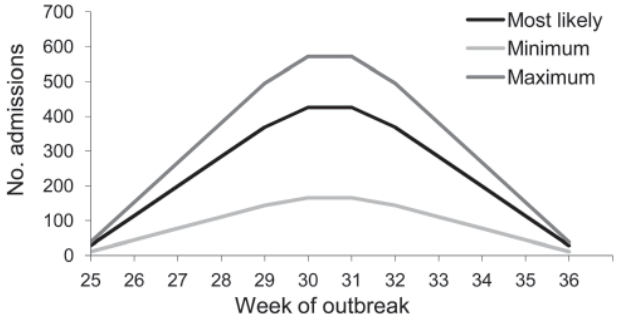

Figure 1. Predicted hospital admissions during an influenza pandemic with 25% attack rate and 12-week duration in Metro North Health Service District, Queensland, Australia, estimated by using FluSurge 2.0.

Follow-up Survey among Users

Twelve (80%) respondents reported they had used the epidemiologists' modeling results to inform pandemic response in their districts. Two broad types of use were identified: specific use in bed planning and general use to enhance awareness about the pandemic. Participants from larger districts (Metro North and SCWB) tended to use the results for preparation purposes in bed use, service demand, and staffing. The districts with smaller populations used the models to enhance general awareness of the pandemic.

Four (36%) of 11 respondents believed the predictions had made a change in the pandemic response, 6 (55%) stated no change was induced, and 1 (9%) was not sure. The changes seemed subjective and indirect, derived mainly from better understanding and more confidence among the planners themselves, which in turn would benefit the health system and society. One respondent described how the modeling provided a robust indication of the impact on hospital resources that facilitated changes of work patterns, transfer of patients, and the delay of elective survey. The curve helped identify outbreak progress and when the peak had passed. However, the perceived changes only occurred in the 2 districts with larger populations, most notably Metro North where most of the epidemiologic support was provided. Respondents from the 2 smaller districts did not report any change attributed to the modeling.

All respondents (n = 11) agreed that the predictions were useful to some extent. Four persons considered the predictions to be "quite useful or very useful"; all were from Metro North or SCWB. Participants from the smaller districts (CQ and CW) only considered the modeling to be little to moderately useful.

Similarly, all respondents (n = 14) stated that the communication (e.g., meetings, telephone calls, and emails) between epidemiologists and districts during the pandemic was important to some extent. Nine rated communication

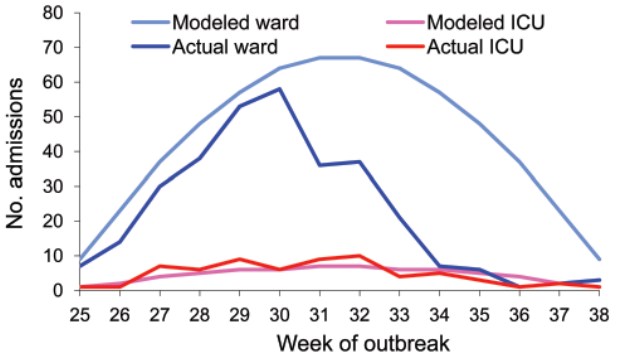

Figure 2. Modeled numbers of total and intensive care unit (ICU) admissions caused by a hypothesized 14-week influenza outbreak in Metro North Health Service District, Queensland, Australia. This model uses assumptions of a 15% attack rate and 0.5% hospitalization rate compared to actual data.

as "quite important" or "very important"; 8 were from the 2 larger districts. All respondents (n = 14) agreed that it is helpful to make some predictions before or at the early stage of a pandemic. Most (11/14) believed it was "quite helpful" or "very helpful"; 9 of these respondents were from the larger districts.

Most (13/14) stated that they would recommend predictive modeling be used in future pandemic responses. The modeling assisted hospital planning and enhanced confidence. One respondent identified the role of having trust in the predictions by understanding the assumptions used by the epidemiologists, and that this trust empowered more sophisticated planning and action to manage the excess demand.

Discussion

We have illustrated a practical framework for epidemiologic modeling in response to a public health emergency, such as a pandemic of infectious disease. The framework could be described as 4 consecutive steps: need identification, modeling and presentation, field use of predictions, and evaluation. This project directly translated existing and emerging knowledge into practice and combined 2 studies to "tell a whole story."

Although models such as FluSurge are readily available and simple to use, our experience suggests modification is required. When we used modified assumptions on the basis of emergent information on the novel pandemic influenza, more reasonable predictions resulted than when we used default assumptions derived from previous pandemics. Although many modeling studies have been conducted using similar tools (*3*,*4*,*10*,*13*–*15*), none of them have been subsequently tested by using actual data and evaluated in terms of the usefulness in practice. Similar to our initial modeling using the default FluSurge model, most of these studies substantially overestimated the impact of the new pandemic compared to the actual 2009 situation. Lack of communication with users and relying only on historical data may contribute to this problem. The interest of users and their involvement appears to be essential for successful epidemiologic modeling.

The post hoc survey is unique to this study. Results indicate that the modeling and associated support by epidemiologists were well received overall. However, the perceived usefulness of the modeling was more notable for the larger districts than for the smaller districts. We speculate that this occurs because there are larger uncertainties regarding modeling and relatively lower consequences in a small population. Although we did not attempt to measure any direct benefits to the communities, the survey data and ad hoc comments received shows that the modeling and consultation services provided by the epidemiologists were used to manage hospital services confidently.

Although they fitted better, the modified projections seemed to still overestimate the impact, especially the total number of hospitalizations (Figure 2). The main reason is that our assumption of hospitalization rate in general population (15% attack rate × 0.5% hospitalization rate = 75/100,000 persons) is far higher than the actual rate in Metro North (308/770,000 = 40/100,000 persons) and the rate in Australia (23/100,000 persons) (*21*). The pandemic (H1N1) 2009 outbreak turned out to be shorter than observed in seasonal influenza (*20*). One plausible explanation is that Metro North is a metropolitan area and disease transmission there may be more intense than in the whole state, which covers a considerable rural population.

It is unknown whether the awareness of the modeling results might have resulted in changes in admission policy and subsequently affected the number of admissions. In this paper we only presented Metro North data as an example to demonstrate our modeling work. For SCWB district with similar number of residents to the Metro North district, similar results were observed (data not shown). However, for the other 2 small districts, the projections were far more serious than the actual events. This, in accordance with the survey data, indicates that the modeling work for small populations is more difficult and less useful.

There are some limitations to the modeling. First, we assumed a normal distribution of patients over a given period, but the epidemic curve is not symmetric (*22*). The curve usually increases quickly, almost exponentially, and then declines with a long tail after the peak. Second, estimates were made on the basis of the population in these Health Service Districts. The actual number of hospitalizations, however, was obtained from all hospitals within this area rather than all episodes occurring in the whole population. In other words, patient flow was not taken

into account in the comparisons. Nevertheless, parallel data obtained from the Queensland Hospital Admitted Patient Data Collection (which records information about episodes of hospital care for all Queensland hospitals) showed that assumptions of patient flows were reasonable and consistent because the net effect was similar. Third, the ventilator capacity was not exactly measured but estimated on the basis of data from another state (*17*), and ICU staff, surge capacity of staff, and absenteeism during a pandemic were not taken into consideration. Furthermore, some major assumptions, such as expected attack rate and hospitalization rate, were somewhat arbitrary. Additionally, many factors that may affect the probability of hospital admission (*23*), such as obesity, pregnancy, and proportion of Indigenous population, were not included in the model. The above limitations would have certain negative effect on the accuracy of the predictions. However, we relied on the best information available at the time. In addition, a series of sensitivity analyses were attached to the base-case estimation. We perceive that the process integrating communication–feedback–presentation is of high value, even higher than the results themselves. The evaluation survey revealed many planners took the opportunity to understand modeling processes and enhance their understanding of the new pandemic.

Another limitation is that only visualization was used to compare projected to actual data, rather than more sophisticated statistical tests. The low response rate (52%) and the small number of respondents (n = 15) may also bias the findings from the survey because nonresponders' views may differ from responders and thus reduce the representativeness of the sample. Although difficult to assess, the role stated by the participants appears to indicate that key informants from each Health Service District are represented in the survey. The survey is also limited in that several months had passed between when the models had first been applied and when the survey was conducted. Consequently, the survey relied on recall and reflection, both of which can be subject to bias.

Given the above limitations, the net robustness of the model and evaluation are less than ideal, and the predictions and modifications cannot be immediately extrapolated to other areas or outbreaks. Despite these limitations, we firmly believe the principles of modeling and partnerships shown here are valuable for epidemiologists and policy makers in practice and research. It is hoped that the framework illustrated in this study may serve as a general model for epidemiologists who provide epidemiologic services in a public health unit context.

Acknowledgments

We thank Martin Meltzer for his advice in using the FluSurge model, and capacity managers and planners, survey participants, and colleagues Daniel Francis and Carolyn Lang for their suggestions.

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Differential Effects of Pandemic (H1N1) 2009 on Remote and Indigenous Groups, Northern Territory, Australia, 2009

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Pandemic (H1N1) 2009 influenza spread through the Northern Territory, Australia, during June–August 2009. We performed 2 cross-sectional serologic surveys on specimens from Northern Territory residents, with 445 specimens obtained prepandemic and 1,689 specimens postpandemic. Antibody titers were determined by hemagglutination inhibition against reference virus A/California/7/2009 on serum samples collected opportunistically from outpatients. All specimens had data for patients' gender, age, and address, with patients' indigenous status determined for 94.1%. Protective immunity (titer ≥40) was present in 7.6% (95% confidence interval [CI] 5.2%–10.1%) of prepandemic specimens and 19.5% (95% CI 17.6%–21.4%) of postpandemic specimens, giving a population-standardized attack rate of 14.9% (95% CI 11.0%–18.9%). Prepandemic proportion of immune persons was greater with increasing age but did not differ by other demographic characteristics. Postpandemic proportion of immune persons was greater in younger groups and around double in indigenous persons. Postpandemic proportion immune was geographically heterogeneous, particularly among remote-living and indigenous groups.

Understanding the epidemiology of pandemic influenza is essential in directing public health responses, not only to the current pandemic, but also for recurrent waves of the same virus and future influenza pandemics. Knowledge of the distribution of protective immunity enables prediction of groups susceptible to reemergence of the virus and thus helps to improve efficacy of vaccine programs. Influenza has uneven effects across demographic and geographic groups, which may contribute to the increases in illness and death sometimes seen with subsequent waves (*1*,*2*). There is an emerging understanding of the effects of the outbreak of pandemic (H1N1) 2009 on indigenous populations, but little is known of the virus's effect on remote and socioeconomically disadvantaged groups.

Direct serologic measures of population immunity are useful in assessing the effect of pandemic influenza, as case or surveillance-based measures of incidence of infection are dependent on recognition of symptoms, use of health services, and subsequent testing (*3*). In remote and ethnically diverse populations, the differential effect of these factors may be particularly marked.

The Northern Territory (NT) is a jurisdiction unique for its large area of 1.35 million km^2 (twice that of Texas) relative to its population of 225,000, of whom 30% are indigenous. The climate ranges from desert and semi-arid in central Australia to tropical in the northern "Top End" where the capital, Darwin, is located. There are also several smaller urban centers and many small, remote indigenous communities of 300–2,000 that may be ≥2 h flight from the nearest hospital. Indigenous Australians of the NT have considerably poorer health than the nonindigenous majority, with a life-expectancy gap of 15–20 years (*4*).

Following recognition of the pandemic (H1N1) 2009 virus in North America in April 2009, Australia experienced a single pandemic wave leading into the Southern

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DOI: http://dx.doi.org/10.3201/eid1709.101196

Hemisphere winter (*5*). Despite enacting carefully prepared nationwide public health measures to delay viral entry and spread, widespread infection followed (*6*,*7*). Australia's first case was reported on May 8, with the first case in the NT reported on June 2 and the first NT death occurring July 9 (*8*). Australia moved to the "protect" phase of its pandemic response on June 17 in an effort to limit illness and death from the virus (*9*), with notifications peaking nationwide and in the NT in July (*10*).

We undertook serosurveys using opportunistically collected outpatient serum specimens from persons across the NT to estimate levels of pre-existing immunity and differential attack rates among demographic groups. Our study included a large proportion of remote-living persons, including Aboriginal and Torres Strait Islanders, enabling assessment of the differential effect of influenza upon these populations.

Methods

Specimens

Specimens were obtained from Western Diagnostic Pathology (Myaree, Western Australia, Australia), which provides outpatient pathology services covering most of the NT. Specimens were eligible for inclusion regardless of indication for testing, provided identifying information was complete and address was within the NT. We accepted only serum tubes with a residual volume ≥0.5 mL and obtained specimens before routine discarding. Baseline specimens were selected during January–May and all postpandemic specimens from September 2009.

Background Information

Data obtained for each specimen consisted of date of collection, patient's age in years at collection, gender, suburb/community of address, and a unique study identifier. Identifying data (name and date of birth) were transferred directly from the laboratory to the Information Services Division of the NT Department of Health and Families for computer-matching to indigenous status. This was successful in 94.1% of cases, and the data were transferred to the investigators linked to the study identifier. Of those cases with a successful match, 59.7% of patients were neither indigenous nor Torres Strait Islander, 39.7% were Aboriginal, 0.1% were Torres Strait Islander, and 0.6% were both Aboriginal and Torres Strait Islander. The suburb of patient's address for each specimen was linked to 2006 Statistical Local Area (SLA), the Australian Bureau of Statistics' general purpose base spatial unit, with 82 of 96 NT SLAs represented (*11*).

After testing, a small number of specimens were redistributed by region, following manual review of suburb of address linkage to SLA. The SLA code was also linked to the 11 statistical subdivisions and 7 health districts in the NT. Three study regions were defined, displayed in Figure 1, consisting of Urban Darwin; Rural Top End (Darwin Rural, East Arnhem, and Katherine districts); and Central Australia (Alice Springs Urban, Alice Springs Rural, and Barkly districts). SLA codes were then linked to the Australian Bureau of Statistics' Socio-Economic Indexes for Area (SEIFA) (*12*). These measures use information from census data relating to material and social resources and ability to participate in society to obtain a broad level of relative socioeconomic status for each SLA. For calculation of attack rates by quintile, the SEIFA index of relative disadvantage was used, while for regression analysis, the SEIFA index of relative advantage and disadvantage was preferred, as this index does not incorporate indigenous status.

Laboratory Methods

Antibody responses to pandemic (H1N1) 2009 influenza were assessed at the World Health Organization Collaborating Centre for Reference and Research on Influenza in North Melbourne, Victoria, Australia. Reactivity of serum against pandemic (H1N1) 2009 influenza was measured in 2140 serum samples by using hemagglutination inhibition (HI). Egg-grown A/California/7/2009 virus was purified by sucrose gradient, concentrated, and inactivated with β-propiolactone to create an influenza zonal pool preparation (a gift from CSL Ltd., Parkville, Victoria, Australia). Serum samples were pretreated with 1:4 vol/vol receptor-destroying enzyme II (Deka Seiken Co. Ltd., Tokyo, Japan) at 37°C for 16 h, then enzyme was inactivated by the addition of an equal volume of 54.4 mmol/L trisodium citrate (Ajax Chemicals, Taren Point, New South Wales, Australia) and incubated at 56°C for 30 min. A total of 25 μL (4 hemagglutinin units) influenza zonal pool preparation A/California/7/2009 virus or 25 μL phosphate-buffered saline ("no virus" control) was incubated at room temperature with an equal volume of receptor-destroying enzyme–treated serum. Serum specimens were titrated in 2-fold dilutions in phosphate-buffered saline from 1:10 to 1:1280. After a 1-h incubation, 25 μL of 1% vol/vol turkey erythrocytes was added to each well. HI was read after 30 min. Any samples that bound the erythrocytes in the absence of virus were adsorbed with erythrocytes for 1 hour and reassayed. Six samples bound erythrocytes in the absence of virus and were excluded from analysis. Titers were expressed as the reciprocal of the highest dilution of serum at which hemagglutination was prevented.

A panel of control and serum samples were run in addition to the test serum samples for all assays. The control panel comprised paired ferret serum samples pre- and postinfection with pandemic (H1N1) 2009; seasonal influeza A (H1N1), A (H3N2), or B viruses; and paired

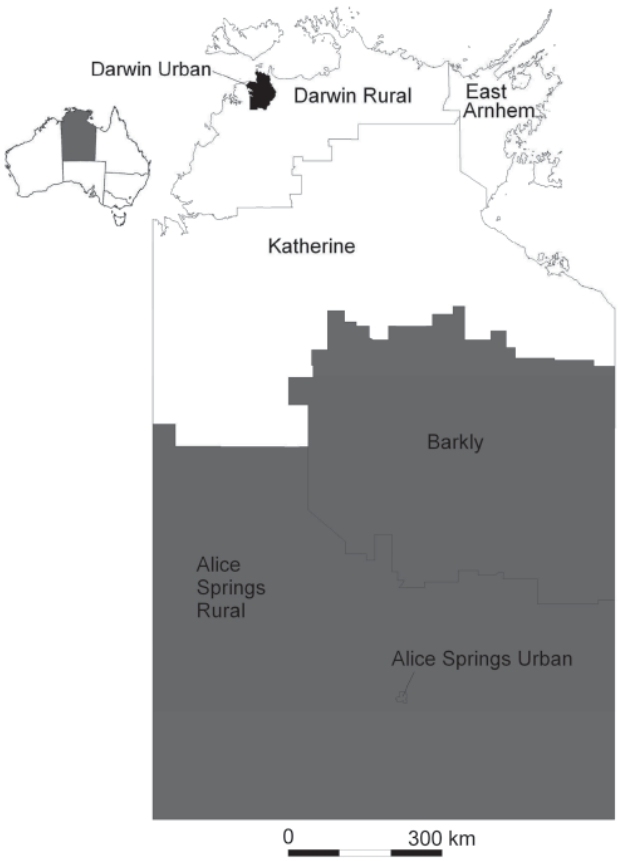

Figure 1. Health districts, by study region, in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, September 2009. Black, Urban Darwin; white, Rural Top End; gray, Central Australia. Inset: Location of the Northern Territory in Australia.

human plasma and serum samples from donors, collected before April 2009 or after known infection with pandemic (H1N1) 2009 or vaccination with the Australian monovalent pandemic (H1N1) 2009 vaccine.

Study Population

We aimed to estimate the proportion of persons with serologic immunity in each of 12 groups in the postpandemic sample, consisting of 4 age groups (≤14, 15–34, 35–54, and ≥55 y) within each of the 3 study regions described. In the postpandemic group, we calculated a required sample size of 195 specimens per group (for a total of 2,340 specimens) on the basis of an estimate of 15% immunity with a 95% confidence interval (CI) of 10%–20%.

In the baseline group, we aimed to provide an age-specific, NT-wide estimate of preexisting immunity and calculated a single sample size for each of the same 4 age groups described. We did not stratify by region and assumed increasing prepandemic immunity with age (2% in those ≤14 y, 5% in those 15–54 y, and 15% in those ≥55 y).

Samples were chosen at random from each stratum and checked for representativeness of the NT population by gender and region before testing. Data on indigenous status were obtained from Information Services only after final selection of specimens.

Analytic Methods

For all analyses, immunity was defined as an HI titer ≥40, consistent with published data (*13*) and the observation that titers of this order develop in 90% of persons ≤21 days of illness (*14*). Attack rates were calculated as the difference in proportion immune between the September category and the total baseline group, except for age-specific attack rates where the age-specific baseline proportion was used as the reference. All attack rate calculations were population standardized, with weights calculated separately for pre- and postpandemic samples, based on the demographic characteristics of the 2009 NT population by age-group, indigenous status, and study region. Regression models and proportions immune are displayed unweighted. Statistical analysis was performed with Stata version 11.0 (StataCorp LP, College Station, TX, USA).

Ethical Approval

We obtained ethical approval from the Menzies School of Health Research Human Research Ethics Committee and the Central Australia Human Research Ethics Committee. We continued to liaise with the Aboriginal and Torres Strait Islander subcommittees of both ethics committees throughout the study.

Results

Baseline Immunity

A total of 445 specimens taken January 10–May 29 were selected from 10,575 available serum tubes (Table 1). Within each age group sampled, the baseline sample was representative of the 2009 NT population (*15*) by gender, indigenous status, and study region, except that higher proportions of indigenous Australians were seen in the 2 older age brackets. There was no tendency toward an increase in the proportion of specimens with protective immunity over the 5 months from which baseline specimens were taken ($p = 0.79$, by χ^2 test for trend).

A total of 34 of 445 baseline specimens (7.6%, 95% CI 5.2%–10.1%) had HI titers ≥40. Multivariate logistic regression revealed no difference in baseline immunity by gender, indigenous status, study region, or index of socioeconomic disadvantage ($p>0.05$), with increasing age in years the only significant independent predictor

Table 1. Demographic characteristics of patients in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, 2009

Characteristic	No. patients	Female, %	Indigenous, %
Baseline, age, y			
≤14	37	54.1	51.4
15–34	91	65.9	43.5
35–54	92	54.4	33.7
≥55	225	44.9	30.3
Total	445	51.9	35.5
September, Urban Darwin, age, y			
≤14	60	53.3	14.0
15–34	194	62.9	13.5
35–54	202	55.5	9.6
≥55	209	48.3	6.7
Total	665	55.2	10.2
September, Rural Top End, age, y			
≤14	25	36.0	44.0
15–34	190	60.5	71.4
35–54	183	47.5	60.8
≥55	190	46.8	42.5
Total	588	51.0	57.8
September, Central Australia, age, y			
≤14	13	46.2	61.5
15–34	84	57.1	82.7
35–54	189	63.5	63.2
≥55	150	51.3	54.9
Total	436	57.6	64.1

of prepandemic immunity (p = 0.003). Although not statistically significant on the regression model, the proportion of specimens with titers ≥40 appeared higher in Central Australia (14.0%) than Urban Darwin and Rural Top End (5.6%). Immunity was nevertheless evenly spread geographically within these regions.

Postpandemic Immunity

A total of 1,689 specimens collected September 3–30, 2009, were selected from 3,228 available. Because of insufficient numbers of specimens, the required sample size was not achieved in 5 of 12 postpandemic groups. The September samples were representative of the 2009 NT population by gender but again included higher proportions of specimens from indigenous Australians in the older age brackets. An HI titer ≥40 was seen in 329 specimens (19.5%, 95% CI 17.6%–21.4%), with proportions by study group shown in Table 2, geometric mean titers in Figure 2, and reverse cumulative distributions in Figure 3. There was a nonsignificant trend toward a decreasing proportion of specimens with protective immunity over the 5 weeks from which the September specimens were taken (p = 0.20, by χ^2 test for trend).

Table 3 shows the results of multivariate logistic regression analysis for the 1,592 postpandemic specimens for which the indigenous status of patients was known. No association was detected between immunity and gender, socioeconomic status, or study region. However, younger age and indigenous status were independently associated with immunity. A measure of remoteness was examined as a possible exposure variable, but colinearity with region meant that it was not a useful predictor variable and therefore was not included in regression analysis (*16*).

The proportion immune in September was geographically heterogeneous across the 3 study regions (p<0.001, by χ^2 test). The same pattern was seen for Statistical Subdivisions (p<0.001, by χ^2 test), with proportionate immunity ranging from 7.5% to 42.9%, as illustrated in Figure 4. The picture of heterogeneity was also seen for the indigenous population considered separately. However, the prevalence of postpandemic immunity was more homogeneous for the nonindigenous population considered by either geographic classification and for urban Darwin considered separately. Figure 5 demonstrates that while postpandemic levels of immunity were relatively homogeneous by SLA in less disadvantaged, generally urban areas, comparatively disadvantaged areas had more variable levels of immunity.

Attack Rates

As shown in Table 4, attack rates by age group were markedly higher in younger groups, reaching approximately 1 in 3 among children ≤14 years of age. Indigenous Australians were also disproportionately affected, with attack rates of ≈1 in 4, which were 1.85-fold higher than

Table 2. Specimens with titers ≥40 in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, September 2009*

Population group	No. positive/ no. tested	% Titers ≥40 (95% CI)
Baseline, age, y		
≤14	0/37	0
15–34	4/91	4.4 (0.1–8.6)
35–54	8/92	8.7 (2.9–14.5)
≥55	22/225	9.8 (5.9–13.7)
Urban Darwin, age, y		
≤14	22/60	36.7 (24.3–49.0)
15–34	34/194	17.5 (12.2–22.9)
35–54	23/202	11.4 (7.0–15.8)
≥55	20/209	9.6 (5.6–13.6)
Rural Top End, age, y		
≤14	5/25	20.0 (4.0–36.0)
15–34	46/190	24.2 (18.1–30.3)
35–54	31/183	16.9 (11.5–22.4)
≥55	35/190	18.4 (12.9–24.0)
Central Australia, age, y		
≤14	7/13	53.9 (25.6–82.1)
15–34	23/84	27.4 (17.8–37.0)
35–54	50/189	26.5 (20.1–32.8)
≥55	33/150	22.0 (15.3–28.7)

*CI, confidence interval.

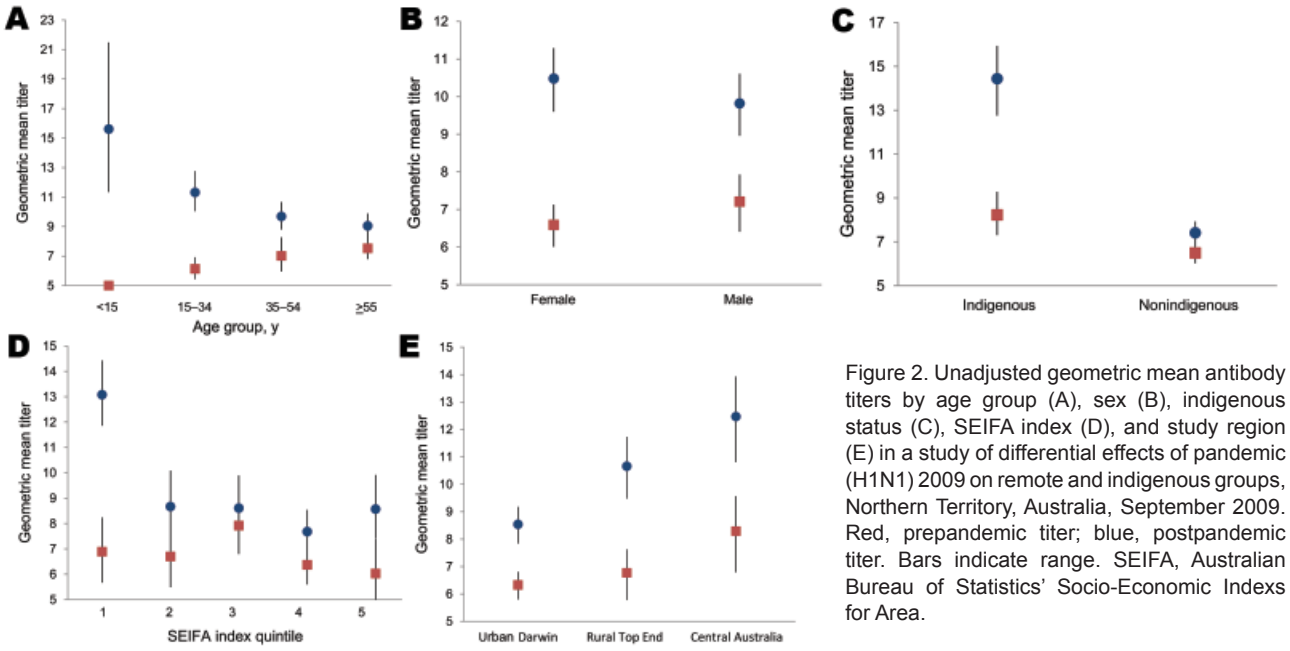

Figure 2. Unadjusted geometric mean antibody titers by age group (A), sex (B), indigenous status (C), SEIFA index (D), and study region (E) in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, September 2009. Red, prepandemic titer; blue, postpandemic titer. Bars indicate range. SEIFA, Australian Bureau of Statistics' Socio-Economic Indexs for Area.

those seen in nonindigenous Australians. No differences in attack rates were seen by gender, region, or socioeconomic quintile. Given these attack rates, we estimate that 15,600 (95% CI 10,900–20,300) of 67,820 indigenous and 19,500 (95% CI 12,700–26,300) of 157,028 nonindigenous persons in the NT acquired pandemic influenza during May–September 2009.

Discussion

Our study is an outpatient-based serologic survey of the impact of pandemic influenza over a large geographic region. Because of our broad sampling base, we have been able to estimate attack rates across the NT population and to assess the differential impact of the virus on the indigenous population. We calculated a population attack rate of ≈15% but found marked differences in patterns of exposure by indigenous status, geographic location, and age. Younger age groups and indigenous Australians were disproportionately affected, with striking geographic variations seen.

Baseline immunity could be overestimated if undetected virus circulation was occurring during our prepandemic period. We believe this is unlikely, as there was no trend toward increasing immunity in samples taken at a later date, no child had a baseline titer >10, and the first confirmed case was not detected in the NT until May 29 (*17*). Similarly, our September sample could have underestimated true postpandemic immunity caused by ongoing infection during this month. However, emergency department presentations of influenza-like illness had returned to baseline by this time, and there were few laboratory-confirmed cases during this period. Similarly, no increase in immunity was observed during September in our study. Because the national pandemic vaccination program in Australia commenced in NT on September 30, testing of specimens before this date would be unaffected by antibodies produced by vaccination (*18*).

Although we attempted to ensure that our sample was demographically representative of the NT population, the prevalence of risk factors for influenza infection may be different in our sample from that of the general population. In particular, chronic disease and pregnancy may have been overrepresented among patients presenting for outpatient pathologic analysis. However, because clinical data, including indication for testing, were not available, the strength of this possible effect cannot be assessed.

We found a prevalence of preexisting immunity of 3.6% in those born after 1980 and of 0% in children. In those born before 1950, the level of pre-existing immunity was 13.7%, which is lower than data from North America (*19*,*20*). This may reflect regional differences or be the result of the 1976 mass-vaccination campaign against swine-origin H1N1 virus because seasonal influenza vaccination does not produce protective titers against the pandemic (H1N1) 2009 virus. Despite this finding, serologic data from a population in China with low seasonal vaccine coverage found lower levels of preexisting immunity (*21*), although comparable levels of preexisting immunity were seen in Singapore (*22*).

Our findings of a postpandemic proportion immune rate of 19.5%, attack rate of 14.9%, and the association with younger age are consistent with other published

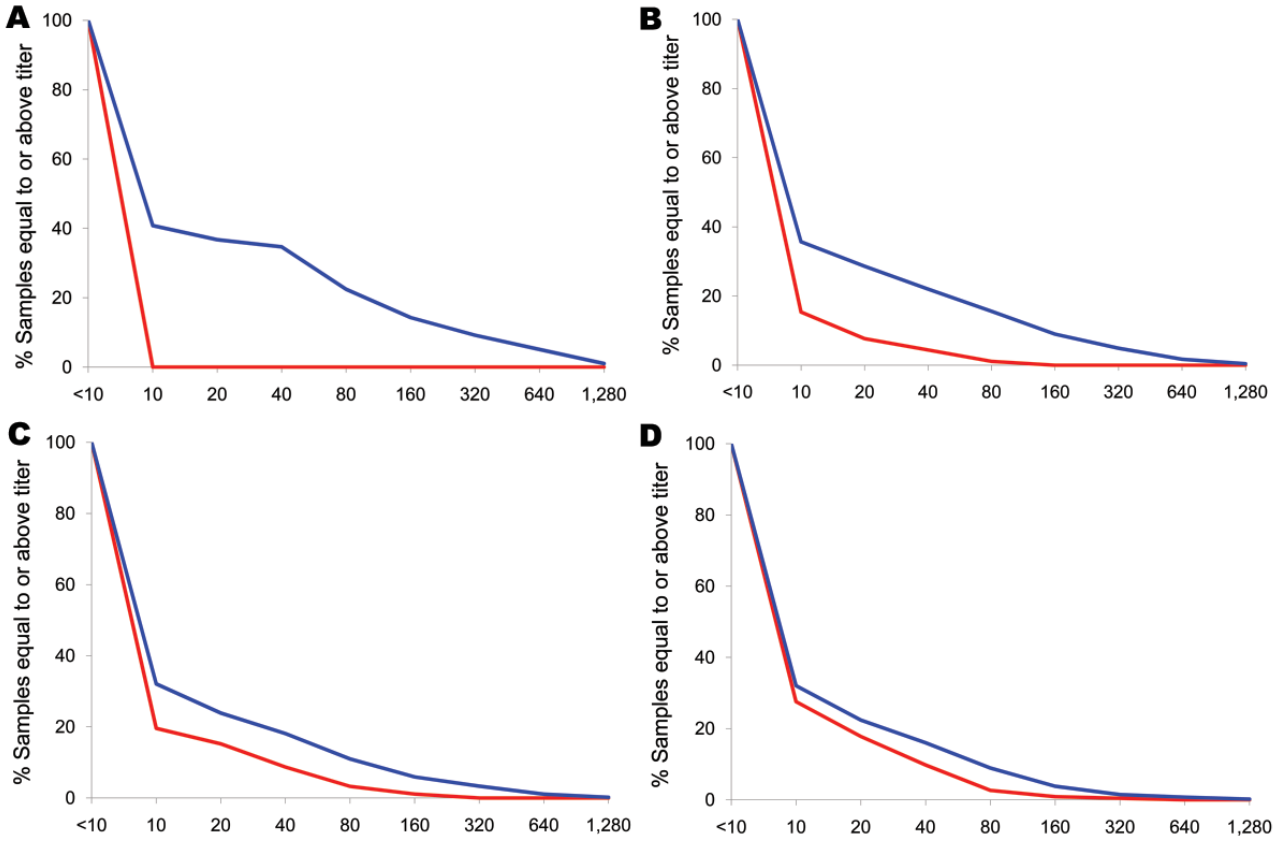

Figure 3. Reverse cumulative distributions by age group in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, September 2009, showing percentage of population with titer at or above each value. A) <15 years of age; B) 15–34 years of age; C) 35–54 years of age; D) ≥55 years of age. Red, prepandemic titer; blue, postpandemic titer.

data (*14*,*22*), although the difference in post-pandemic immunity in the Australian indigenous population has not been reported. Our overall attack rate was notably higher than the estimated clinical attack rate of 7.2% extrapolated from surveillance data (*23*). Moreover, the incidence rate ratio between indigenous and nonindigenous populations

Table 3. Multivariate logistic regression for exposures associated with titer ≥40 in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, September 2009

Characteristic	Odds ratio (95% confidence interval)	p value
Female sex	1.06 (0.82–1.37)	0.65
Aboriginal and Torres Strait Islander	2.32 (1.63–3.31)	<0.001
Age, y		<0.001
≥55	Reference	
35–54	1.05 (0.76–1.45)	
15–34	1.28 (0.91–1.79)	
≤14	2.98 (1.80–4.92)	
Region		0.05
Urban Darwin	Reference	
Rural Top End	0.83 (0.56–1.23)	
Central Australia	1.23 (0.80–1.90)	
Socioeconomic quintile*		0.43
5 (least disadvantaged)	Reference	
4	0.91 (0.55–1.51)	
3	1.16 (0.73–1.86)	
2	1.41 (0.84–2.36)	
1 (most disadvantaged)	1.21 (0.70–2.12)	

*Australian Bureau of Statistics' Socio-Economic Indexes for Area index of relative socioeconomic advantage and disadvantage.

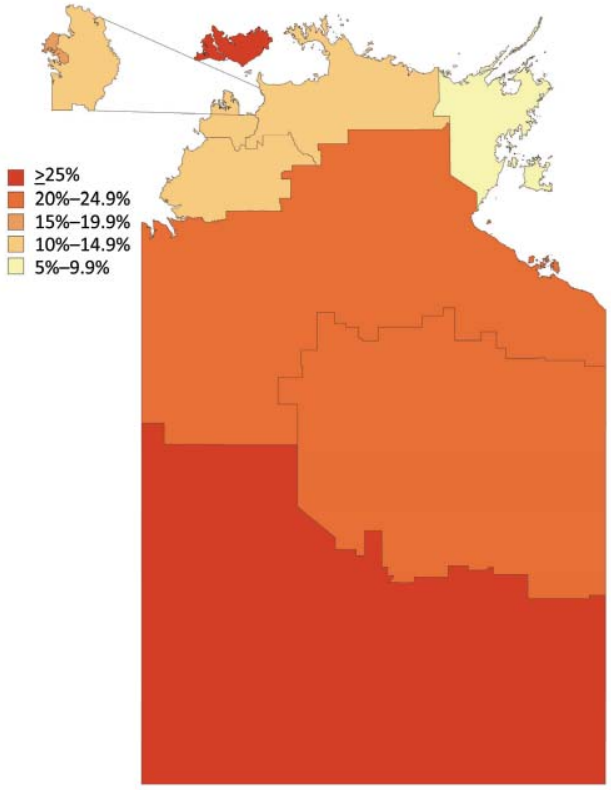

Figure 4. Postpandemic proportion immune by statistical subdivision in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, September 2009. Inset represents Urban Darwin.

based on the number of laboratory-confirmed cases was 4.9, notably greater than our 2-fold ratio. However, the ratio in serologic attack rates between Central Australia and the Top End was ≈1.5 and consistent with the ratio from laboratory-confirmed cases (*23*). Data from notifications and hospitalizations in the Top End indicate that the prevalence of risk factors in patients admitted with pandemic influenza was similar between indigenous and nonindigenous patients (*24*), suggesting that the increased frequency of admissions in indigenous persons was because of the greater prevalence of risk factors for severe disease.

Australian indigenous populations have more respiratory infections than nonindigenous groups (*25*), and the higher pandemic attack rate in this group is also consistent with their overrepresentation in admissions to intensive care units (*26*). North American indigenous persons also have a greatly increased risk for hospitalization and death from pandemic (H1N1) 2009 influenza compared with their nonindigenous counterparts, particularly at extremes of age (*27*). However, Australia has among the greatest differences in rates of hospitalization and mortality between indigenous and nonindigenous populations in the Americas and Pacific regions (*28*). For this reason, the NT Centre for Disease Control has identified this group as a particular focus of the univalent pandemic influenza vaccination program, achieving coverage of 24% overall and 41% in the indigenous population.

We used the Australian Bureau of Statistics SEIFA index as our measure of relative socioeconomic disadvantage (*12*). By this measure, the most disadvantaged quintile appeared to have higher rates of infection. However, this finding was not borne out by multivariate analysis, suggesting confounding by other variables, particularly indigenous status and remoteness, which are highly correlated in NT. Moreover, accurate estimates of socioeconomic disadvantage are notoriously difficult to attain (*29*) and, when measured by area, are at best at an average level of deprivation.

We observed marked differences in the postpandemic proportion immune between Statistical Subdivisions, with the degree of heterogeneity being particularly prominent among indigenous and remote populations. This variability in influenza infections has been noted from surveillance data (*30*) and serologic survey data (*14*). Although many Aboriginal communities in the NT are remote and isolated, a large proportion of persons from remote communities demonstrate intercommunity mobility (*31*). The Aboriginal population of the NT is known to have high rates of chronic diseases, including conditions identified as increasing susceptibility to influenza (*4*,*32*), such as poor housing (*33*) and sanitation (*34*). These factors, in particular overcrowding, are likely to facilitate transmission of influenza once the disease is present within a community.

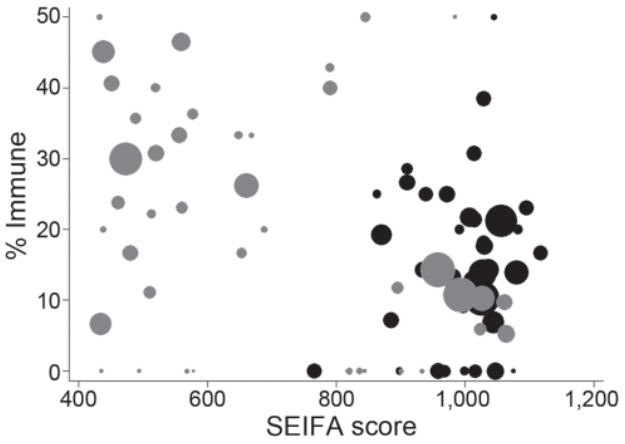

Figure 5. Postpandemic proportion of Statistical Local Area (SLA) demonstrating titers ≥40 by Socio-economic Index for Area (SEIFA) of relative socioeconomic disadvantage. Gray circles, Urban Darwin; black circles, Rural Top End and Central Australia. Circle size proportional to number of specimens in group. Lower score indicates greater degree of relative socioeconomic disadvantage. One SLA containing 1 observation with a proportion immune of 100% is not displayed.

Table 4. Attack rates standardized to Northern Territory population, by age group, indigenous status, and geographic region, in a study of differential effects of pandemic (H1N1) 2009 on remote and indigenous groups, Northern Territory, Australia, September 2009

Demographic characteristics	Adjusted attack rate, % (95% Confidence interval)
Overall	14.9 (11.0–18.9)
Sex	
F	15.4 (10.7–20.0)
M	14.4 (9.1–19.7)
Aboriginal and Torres Strait Islander	22.9 (16.0–29.9)
Nonindigenous	12.4 (8.1–16.8)
Age, y	
≤14	36.0 (25.5–46.4)
15–34	15.3 (9.8–20.9)
35–54	4.3 (–3.2 to 11.8)
≥55	3.5 (–1.2 to 8.2)
Geographic region	
Urban Darwin	12.8 (8.4–17.2)
Rural Top End	14.2 (8.0–20.4)
Central Australia	21.4 (12.8–30.1)
Socioeconomic quintile*	
5 (least disadvantaged)	13.6 (7.5–19.8)
4	10.0 (4.3–15.7)
3	14.6 (7.5–26.8)
2	24.0 (14.6–33.5)
1 (most disadvantaged)	13.8 (6.9–20.6)

*Australian Bureau of Statistics' Socio-Economic Indexes for Area index of relative socioeconomic advantage and disadvantage.

Our results suggest that although some communities were severely affected, others may have been less affected by the pandemic because of their isolation. These communities are likely to be particularly susceptible to subsequent waves of infection because East Arnhem communities were particularly hard hit by pandemic (H1N1) 2009 in 2010, and Central Australia communities were relatively spared. Moreover, the first cluster of laboratory-confirmed cases since the first pandemic wave occurred in June and July 2010 in the SLA with the lowest postpandemic proportional immunity of any SLA represented by ≥20 specimens (2/38, 5.4%).

Our serosurvey indicates that the full effect of the influenza pandemic on the NT may have been underestimated and highlights the differential impact of the virus on vulnerable groups, including children and indigenous populations. Our findings show similarities to other published data, but the results are more likely to be applicable to remote-living and ethnically diverse populations. Given that in all groups, the majority of the population is likely to remain susceptible to the virus following the pandemic, vaccination campaigns and public health responses are essential and should focus on high-risk groups, which requires respectful engagement with communities.

Acknowledgments

We gratefully acknowledge the assistance of the staff of Western Diagnostic Pathology in Darwin, Alice Springs, and Perth, including Adrian Sutton, Brian Tucker, Miles Beaman, Philip Suhr, Ian Meyer, Tracy Horner, Caroline Maxwell, Rachel Tuck, and Holly Steers. We also thank Louise Carolan, Rob Shaw, and Chantal Baas for performing the HI assays.

The study was funded by the Centre for Disease Control of the Northern Territory Department of Health and Families. Western Diagnostic Pathology sorted and provided the samples at no cost. The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health and Ageing.

Dr Trauer is a public health registrar at the Centre for Disease Control, a division of the Northern Territory Department of Health and Families. His research interests include tuberculosis and mycobacterial disease, pandemic influenza, and chronic obstructive pulmonary disease.

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Multiple Reassortment between Pandemic (H1N1) 2009 and Endemic Influenza Viruses in Pigs, United States

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As a result of human-to-pig transmission, pandemic influenza A (H1N1) 2009 virus was detected in pigs soon after it emerged in humans. In the United States, this transmission was quickly followed by multiple reassortment between the pandemic virus and endemic swine viruses. Nine reassortant viruses representing 7 genotypes were detected in commercial pig farms in the United States. Field observations suggested that the newly described reassortant viruses did not differ substantially from pandemic (H1N1) 2009 or endemic strains in their ability to cause disease. Comparable growth properties of reassortant and endemic viruses in vitro supported these observations; similarly, a representative reassortant virus replicated in ferrets to the same extent as did pandemic (H1N1) 2009 and endemic swine virus. These novel reassortant viruses highlight the increasing complexity of influenza viruses within pig populations and the frequency at which viral diversification occurs in this ecologically important viral reservoir.

Since its detection in humans in April 2009 (*1*), the pandemic influenza A (H1N1) 2009 virus spread quickly throughout the world. The pandemic virus was first detected in pigs in Canada in early May 2009 (*2*), and at least 14 countries have reported pigs infected with pandemic (H1N1) 2009 viruses (*3*), a few of which have been thoroughly described in the literature: in the Americas during summer 2009 (*4*,*5*); in Norway and Italy during fall 2009 (*6*,*7*); in India in May, June, and November 2009 (*8*); in Hong Kong Special Administrative Region, People's Republic of China, during October 2009–January 2010 (*9*); and in South Korea and Thailand during December 2009 (*10*,*11*). All of these infections were caused by human-to-pig transmission.

In the United States, H1N1 subtypes of the classical swine influenza lineage (now designated as H1α) dominated from 1918 through 1998. In ≈1998, influenza (H3N2) triple reassortant viruses appeared, accompanied by a transient increase in disease severity (*12*,*13*). These triple reassortants contained polymerase acidic (PA) and polymerase basic 2 (PB2) genes of avian virus origin; hemagglutinin (HA), neuraminidase (NA), and polymerase basic 1 (PB1) genes of human virus origin; and matrix (M), nucleoprotein (NP) and nonstructural (NS) genes of classical swine virus origin (*13*). After these viruses appeared, multiple reassortment occurred that combined different HA and NA genes with the triple reassortant internal gene (TRIG) cassette (PA, PB1, PB2, NP, M, and NS) (*14*–*20*).

In addition to H1α, 3 distinct lineages of H1 hemagglutinin have been defined and characterized: H1β strains, first detected in 2001–2002; H1δ (or "seasonal human-like" swine H1) strains in 2003–2005; and H1γ strains in 1999–2000 (*19*,*21*). Soon after the appearance of pandemic (H1N1) 2009 viruses (whose HA clusters with the swine H1γ viruses) in pigs, the first reassortment event with an endemic swine influenza virus was reported in pigs in Hong Kong. This virus, A/swine/201/2010, contained a Eurasian swine lineage HA, a pandemic (H1N1) 2009 NA, with the TRIG cassette (*9*). Subsequently, a reassortant with 7 pandemic (H1N1) 2009 gene segments and a swine N2 gene was found in Italy (*22*), and a reassortant with 7 pandemic (H1N1) 2009 gene segments and a swine N1

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DOI: http://dx.doi.org/10.3201/1709.110338

gene was found in Germany (*23*). Considering the known circulation of TRIG-containing endemic and pandemic (H1N1) 2009 viruses in pigs, the chance for similar reassortment to occur in the United States also seemed high.

We describe the isolation of 9 pandemic (H1N1) 2009/endemic swine reassortant influenza viruses representing 7 distinct genotypes in pigs in the United States. Our study highlights the effect of reverse zoonotic transmission of the pandemic virus on this population.

Materials and Methods

Samples

Samples used in this study were nasal swabs or lungs collected from pigs with clinical signs of respiratory disease, with the exception of A/swine/Indiana/240218/2010, which was isolated from a healthy pig within the framework of an active swine influenza surveillance program. In this program, nasal swab specimens had been randomly collected on a monthly basis since June 2009 from commercial farms in Iowa, Indiana, Minnesota, North Carolina, and Illinois. A/swine/Indiana/240218/2010 was 1 of 176 viruses detected. Vaccination status of the pigs was unknown. The specimens were transported in virus transport media at 4°C to the laboratory (Newport Laboratories, Worthington, MN, USA, or St. Jude Children's Research Hospital, Memphis, TN, USA) for influenza screening. Samples were either tested within 48 h or frozen at −80°C before being processed.

RNA Extraction and Real-time Reverse Transcription PCR

RNA was extracted either with a MagMAX–96 AI/ND viral RNA isolation kit (Applied Biosystems/Ambion, Austin, TX, USA) on a Kingfisher Flex (Thermo Scientific, Rockford, IL, USA), or with QIAGEN viral RNA kit (QIAGEN, Valencia, CA, USA), following the manufacturers' instructions. Real-time reverse transcription PCR (rRT-PCR) was performed to initially screen for all influenza A viruses (*24*). Positive samples were then screened specifically for swine H3 and H1 HA genes (*25*) and the pandemic (H1N1) 2009 M gene (*24*). One-step RT-PCR was performed by using the QIAGEN 1-step RT-PCR kit (QIAGEN), 600 nmol/L of each primer, 300 nmol/L of the probe, and 1.4 mmol/L $MgCl_2$. The ABI Fast real-time PCR system 7500 thermocycler and corresponding software (Applied Biosystems, Foster City, CA, USA) were used with the following cycling conditions: 50°C for 30 min, 95°C for 15 min, followed by 40 cycles of 95°C for 10 sec, and 60°C for 30 sec. For the growth curves, viral titers were monitored by rRT-PCR by using the method described by Harmon et al. (*26*).

Virus Isolation and Growth

Swab samples were added to MDCK or swine testicle (ST) cells (American Type Culture Collection) as described (*27*,*28*). Virus isolates were identified by HA assay, as described in the World Health Organization manual on animal influenza diagnosis and surveillance (*28*). Specimens were plaque purified 2× on either MDCK or ST cells. Isolates were then characterized by full genome sequencing. Virus growth characteristics were compared on ST cells. Approximately 1.0 50% tissue culture infectious dose per milliliter of each virus was added to a confluent monolayer of ST cells. An aliquot was immediately removed after inoculation and every 24 hours through 4 days postinoculation. Samples were analyzed by using rRT-PCR that targeted the M gene and by titration on ST cells by using the method of Spearman-Karber.

Sequencing and Sequences Analysis

Specimens were sequenced by using an Illumina Genome Analyzer (Illumina, Inc., San Diego, CA, USA). An RT-PCR was performed on RNA templates by using Uni-12 and Uni-13 primers to amplify all 8 segments in 1 reaction with Invitrogen SuperScript III One-Step Reverse Transcriptase and Platinum *Taq* HiFi (Invitrogen, Carlsbad, CA, USA). Polymerase gene primers were added to optimize the sequencing reaction (*29*). The obtained double-stranded DNA was sonicated in a Covaris AFA (Covaris, Woburn, MA, USA) until a broad peak at 200 bp appeared. The 3′ overhangs were removed from the sheared DNA by end repair, a Poly-A tail was added, and adapters were then ligated to the DNA fragments by using New England Biolabs (NEB) kits E6050L, E6053L, and E6056L (NEB, Ipswich, MA, USA). The ligation products were purified by gel electrophoresis by using E-Gel SizeSelect 2% agarose precast gels (Invitrogen). Index sequences were added to the DNA samples by Phusion DNA polymerase (NEB) before they were loaded on the illumina sequencer.

For sequence analyses, samples were de-multiplexed and each genome was assembled by using CLC Genomics Workbench software (CLC bio, Germantown, MD, USA) by running a high stringency de novo assembly. Sequences were compared by using BioEdit (*30*) and ClustalW (*31*). Phylogenetic analyses were performed by using MEGA version 4.0.2 (*32*). The sequences of the 9 influenza viruses we studied were submitted to GenBank under accession nos. CY086877–CY086942.

Assessment of Virus Pathogenicity in Ferrets

The pathogenicity of selected influenza viruses was tested in male ferrets (*Mustela putorius furo*) 3–4 months of age obtained from Triple F Farms (Sayre, PA, USA). All ferrets were seronegative for seasonal influenza A (H1N1) and (H3N2) viruses, pandemic (H1N1) 2009,

and influenza B viruses by hemagglutination-inhibition assay. Five ferrets were inoculated intranasally under light isoflurane anesthesia with 10^6 50% egg infectious dose (EID_{50}) of the subtype H3N2 reassortant, A/swine/Minnesota/239105/2009, in 1 mL of sterile phosphate-buffered saline. Pandemic (H1N1) 2009 strain A/Tennessee/560–1/2009 (TN/560/09) and influenza (H3N2) strain A/swine/Texas/4199–2/98 (sw/TX/4199/98) were used as controls for comparison. Clinical signs of infection, relative inactivity index (*33*), weight, and temperature were recorded throughout the 12-day study period.

To monitor virus shedding, nasal washes were collected from ferrets on days 3, 5, and 7 postinoculation as described (*34*). The virus titers were determined as $\log_{10}$ EID_{50}/mL. The limit of virus detection was 0.5 $\log_{10}$ EID_{50}/mL. For calculation of the mean, samples with a virus titer <0.5 $\log_{10}$ EID_{50}/mL were assigned a value of 1.

All animal experiments were performed in BioSafety level 2+ facilities at St. Jude Children's Research Hospital (Memphis, TN, USA). All animal studies were approved by the St. Jude Children's Research Hospital Animal Care and Use Committee and were conducted according to applicable laws and guidelines.

Results

Identification of Endemic Swine–Pandemic (H1N1) 2009 Influenza Virus Reassortants

During routine surveillance for influenza viruses in pigs, 9 reassortant viruses were detected during 2009 and 2010. These viruses came from asymptomatic animals or from animals showing classic influenza symptoms, including coughing, respiratory distress, fever, or nasal discharge. These viruses were detected in Minnesota, Indiana, and North Carolina. Complete genome sequences were obtained for 7 strains: A/swine/Indiana/240218/2010(H1N2) (sw/IN/240218/10), A/swine/Minnesota/239105/2009(H3N2) (sw/MN/239105/09), A/swine/Minnesota/239106/2010(H1N2) (sw/MN/239106/10), A/swine/North Carolina/239108/2010(H1N2) (sw/NC/239108/10), A/swine/North Carolina/226124/2010 (H1N2) (sw/NC/226124/10), A/swine/North Carolina/226125/2010(H1N2) (sw/NC/226125/10), and A/swine/North Carolina/226126/2010(H1N2) (sw/NC/226126/10). Partial sequences were obtained from 2 additional samples, A/swine/Minnesota/340304/2010(H1N2)(sw/MN/340304/10), and A/swine/Minnesota/226128/2010(H1N1) (sw/MN/226128/10). Of the 9 viruses, 8 displayed HA and NA genes of endemic swine influenza viruses and pandemic (H1N1) 2009 M gene segments; the origin of the remaining gene segments differed depending on the virus (Figure 1). The phylogeny of HA, NA, and M genes were compared with reference strains (Figure 2, panels A, B, C, respectively). Only sw/NC/226124/10, sw/NC/226125/10, and sw/NC/226126/10 had identical genotypes with pandemic (H1N1) 2009 NP, M, and NS genes. These 3 viruses were isolated during a short period from the same general location: sw/NC/226124/10 and sw/NC/226125/10 came from the same farm, and sw/NC/226126/10 was isolated 48 hours later at a neighboring farm.

Eight of the 9 reassortant viruses were successfully isolated on either MDCK or ST cells. Sw/MN/340304/10 could not be isolated, most likely because of lack of initial material, because this specimen had the highest cycle threshold value by rRT-PCR targeting the M gene (cycle threshold value 36). Isolates were plaque-purified on MDCK or ST cells, and the genotypes were all confirmed. Taken together these data show that several novel genotypes of swine influenza viruses had been generated after the reverse zoonotic transmission of pandemic (H1N1) 2009 virus to pigs.

Replication and Pathogenicity of Pandemic Reassortant Influenza Virus In Vitro and In Vivo

To understand whether the increased genetic diversity created through the reassortment was associated with an increase in phenotypic diversity, select reassortants were assessed for growth in vitro and in ferrets. The growth characteristics of 6 endemic viruses from 2009–2010 and 3 pandemic reassortant viruses (sw/MN/239105/09, sw/MN/239106/10, and sw/NC/239108/10) were compared on ST cells. We found no difference in replication potential between any of these viruses, which suggests that no selective growth advantages had occurred through reassortment (Figure 3, panel A).

Because of the recent zoonotic transmissions of triple reassortant swine influenza (H3N2) viruses in the United States during 2010 (*36*), we also sought to determine whether reassortment had the potential to lead to a more pathogenic virus than previously circulating swine influenza strains. We used the ferret model to assess this possibility. Each of 5 ferrets was inoculated with 10^6 EID_{50} of sw/MN/239105/09 (H3N2). This reassortant virus caused only mild clinical signs (relative inactivity index ≈0.1) without marked weight changes or body temperature elevation (maximum of 3% weight loss, and 0.4°C increase in temperature; data not shown). The virus replicated to similar titers, as did the pandemic (H1N1) 2009 virus, TN/560/09, and the triple reassortant swine subtype H3N2 virus, sw/TX/4199/98, with a peak titer of infection of 5–6 $\log_{10}$ of virus and with viral clearance occurring ≈1 week postinfection (Figure 3, panel B). The similar disease and growth property of these viruses in ferrets again suggested that no unusual biologic properties had been inherited upon reassortment.

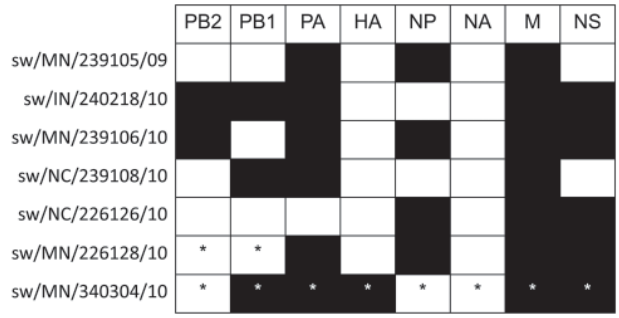

Figure 1. Lineages of North American reassortant swine influenza viruses identified through phylogenetic analyses. Pandemic and endemic gene segments are represented in black and white, respectively. *Denotes partial sequences. Isolates sw/NC/226124/10 and sw/NC/226125/10 (not shown) have the same genotype as sw/NC/226126/10. PB2, polymerase basic 2; PB1, polymerase basic 1; PA, polymerase acidic; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

Discussion

During 1998–2009, reassortment of influenza viruses in US pigs occurred relatively frequently (*37*). The genotypes of the viruses generated through these reassortments typically contained different swine or human influenza virus HA and NA genes in combination with the TRIG cassette (*13–20*). These reassortant viruses provided 6 of the 8 gene segments to the pandemic (H1N1) 2009 virus (*38*). Thus, and as indicated by similar events in other geographic locations, it was not unexpected that reassortment between pandemic (H1N1) 2009 and endemic swine influenza viruses would occur in US pigs after identification of the former virus in this population.

Somewhat unpredicted, however, was the number of reassortants that we identified in this study; 7 distinct viral genotypes were characterized. Although different genotypes were detected, each had an M gene of pandemic (H1N1) 2009 origin, a novel gene segment introduced into this animal population after human-to-pig transmission of the pandemic strain. The TRIG cassette in the reassortant viruses, a cassette that had remained relatively unchanged since 1998, was disrupted to include not only the M gene segment but also variably the NS, NP, and PA genes of pandemic (H1N1) 2009 virus. Because the pandemic virus contains M and NA gene segments from Eurasian-lineage swine influenza viruses and PB2, PB1, PA, NP, and NS gene segments from TRIG viruses, it is not surprising that several pandemic (H1N1) 2009 genes could be introduced into endemic US swine influenza viruses without altering the viability of the progeny viruses.

The inclusion of the pandemic (H1N1) 2009 M gene in the reassortants suggests a selective advantage to viruses containing it, although we were unable to measure any phenotypic differences in these viruses in our in vitro and in vivo assays. One phenotype that we did not measure was transmission, and it is tempting to speculate that the pandemic (H1N1) 2009 M gene segment could play a

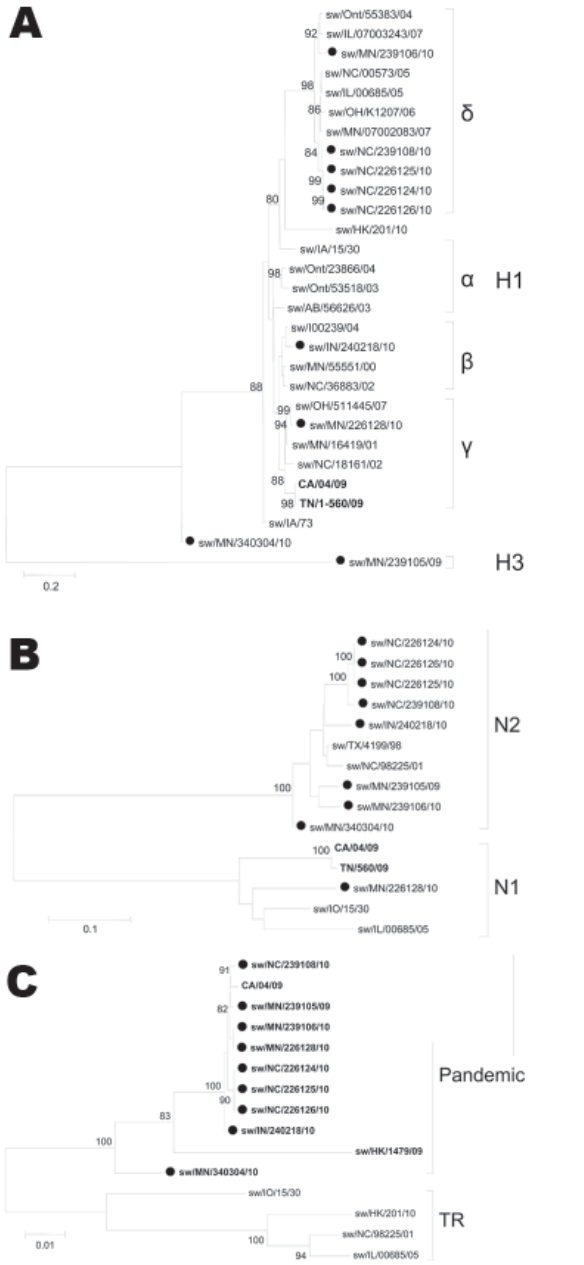

Figure 2. Phylogenetic trees of pandemic reassortant swine influenza viruses compared with currently circulating swine influenza strains: A) hemagglutinin (H); B) neuraminidase (N); C) matrix genes. The trees were constructed by using the neighbor-joining method (Kimura 2-parameter) with 1,000 bootstrap replicates. Only bootstrap values >74 are shown. Swine reassortant strains characterized in this study are indicated with a closed circle. **Boldface** indicates pandemic segments. Greek letters indicate virus genogroups; α represents classical swine influenza virus and δ seasonal human-like swine influenza virus. TR indicates swine triple reassortant influenza virus. Scale bars indicate nucleotide substitutions per site.

role here, both in terms of its selection in the reassortants described and in the human pandemic virus itself. Further studies are required to test this hypothesis.

The effect of these reassortants on the US pig industry is somewhat difficult to predict, although on the basis of the data generated here it is not likely to be great in terms of animal health. Antigenically, the reassortant influenza viruses carried HA genes already within the population (either endemic or pandemic (H1N1) 2009 viruses), and we were unable to detect any replication differences or unusual clinical signs in the field. Thus, and because HA-specific immunity is the target of current vaccines, the generation of these viruses is not expected to have any adverse effect on vaccine efficacy levels or disease severity in the field unless further adaptive changes occur as a result of continued circulation of these viruses.

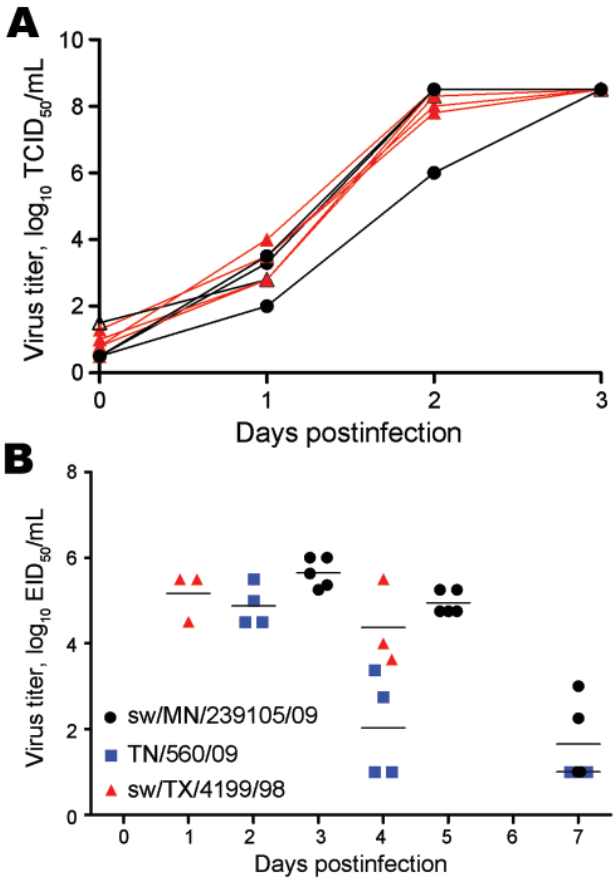

Figure 3. Replication of North American reassortant and endemic swine influenza viruses on swine testicle (ST) cells and in ferrets. A) The growth of 6 endemic swine viruses and 3 reassortant viruses (sw/MN/239105/09, sw/MN/239106/10, and sw/NC/239108/10) from 2009–2010 were analyzed in vitro. One curve corresponds to 1 isolate. Black lines and symbols indicate swine pandemic influenza reassortant viruses; red lines and symbols indicate swine triple reassortant (TR) influenza viruses. The progeny viruses released from infected ST cultures were collected at the indicated time points and titrated in ST cells by performing a 50% tissue culture infectious dose ($TCID_{50}$) assay. Negative $TCID_{50}$ titers were given the value 0.5; $\log_{10}$ $TCID_{50}$ ≥8.5 were given the value 8.5. B) Virus titers in the upper respiratory tract (nasal washes) of ferrets infected with 10^6 50% egg infectious dose (EID_{50}) pandemic (H1N1) 2009 TN/560/09 (*35*); 10^6 $TCID_{50}$ swine TR influenza virus sw/TX/4199/98; or 10^6 EID_{50} swine pandemic reassortant virus sw/MN/239105/09. Values are the mean ± SD for 4, 3, and 5 ferrets for TN/560/09, sw/TX/4199/98, and sw/MN/239105/09, respectively.

Although the reassortants invariably contained the pandemic virus M gene and, with the exception of 1 reassortant, endemic virus HA and NA genes, the fact that we only saw viruses of the exact same genotype in limited spatial and temporal space suggests that there is no single dominant reassortant yet. Indeed, it is possible that these reassortants are generated but quickly displaced by other influenza viruses. Nevertheless, the data presented here once again highlight the dynamic nature of influenza viruses in pig populations and the continued monitoring of viruses in US pigs at the level of full genome sequencing is absolutely required.

Acknowledgments

We thank Susan Detmer for her help with phylogeny, and the Hartwell Center, Department of Bioinformatics, St. Jude Children's Research Hospital, Memphis, Tennessee, for assistance with the sequencing. We acknowledge Nicole Grate-Hurt, Jessi Woods, Jess Waddell, Chase Stahl, Micah Taylor, Britni Miller, Samantha Doyle, Dayle Dillon, Mike Kuhn, Max Rodibaugh, and Darryl Ragland for collecting and shipping specimens. We thank Emily Collin and Luke Kroger for assistance with virology and molecular analysis.

This study was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, US Department of Health and Human Services, under contract no. HHSN266200700005C; and by the American Lebanese Syrian Associated Charities.

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Role of *Chlamydia trachomatis* in Miscarriage

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To determine the role of *Chlamydia trachomatis* in miscarriage, we prospectively collected serum, cervicovaginal swab specimens, and placental samples from 386 women with and without miscarriage. Prevalence of immunoglobulin G against *C. trachomatis* was higher in the miscarriage group than in the control group (15.2% vs. 7.3%; p = 0.018). Association between *C. trachomatis*–positive serologic results and miscarriage remained significant after adjustment for age, origin, education, and number of sex partners (odds ratio 2.3, 95% confidence interval 1.1–4.9). *C. trachomatis* DNA was more frequently amplified from products of conception or placenta from women who had a miscarriage (4%) than from controls (0.7%; p = 0.026). Immunohistochemical analysis confirmed *C. trachomatis* in placenta from 5 of 7 patients with positive PCR results, whereas results of immunohistochemical analysis were negative in placenta samples from all 8 negative controls tested. Associations between miscarriage and serologic/molecular evidence of *C. trachomatis* infection support its role in miscarriage.

The incidence of *Chlamydia trachomatis* infection has dramatically increased during the past 10 years (*1*). Mostly asymptomatic, untreated *C. trachomatis* infections are responsible for a large proportion of salpingitis, pelvic inflammatory disease, ectopic pregnancy, and infertility in women. *C. trachomatis* is a recognized agent of preterm labor and premature rupture of membranes (*2*,*3*). However, its role in miscarriage is unclear (*2*,*3*).

C. trachomatis has been isolated or detected in cervical smear, urine (*4*–*6*), or products of conception (*7*,*8*). Nevertheless, none of these studies demonstrated association between isolation of *C. trachomatis* and miscarriage. However, culturing *C. trachomatis* is technically difficult, given its strict intracellular life cycle. Even with molecular approaches, detecting *C. trachomatis* can be difficult because of PCR inhibitors or low number of copies often present in the lesions (*4*–*7*). Moreover, infection could be localized at deeper sites not amenable to sampling (*9*).

Several studies have reported a higher prevalence of *C. trachomatis* antibodies in spontaneous (*10*,*11*) or recurrent (*2*,*9*,*11*,*12*) miscarriages. The inability to detect immunoglobulin (Ig) M or to isolate *C. trachomatis* from any of these seropositive patients might suggest that *Chlamydia* spp. are not directly associated with miscarriage (*9*,*12*). Other seroepidemiologic studies have failed to find any correlation between *C. trachomatis* and spontaneous (*13*–*16*) or recurrent miscarriage (*17*,*18*).

The main purpose of this study was to investigate whether *C. trachomatis* is associated with miscarriage. We used molecular, serologic, and immunohistochemical approaches to compare evidence of present and past *C. trachomatis* infection in women with or without miscarriage.

Materials and Methods

During November 2006–June 2009, a total of 386 women were prospectively enrolled at the obstetric department of the University Hospital of Lausanne (Lausanne, Switzerland). The miscarriage group comprised 125 women consulting at the emergency gynecology ward for an acute miscarriage. The control group comprised 261 women attending the labor ward with an uneventful pregnancy and without any history of miscarriage, stillbirth, or preterm labor. All women gave written consent, and the local ethical committee approved the study.

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DOI: http://dx.doi.org/10.3201/eid1709.100865

We collected demographic and obstetric data prospectively. Placenta (or products of conception in cases of miscarriage), cervicovaginal swab specimens, and serum were sampled at the time of labor and of miscarriage.

All serum samples were tested for IgG and IgA against *C. trachomatis* with the Ridascreen *Chlamydia* IgG/IgA Kit (R-biopharm, Darmstadt, Germany) according to the manufacturer's instructions and by using Dynex DSX (Magellan Biosciences, Chantilly, VA, USA). Cervicovaginal swabs and placenta were extracted by using QIAamp DNA Mini kit (QIAGEN, Hilden, Germany). Samples were screened for *C. trachomatis* DNA by using a TaqMan real-time PCR specific for the cryptic plasmid of *C. trachomatis*, as described (*19*). A PCR inhibition control was used to verify that absence of amplification was not caused by PCR inhibitors. Only 1 of the 386 PCR inhibition controls was negative. This sample was thus retested at a 1:10 dilution.

Hematoxylin and eosin–stained histologic sections of all placentas were investigated for deciduitis, vasculitis, endometritis, or chorioamnionitis. Histologic samples were read blindly by a pedopathologist (M.-C.O.). Samples positive for *C. trachomatis* by real-time PCR were tested by immunohistochemical analysis (IHC). Presence of *C. trachomatis* on histologic sections was assessed by using a specific mouse monoclonal antibody, as described (*20*). To test the placental specimens, we used a commercial *Chlamydiaceae* family–specific monoclonal antibody directed against the chlamydial lipopolysaccharide (clone AC1-P; Progen, Heidelberg, Germany) at a dilution of 1:200. Detection was performed with the Dako ChemMate detection Kit (Dako, Glostrup, Denmark) according to the manufacturer's instructions. Antigen retrieval was performed by 10-min enzyme digestion (proteinase K; Dako). Immersion of the slides in peroxidase-blocking solution for 5 min at room temperature resulted in blocking of endogenous peroxidase activity. Specimens were incubated with primary antibody for 1 h. Sections were incubated for 10 min at room temperature with the link-antibody (Dako), followed by 10 min incubation with horseradish peroxidase (Dako) and finally developed in 3-amino, 9-ethyl-carbazole substrate solution for 10 min at room temperature and counterstained with hematoxylin. Using the antibody diluent instead of the primary antibody, we performed a negative control of each section. Moreover, 8 placentas from *C. trachomatis* PCR-negative patients were randomly selected as negative controls. IHC was blindly read by 2 pathologists with experience in chlamydial IHC (S.B., N.B.) and confirmed by a pedopathologist (M.-C.O.).

We compared demographic data and risk factors of patients with and without miscarriage or *C. trachomatis* infection by the Pearson χ^2 test (or the Fisher exact test when indicated) for categorical variables. For continuous variables, medians were compared by the Wilcoxon-Mann-Whitney test. Multivariate logistic regression was performed to identify factors independently associated with miscarriage or with *C. trachomatis* infection. Statistical analyses were performed by using Stata version 10.0 (StataCorp LP, College Station, TX, USA).

Results

Of 395 patients, 9 (2.3%) were excluded because of missing serum or vaginal swab samples. Sociodemographic data for the remaining 386 women are shown in Table 1.

A total of 16 (4.2%) patients were positive for IgG and IgA against *C. trachomatis*, 22 (5.7%) were positive only for IgG against *C. trachomatis*, and 4 (1.0%) were positive only for IgA against *C. trachomatis*. Prevalence of IgG against *C. trachomatis* was higher in the miscarriage group (15.2%) than in the control group (7.3%; p = 0.018) (Table 1). This association between miscarriage and IgG against *C. trachomatis* remained significant, even after adjustment for age, origin, education, and number of sex partner (odds ratio [OR] 2.3, 95% confidence interval [CI] 1.1–5.1). Similarly, prevalence of IgA against *C. trachomatis* was higher in the miscarriage group (8.0%) than in the control group (3.8%), but this trend was not significant (p = 0.091) by univariate analysis. When adjusted for age, origin, education, and number of sex partners, the association between miscarriage and IgA against *C. trachomatis* was significant (OR 2.7, 95% CI 1.1–7.4).

Multivariate logistic regression including all sociodemographic variables (Table 1) and *C. trachomatis* IgG serologic results identified 5 independent factors positively or negatively associated with miscarriage: *C. trachomatis* IgG–positive serologic results (OR 2.3, 95% CI 1.1–4.9), age ≥35 years (OR 2.7, 95% CI 1.6–4.4), European origin (OR 0.3, 95% CI 0.2–0.5), marriage (OR 0.4, 95% CI 0.2–0.7), and 1 lifetime sex partner (OR 0.4, 95% CI 0.2–0.7).

C. trachomatis DNA was more frequently amplified from products of conception or placenta from women with miscarriage (5 [4.0%] women) than from controls (2 [0.7%], p = 0.026). Most patients with a positive PCR result for placenta also had a positive result for vaginal swab specimens (Table 2). Six of the 7 patients with *C. trachomatis* DNA in the cervicovaginal swab specimen also had positive findings in the placenta. Thus, again, cervicovaginal *C. trachomatis* DNA was more often detected in patients from the miscarriage group (n = 5, 4.0%) than from the control group (n = 2, 0.7%; p = 0.026). All 7 patients with *C. trachomatis* DNA in the cervicovaginal swab also exhibited IgG against *C. trachomatis*, whereas all patients but 1 with *C. trachomatis* DNA in the placenta exhibited IgG against *C. trachomatis* (Table 2). Both

patients with *C. trachomatis* DNA and IgG and IgA against *C. trachomatis* belonged to the miscarriage group.

All placentas were analyzed for inflammation (Figure 1). In the basal plate, inflammatory cells (deciduitis) were present in 15 (39.5%) of 38 patients and 91 (26.1%) of 348 patients with and without *C. trachomatis* IgG–positive serologic results, respectively (p = 0.081). This trend was observed to a lesser extent when *C. trachomatis* IgA serologic results were considered (7 [35.0%] of 20 vs. 99 [26.3%] of 376; p = 0.446).

All 8 persons with samples positive for *C. trachomatis* by real-time PCR in the placenta (n = 7) or cervicovaginal swab specimen (n = 7) were tested by IHC (Table 2; Figure 2). *C. trachomatis* was confirmed in 4 of 6 placentas from

Table 1. Characteristics of 386 women, by miscarriage history, in a study of the role of *Chlamydia trachomatis* in miscarriage, University Hospital of Lausanne, Lausanne, Switzerland, November 2006–June 2009*

Characteristic	Control group, no. (%), n = 261	Miscarriage group, no. (%), n = 125	p value
Age, y†			
<35	194 (74.3)	71 (56.8)	0.001
≥35	67 (25.7)	54 (43.2)	
No. pregnancies‡			
1	141 (54.0)	38 (30.4)	<0.001
2	78 (29.9)	32 (25.6)	
>2	42 (16.1)	55 (44.0)	
Parity§			
0	160 (61.3)	62 (49.6)	0.066
1	72 (27.6)	41 (32.8)	
>1	29 (11.1)	22 (17.6)	
Origin			
European	217 (83.1)	69 (55.2)	<0.001
Non-European	44 (16.9)	56 (44.8)	
Marital status			
Married	201 (77.0)	90 (72.0)	0.193
Single	49 (18.8)	24 (19.2)	
Divorced	11 (4.2)	11 (8.8)	
Education			
Non–university studies	170 (65.1)	96 (76.8)	0.025
University studies	91 (34.9)	29 (23.2)	
No. lifetime sex partners			
1	58 (22.2)	37 (29.6)	0.031
2 or 3	43 (16.5)	24 (19.2)	
4–6	45 (17.2)	10 (8.0)	
>6	36 (13.8)	10 (8.0)	
No answer	79 (30.3)	44 (35.2)	
Previously used contraceptive method			
Pill	101 (38.7)	36 (28.8)	0.093
Condoms	68 (26.1)	34 (27.2)	
Other	19 7.3)	6 (4.8)	
Never used contraception	73 (28.0)	49 (39.2)	
Smoking status			
Nonsmoker	224 (85.8)	106 (84.8)	0.877
Smoker	37 (14.2)	19 (15.2)	
C. trachomatis serologic results			
IgG+	19 (7.3)	19 (15.2)	0.018
IgA+	10 (3.8)	10 (8.0)	0.091
IgG+ and IgA+	7 (2.7)	9 (7.2)	0.037
IgG+ or IgA+	22 (8.4)	20 (16.0)	0.025
C. trachomatis PCR			
Cervicovaginal swab	2 (0.8)	5 (4.0)	0.026
Placenta	2 (0.8)	5 (4.0)	0.026
≥1 PCR positive	2 (0.8)	6 (4.8)	0.009

*Ig, immunoglobulin.
†Age, y, mean ± SD: controls, 31.5 ± 5.0; women with miscarriage, 33.3 ± 6.1; p = 0.002.
‡No. pregnancies, mean ± SD: controls, 1.7 ± 0.9; women with miscarriage, 2.6 ± 0.5; p<0.001.
§Parity, mean ± SD: controls, 0.5 ± 0.8; women with miscarriage, 0.8 ± 1.0; p = 0.008.

Table 2. Clinical history and serologic, PCR, and IHC results of 8 women with samples positive for *Chlamydia trachomatis* by real-time PCR, University Hospital of Lausanne, Lausanne, Switzerland, November 2006–June 2009*

Study group, patient no.	No. pregnancies	Parity	Pregnancy, wk	*C. trachomatis* PCR					Placental histology
				IgG	IgA	Placenta PCR	Vagina PCR	IHC	
Miscarriage group									
235	2	0	8	+	–	+	+	+	Lymphocytes in chorion, acute endometritis
355	1	0	7	+	–	+	+	–	Polymorphonuclear cells in decidua
518	2	0	6	+	–	+	+	+	Subchorial fibrin, lymphocytes in decidua
564	5	2	12	+	+	+	+	+	Lymphocytes in decidua
568	2	1	6	-	–	+	–	–	Lymphocytes in decidua, hemorrhagic necrosis
460	1	0	11	+	+	–	+	+	Presence of eosinophils
Control group									
35	2†	1	37	+	–	+	+	–	Histiocytes, rare calcifications
390	1	1	40	+	–	+	+	+	Chronic deciduitis

*Ig, immunoglobulin; IHC, Immunohistochemical analysis; +, positive; –, negative.
†One previous termination of pregnancy.

women with miscarriage and in 1 of 2 placentas from women with uneventful pregnancies, whereas none of the 8 *C. trachomatis* DNA–negative controls randomly selected exhibited the bacteria by IHC. *C. trachomatis* predominantly localized around endometrial glands of the chorion (Figure 2), associated with different degree of inflammation (Figure 1).

We also compared characteristics of patients with (n = 38) and without (n = 348) *C. trachomatis* IgG–positive serologic results. Number of pregnancies, parity, marital status, education, number of lifeltime sex partners, and smoking status were all associated with *C. trachomatis* IgG–positive serologic results by univariate analysis. Women who declined to provide information on the number of sex partners had a *C. trachomatis* IgG prevalence of 12.2%, whereas none of the 95 women who reported having 1 sex partner had *C. trachomatis* IgG–positive serologic result. In multivariate analyses, independent factors positively or negatively associated with *C. trachomatis* IgG–positive serologic results were ≥2 lifetime sex partners (OR 3.3, 95% CI 1.4–7.7), divorced women (OR 4.9, 95% CI 1.7–14.3), European origin (OR 0.4, 95% CI 0.2–0.9), and attending a university (OR 0.2, 95% CI 0.1–0.6). Age and smoking were not independently associated with *C. trachomatis* IgG–positive serologic results.

Discussion

We found an association of spontaneous miscarriage with serologic (p = 0.018) and molecular (p = 0.026) evidence of *C. trachomatis* infection. Moreover, *C. trachomatis* in the placenta was documented by specific IHC. *C. trachomatis* was mainly localized in the epithelial cells of endometrial glands.

Several studies have failed to document an association between *C. trachomatis* and spontaneous (*13*–*16*) or recurrent miscarriage (*17*,*18*). However, these studies were conducted >10 years ago, i.e., before the recent dramatic increase in the prevalence and incidence of *C. trachomatis* infection (*1*,*21*,*22*). Because of improved statistical power, such increased prevalence might indicate an association between *C. trachomatis* infection and adverse pregnancy outcomes. Second, sensibility and specificity of diagnostic

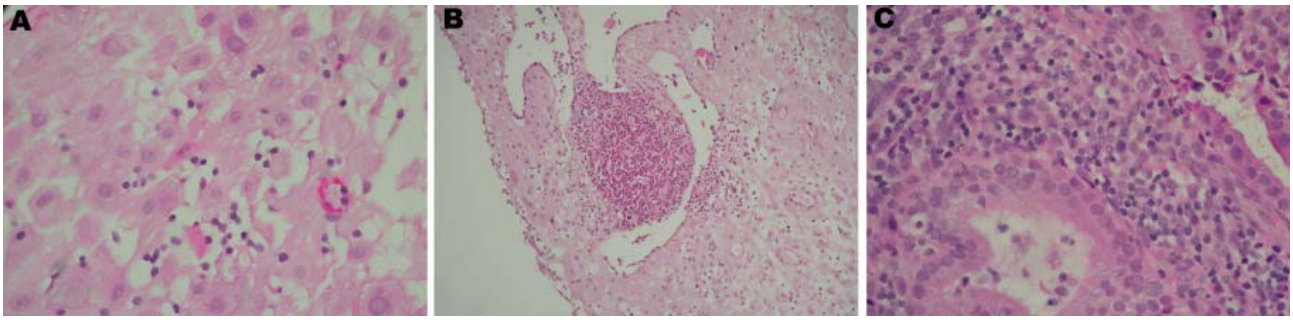

Figure 1. Placental histologic results from 3 women with real-time PCR–positive results for *Chlamydia trachomatis* (Table 2). A) Case-patient 390; B) case-patient 235; C) case-patient 564. Histologic analysis shows different degree of periglandular lymphocytes infiltration, with a microabscess in panel B. Original magnifications ×600 except panel B (×400).

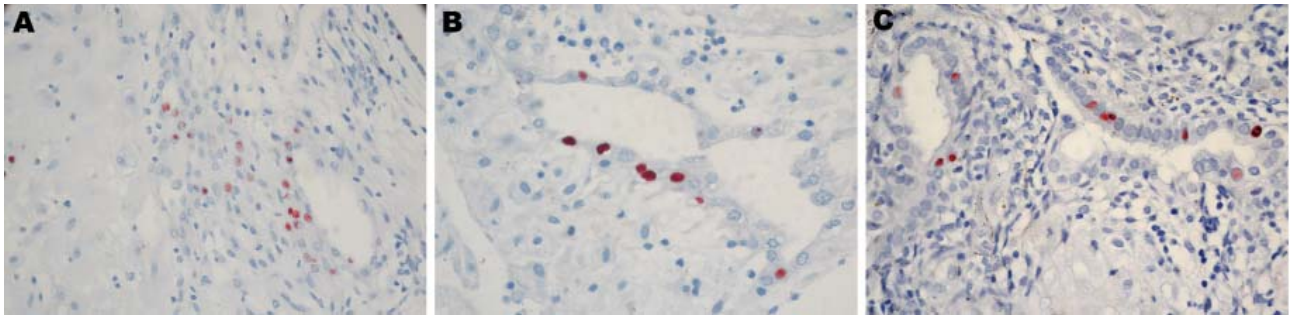

Figure 2. Immunohistochemical analysis of placentas in Figure 1. These placentas were obtained from 3 patients positive for *Chlamydia trachomatis* by real-time PCR. A) Case-patient 390; B) case-patient 235; C) case-patient 564. Immunohistochemical analysis demonstrated *C. trachomatis*–infected cells from endometrial glands. Original magnification ×600.

methods have also improved during the past decade. Thus, the high *C. trachomatis* seroprevalence observed in the control group of several older studies, ranging from 28% to 53% (*16*,*17*) was likely to have resulted from a low specificity of the serologic test used at that time. The *Chlamydia* IgG/IgA kit from R-biopharm we used in the present study exhibited better specificity than did 4 other commercially available tests for detecting IgG against *C. trachomatis* (*23*) and is thus more likely to identify a slight but true association. Moreover, the sensitivity of the *C. trachomatis* TaqMan real-time PCR we used here is high, detecting even <10 DNA copies. This validated assay also detects strains that contain a recently identified 350-bp deletion in the cryptic plasmid (*24*,*25*) because the 71-bp DNA fragment amplified is 93 bp downstream from the deletion (*19*).

The serologic association we observed is unlikely to be due to cross-reactivity with other chlamydial species such as *C. abortus* (previously classified as *C. psittacci* senso lato) because we also observed a molecular association with miscarriage. Moreover, the PCR we used was specific at species level because the *C. abortus* genome contains no cryptic plasmid. Finally, *C. abortus* has been only infrequently associated with miscarriages in humans (*26*), mostly after zoonotic exposure.

Miscarriage could be induced by a persistent asymptomatic *C. trachomatis* infection spreading to the fetal tissue or endometrium. Relatively few miscarriages occur during *C. trachomatis* primary infection, which explains the absence of association with IgA. That several patients exhibited *C. trachomatis*–positive serologic results without *C. trachomatis* DNA suggests that miscarriage might also occasionally be induced by damage from a past chlamydial infection or persistent *C. trachomatis* antibodies that might interfere with embryonic antigens (*2*).

A limitation of our study was the absence of investigation of other infectious etiology of miscarriage. Some viruses can produce chronic or recurrent maternal infection. In particular, cytomegalovirus during pregnancy can reach the placenta by hematogenous spread or by ascending route from the cervix. Parvoviruses also have been implicated in the development of repeated fetal loss. Among bacterial infections, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and bacterial vaginosis have been mostly associated with miscarriages (*27*). In addition, several intracellular bacteria such as *Coxiella burnettii* (*28*), *Brucella abortus* (*29*), and *Waddlia chondrophila* (*11*) have been associated with miscarriage.

Our study shows an association between miscarriage and molecular and serologic evidence of *C. trachomatis* infection. Several previous studies failed to document such an association probably because of the limited number of patients in some of these studies resulting from the lower prevalence of *C. trachomatis* infection in the late 20th century and to lower sensitivity or specificity of diagnostic methods available at that time. The results of our study suggest that all women experiencing a miscarriage should be screened for *C. trachomatis* infection and, if positive, adequately treated to prevent recurrent miscarriages. Moreover, preconceptional screening might be proposed to reduce the prevalence of this adverse pregnancy outcome.

Acknowledgments

We thank the midwives and doctors who participated to the sampling of this study. Their involvement was essential to the whole process, and they enthusiastically gave their time to provide information and samples. We thank Francoise Damnon and Andre Baud for computer assistance, Sebastien Aeby for technical help, and Carine Groux and Corinne Grau for *C. trachomatis* ELISA. We thank Carmen Kaiser and Belinda Senn for performing the chlamydial IHC.

This project was supported mainly by an interdisciplinary grant from the University of Lausanne and partially by grants from the Swiss National Science Foundation (32C0B0-116445 and 310030-130466); by the Institute of Microbiology; and by the Department of Obstetrics and Gynecology. G.G. is supported by the Leenards Foundation through a career award entitled "Bourse Leenards pour la relève académique en médecine clinique à

Lausanne." D.B. is supported by the "Société Académique Vaudoise" through the "Paul Blanc" grant and the SICPA Foundation.

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Classical Bovine Spongiform Encephalopathy by Transmission of H-Type Prion in Homologous Prion Protein Context

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Bovine spongiform encephalopathy (BSE) and BSE-related disorders have been associated with a single major prion strain. Recently, 2 atypical, presumably sporadic forms of BSE have been associated with 2 distinct prion strains that are characterized mainly by distinct Western blot profiles of abnormal protease-resistant prion protein (PrP^{res}), named high-type (BSE-H) and low-type (BSE-L), that also differed from classical BSE. We characterized 5 atypical BSE-H isolates by analyzing their molecular and neuropathologic properties during transmission in transgenic mice expressing homologous bovine prion protein. Unexpectedly, in several inoculated animals, strain features emerged that were highly similar to those of classical BSE agent. These findings demonstrate the capability of an atypical bovine prion to acquire classical BSE–like properties during propagation in a homologous bovine prion protein context and support the view that the epidemic BSE agent could have originated from such a cattle prion.

Transmissible spongiform encephalopathies, or prion diseases, are a group of neurodegenerative disorders that include Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep and goats, and bovine spongiform encephalopaty (BSE) in cattle. Prion diseases are characterized by specific histopathologic lesions and deposits of an abnormal conformational isoform (PrP^{Sc}) of the host-encoded physiologic prion protein (PrP^{C}) in the central nervous system. PrP^{Sc} but not PrP^{C} is partially resistant to digestion by proteinase K, resulting in an N terminally truncated prion protein termed PrP^{res} that can be detected by Western blot and showing a characteristic banding pattern that reflects the 3 PrP^{res} glycoforms. The apparent molecular masses and relative quantities of these glycoforms are used in biochemical PrP^{res} typing as the criteria to differentiate between prion diseases.

BSE is a prion epidemic that has caused the deaths of ≈200,000 cattle in Europe, mainly in the United Kingdom, since it emerged in 1985. Although multiple agent strains have been identified in sheep scrapie (*1*,*2*) and human CJD (*3*,*4*), early evidence showed that BSE was caused by a single major strain (*5*,*6*) with the ability to efficiently cross the species barriers and showing stable features even when transmitted to other species. Transmission of BSE to humans through contaminated food is believed to be responsible for variant CJD (vCJD) (*7*,*8*). Several authors reported that BSE and vCJD prions share similar strain-specific features, including a unique PrP^{res} molecular signature (*6*,*9*,*10*), after transmission to mice or macaques,. However, other studies described the production of different PrP^{res} molecular signature after BSE and vCJD prions transmission in wild-type (*11*) and human PrP transgenic mice (*12*,*13*).

Epidemiologic investigations identified contaminated meat and bone meal as the vehicle that recycled the BSE agent in the cattle population (*14*). However, the origin of BSE remains under debate, and the disease has been hypothesized to have derived either from sheep scrapie

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DOI: http://dx.doi.org/10.3201/eid1709.101403

or from a spontaneous bovine prion disease analogous to sporadic forms of CJD in human (*15*) or even from human transmissible spongiform encephalopathy (*16*).

More recently, 2 atypical forms of BSE have been identified in several European countries (*17*), Japan (*18*,*19*), the United States (*20*), and Canada (*21*). Several studies suggest that these atypical disorders are associated with 2 distinct prion strains that are mainly characterized by distinct PrP^{res} profiles, named high-type (H-type) and low-type (L-type) according to the electrophoretic migration of the unglycosylated PrP^{res}, which is higher (BSE-H) or lower (BSE-L) than classical BSE (BSE-C) (*22*). An additional distinctive signature of H-type and L-type PrP^{res} is the smaller proportion of the diglycosylated PrP^{res} compared with the classical-type (C-type) PrP^{res}, more obvious in L-type BSE (*23*–*25*).

All epidemiologic and biologic evidence strongly suggests that BSE-H and BSE-L represent sporadic forms of BSE (*23*,*24*) associated with 2 distinct prion strains. Transmission experiments in different mouse models, including transgenic mice expressing bovine PrP, showed that BSE-H and BSE-L exhibited strain-specific features clearly distinct between each other that also differed from BSE-C (*13*,*25*–*28*). However, BSE-L isolates unexpectedly showed transmission of a disease with some phenotypic features that resembled those of the BSE-C agent when inoculated in either transgenic mice expressing ovine PrP (*28*) or inbred wild-type mouse lines (*25*), suggesting that atypical bovine strains can modify their properties, at least after species barrier passages, converging with those of BSE-C.

We show that the transmission of atypical BSE-H isolates in transgenic mice expressing homologous bovine prion protein (PrP) led to emergence of a clearly distinct prion with strain features similar to those of the BSE-C agent and that such similarities were maintained on subsequent passages. These observations provide new insights into the nature of the events that could have led to the BSE epizootic.

Materials and Methods

Transgenic Mice

We used *Tg110* transgenic mice in all inoculation experiments. This mouse line expresses bovine PrP^{C} (≈8× that of the level of PrP^{C} in cattle brain) under the control of the mouse *prnp* gene promoter in a mouse $PrP^{0/0}$ background (*29*).

BSE Isolates

The 5 BSE-H isolates used in this study comprised brainstem samples from naturally affected cows, diagnosed as atypical H-type BSE on the basis of the molecular analyses of PrP^{res} (*23*). All cows were healthy and killed at 8–15 years of age. Four (isolates 07-644, 03-440, 03-2095, and 02-2695) were provided by the Agence Française de Sécurité Sanitaire des Aliments (Lyon, France). Isolate 45 was obtained from the Polish National Veterinary Research Institute (Pulawy, Poland). For comparative studies, material obtained from brainstem of 1 cow naturally infected with BSE-C (RQ 225:PG1199/00), supplied by the Veterinary Laboratories Agency (New Haw, Addlestone, Surrey, UK), was used as BSE-C control. For mouse inoculations, all isolates were prepared from brain tissues as 10% (wt/vol) homogenates. For subpassages, 10% brain homogenates from *Tg110* mice collected from primary passage were used as inocula.

All inocula were prepared in sterile 5% glucose as 10% homogenates. Each inoculum was prepared separately in a biosafety cabinet according to a strict protocol to avoid cross-contamination. To diminish the risk for bacterial infection, the inocula were preheated for 10 min at 70°C before inoculation.

Mouse Transmission Studies

Groups of 6–12 mice (6–7 weeks of age, weighing ≈20 g) were inoculated with 20 μL of the appropriate sample in the right parietal lobe by using 25-gauge disposable hypodermic syringes. UNO MICRO ID-8 ISO transponders (Roestvaststaal BV, Zevenaar, the Netherlands) were used for individual identification of mice. After inoculation, mice were observed daily and their neurologic status were assessed 2×/wk. When progression of the disease was evident, animals were euthanized. All animals were housed in accordance with guidelines of the Code for Methods and Welfare Considerations in Behavioral Research with Animals of the European Union directive 86/609EC). Necropsy was performed, and brain and spleen were taken. Part of the sample was frozen at –20°C for biochemical analysis, and the remaining part was fixed for histopathologic studies.

Survival times were calculated for each inoculum as the time between inoculation and euthanasia in days and expressed as the mean of the survival days postinoculation (dpi) of all the inoculated mice with its correspondent standard error of the mean. Data were processed by using SigmaPlot 2001 software (Systat Software, San Jose, CA, USA).

PrP^{res} Western Blotting

Frozen mouse brain samples were prepared as 10% (wt/vol) homogenates in 5% glucose in distilled water in grinding tubes (Bio-Rad, Hercules, CA, USA) by using a TeSeE Precess 48 homogenizer (Bio-Rad) following the manufacturer's instructions. All samples were analyzed by Western blot by using the kit TeSeE Western Blot

355 1169 (Bio-Rad) but with some adjustments for the different amounts of samples used. To achieve the volume proposed in the manufacturer's recommendations, 100 µL of the brain homogenates to be tested were supplemented with 100 µL of a 10% brain homogenate from PrP null mice (*30*). Processed samples were loaded on Criterion 12% polyacrylamide gels from Bio-Rad (165.6001) and electrotransferred to immobilon membranes (IPVH 000 10 [Millipore, Billerica, MA, USA]). For the immunoblotting experiments, Sha31 (*31*), Saf84 (Cayman Chemical, Ann Arbor, MI, USA) and 12B2 (*32*) monoclonal antibodies (mAbs) were used at a concentration of 1 µg/mL. Sha31 recognizes $_{156}$YEDRYYRE$_{163}$ epitope, and Saf84 recognizes $_{171}$QVYYRPVDQYS$_{181}$ epitope and 12B2 recognizes $_{101}$WGQGG$_{105}$ epitope of the bovine PrP sequence. Immunocomplexes were detected by horseradish peroxidase–conjugated antimouse immunoglobulin G (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Immunoreactivity was visualized by chemiluminescence (Amersham Pharmacia Biotech) and obtained after exposition with medical radiographic film (Agfa, Mortsel, Belgium).

Histopathologic Analysis

All procedures involving mouse brains and spleens were performed as described (*33*). Briefly, samples were fixed in neutral-buffered 10% formalin (4% formaldehyde) before embedding in paraffin. Once deparaffinized, 2-µm–thick tissue sections were stained with hematoxylin and eosin. Lesion profiles of the brains were established according to the standard method described by Fraser and Dickinson (*34*). For paraffin-embedded tissue blots, the protocol described by Andréoletti et al. (*35*) was used.

Results

The transmission dynamic of BSE-H agent into *Tg110* mice is similar to that of BSE-C. The 4 BSE-H isolates from France and 1 from Poland that were intracerebrally inoculated into transgenic mice expressing bovine PrP (*Tg110* mice) induced a typical neurologic disease on primary transmission, with a 100% attack rate (Figure 1). Remarkably, the survival times (mean ± SD 274 ± 3 to 346 ± 6 dpi) were similar than those produced by several BSE-C isolates ≈300 days) on the same *Tg110* mouse line (*29*,*36*,*37*). The longest mean survival times observed for mice infected with isolates 03–440 (mean ± SD 346 ± 6 dpi) and 02–2695 (330 ± 14 dpi) could reflect a lower infectivity of these isolates, consistent with its comparatively lower PrP^{res} content (data not shown). Moreover, the survival time of mice infected with these 2 isolates was reduced on subpassage, approaching that for BSE-C or BSE-H isolates of presumably higher titer (i.e., producing no substantial reduction of survival time on subpassage: isolates 07-644, 03-2095, and 45).

PrP^{res} Molecular Profiles of BSE-H–Inoculated *Tg110* Mice

The brains of inoculated mice were examined for PrP^{res} by Western blot analysis with Sha31 mAb. Consistent with the efficient transmission observed, PrP^{res} was readily detected from the first passage in all *Tg110* mice inoculated with the different BSE-H isolates. In 3 BSE-H isolates (07-644, 03-440, and 03-2095), the totality (100%) of the inoculated *Tg110* mice produced a PrP^{res} profile similar to that in cattle (H-type PrP^{res}) but clearly distinct from that produced by BSE-C agent (C-type PrP^{res}) in cattle (Figure 2, panel A; data not shown). Compared with C-type PrP^{res}, H-type PrP^{res} was characterized by a significantly higher apparent molecular mass of the unglycosylated band. The results obtained with these 3 isolates were comparable with those obtained by other authors with 2 BSE-H isolates inoculated in a different bovine PrP mouse line (*27*), where they concluded that the H-type agent essentially retained its biochemical phenotype upon serial transmission to bovine PrP transgenic mice.

We observed a different situation for the other 2 BSE-H isolates (02-2695 from France and 45 from Poland), where 3 and 2, respectively, of infected mice (Figure 1) showed a PrP^{res} profile clearly distinct from that of BSE-H in cattle (Figure 2, panel B). These 5 mice produced a PrP^{res}

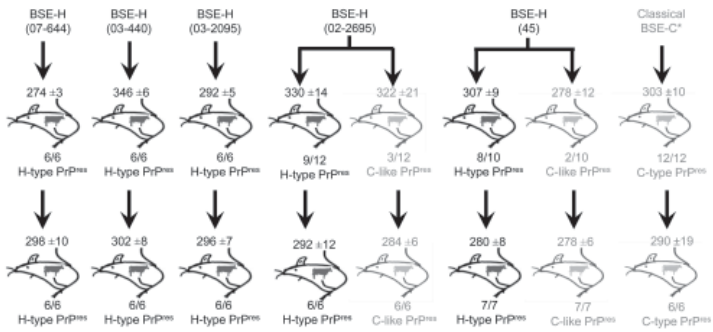

Figure 1. Overview of transmission of BSE-H isolates in *tg110* mice. Five different isolates were intracerebrally inoculated into groups of 6–12 mice per isolate. Survival times at different serial passages are indicated as mean ± SD days postinoculation. Molecular profiles exhibited in the brains of inoculated mice are indicated as H-type, C-type, or C-like PrP^{res}, and proportion of mice showing each profile. Previously reported data on BSE-C transmission in these mice (*36*) are included here only for comparison. BSE, bovine spongiform encephalitis; BSE-H, unglycosylated PrP^{res} that is higher than BSE-C; H-type, high-type Western blot profile of PrP^{res}; C-type, classical-type Western blot profile of PrP^{res}; C-like, classical BSE–like; PrP^{res}, protease-resistant prion protein; BSE-C, classical BSE.

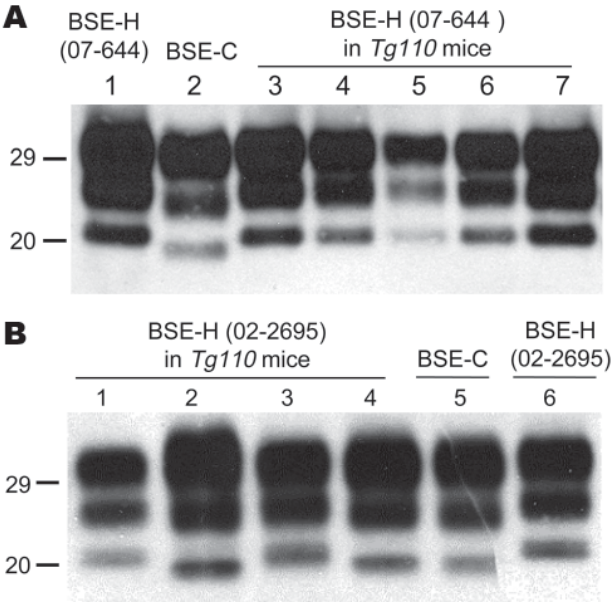

Figure 2. Western blot analyses of brain protease-resistant prion protein (PrP^{res}) from BSE-H infected mice by using Sha31 monoclonal antibody. A) Mice infected with isolate 07-644 at first passage (lanes 3–7) showing a homogeneous high-type (H-type) PrP^{res} molecular profile; BSE-H isolate 07-644 (lane 1) and a BSE-C isolate (lane 2) were included for comparison. B) Mice infected with isolate 02-2695 at first passage showing either H-type (lanes 1 and 3) or classical BSE–like (C-like) PrP^{res} molecular profile (lanes 2 and 4); BSE-H isolate 02-2695 (lane 6) and a BSE-C isolate (lane 5) were included for comparison. In panel B, a 10-fold equivalent brain tissue mass was loaded for brains from mice showing H-type PrP^{res} molecular profile (lanes 1 and 3) than from those with a C-like PrP^{res} molecular profile (lanes 2 and 4) to obtain equivalent PrP^{res} signals. Values to the left indicate molecular mass in kDa. BSE, bovine spongiform encephalopathy; BSE-H, unglycosylated PrP^{res} that is higher than BSE-C; BSE-C, classical BSE.

with lower ≈1.5 kDa) apparent molecular mass of the 3 PrP^{res} glycoforms, which was indistinguishable from that produced by BSE-C agent in these mice (Figure 2, panel B, and data not shown). Further characterization of this PrP^{res} with other antibodies showed that the PrP^{res} produced by these mice was not recognized by 12B2 mAb (Figure 3). However, 12B2 immunoreactivity against the H-type PrP^{res} produced by other mice (inoculated at the same time with the same isolates) remains essentially similar to that in cattle BSE-H (Figure 3). Furthermore, PrP^{res} immunolabeling with Saf84 mAb showed that these mice, contrary to mice with H-type PrP^{res}, did not retain the characteristic PrP^{res} band profile (4 bands) of cattle BSE-H but showed a PrP^{res} profile (3 bands) similar to that of BSE-C in *Tg110* mice (Figure 4).

In addition, the proportion of the diglycosylated PrP^{res} increased in comparison with the mice with H-type features, as shown by using Sha31 mAb (Figures 2, 3). Another difference was that the PrP^{res} level in brain was much higher than that in mouse brains with H-type PrP^{res} but similar to that in mouse brains inoculated with BSE-C, as shown by comparative Western blot analysis by using Sha31 mAb (Figure 5) and by the different equivalent brain tissue masses loaded to obtain equivalent PrP^{res} signals (Figures 2–4). A 10-fold equivalent brain tissue mass was loaded for brains from mice showing H-type PrP^{res} molecular profile than from those with a classical-like (C-like) PrP^{res} molecular profile to obtain equivalent PrP^{res} signals (Figures 2–4). These mice thus showed PrP^{res} molecular features indistinguishable from those in *Tg110* mice infected with C-like features.

For these 2 isolates, a second passage was performed in *Tg110* mice by using a brain homogenate derived from a mouse with either H-type or C-like PrP^{res} (Figure 1). Survival times did not differ substantially when the different inocula were compared (H-type vs. C-like PrP^{res} brain homogenate) or when compared with second passages

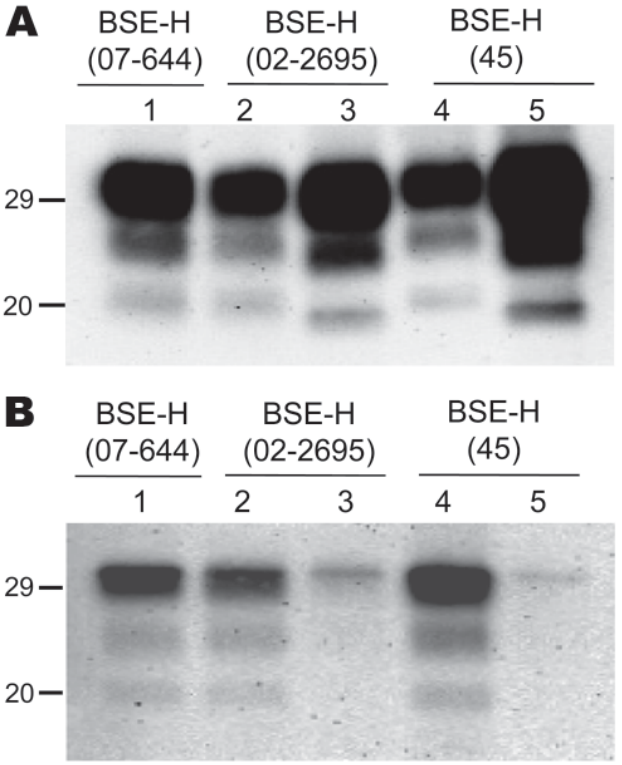

Figure 3. Comparative Western blot analyses with Sha31 and 12B2 monoclonal antibodies (mAbs) of brain protease-resistant prion protein (PrP^{res}) from BSE-H–infected mice. Mice infected with isolate 07-644 (lane 1), 02-2695 (lanes 2 and 3), or 45 (lanes 4 and 5) at first passage showing either high-type (lanes 1, 2, and 4) or classical BSE–like PrP^{res} molecular profile (lanes 3 and 5). Panel A was shown with Sha31 mAb; panel B was shown with 12B2 mAb. The same quantities of PrP^{res} were loaded in both panels A and B. Values to the left indicate molecular mass in kDa. BSE, bovine spongiform encephalopathy; BSE-H, unglycosylated PrP^{res} that is higher than classical BSE.

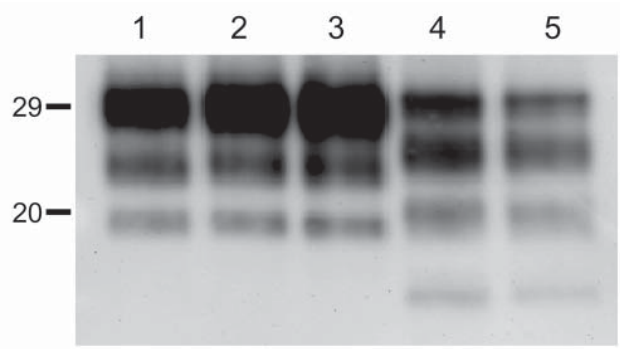

Figure 4. Western blot analyses of brain protease-resistant prion protein (PrP^{res}) from BSE-H infected mice by using Saf84 monoclonal antibody. *Tg110* mice infected with isolate 02-2695 (lanes 2 and 3) or 45 (lane 4) at first passage showing either high-type (lane 2) or classical BSE–like PrP^{res} molecular profile (lanes 3 and 4). The BSE-H isolate (02–2695) (lane 1) and a BSE-C isolate (lane 5) were included for comparison. Similar quantities of PrP^{res} were loaded in each lane. Values to the left indicate molecular mass in kDa. BSE, bovine spongiform encephalopathy; BSE-H, unglycosylated PrP^{res} that is higher than BSE-C; BSE-C, classical BSE.

of BSE-C in these mice. All the mice inoculated with the H-type brain homogenates showed H-type PrP^{res} features, whereas all the mice inoculated with the C-like brain homogenates exhibited C-type PrP^{res} molecular features indistinguishable from that of BSE-C (data not shown).

Lesion Profiles and PrP^{Sc} Deposition Patterns in BoPrP-*Tg110* Mice

We next examined vacuolation and PrP^{Sc} distribution in the brain, which are known to vary by strain (*10*,*34*,*38*). In general, mice brains exhibiting H-type PrP^{res} correlated with overall more intensive vacuolation that is pronounced in areas such as the hypothalamus, medial thalamus, and mesencephalic tegmentum (Figure 6). However, a different situation was observed when we studied the brains of BSE-H–infected mice exhibiting C-like PrP^{res} features. All these mice showed a lesion pattern comparable with that in BSE-C–infected mice, in which slight differences are found only in the mesencephalic tegmentum (Figure 6). These differences consisted of moderate lesions, whereas in BSE-C–infected mice, no lesions were found in this area. These features were conserved on secondary transmissions where no remarkable differences were found when compared with primary transmissions (data not shown).

Moreover, PrP^{Sc} deposits were distinctly distributed after both primary and secondary transmissions when BSE-H–infected mice exhibiting C-type PrP^{res} features were compared with those with H-type PrP^{res}, as assessed by paraffin-embedded tissue blot on brain coronal sections (Figure 7). However, the PrP^{Sc} deposition patterns were clearly similar when these mice were compared with those infected with BSE-C, after both primary and secondary transmission.

Transmission experiments (*27*) showed that, contrary to BSE-C, BSE-H is poorly lymphotropic in mouse models. The comparative study of PrP^{Sc} accumulation in spleen from our *Tg110* mice infected with both agents showed that BSE-H infected mice exhibiting H-type PrP^{res} in their brain were consistently scored as negative for PrP^{Sc} detection by paraffin-embedded tissue blot. In contrast, clear PrP^{Sc} deposits were always detected in BSE-H–infected mice exhibiting C-like features, as in mice infected with BSE-C (Figure 7). Similar results were obtained after secondary transmissions.

Discussion

We studied the behavior and stability of the atypical BSE-H during propagation into a bovine PrP background, thus in the absence of a species barrier. We used *Tg110* mice (*29*,*36*) because they express a PrP^{C} homologous to that of the donors, thus providing a relevant context for comparing atypical BSE-H and epizootic BSE-C isolates.

Our results showed that all BSE-H isolates induced a typical neurologic disease on primary transmission, with a 100% attack rate and survival times similar to those produced by several BSE-C isolates in this mouse line (*29*,*36*) (Figure 1). The longer survival times for some mice infected with BSE-H isolates could reflect a lower

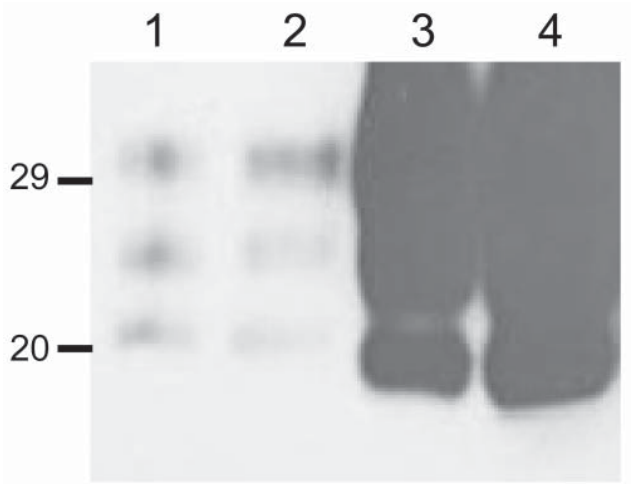

Figure 5. Comparison of the amount of protease-resistant prion protein (PrP^{res}) in brain sample from mouse inoculated with BSE-H (isolate 02-2695) showing either high-type (lane 1 and 2, first and second passages, respectively) or classical BSE–like PrP^{res} molecular profile (lanes 3 and 4, first and second passages, respectively). Identical amounts of 10% brain homogenate were loaded in each lane. Western blot was shown with Sha31 monoclonal antibody. Values to the left indicate molecular mass in kDa. BSE, bovine spongiform encephalopathy; BSE-H, unglycosylated PrP^{res} that is higher than classical BSE–like.

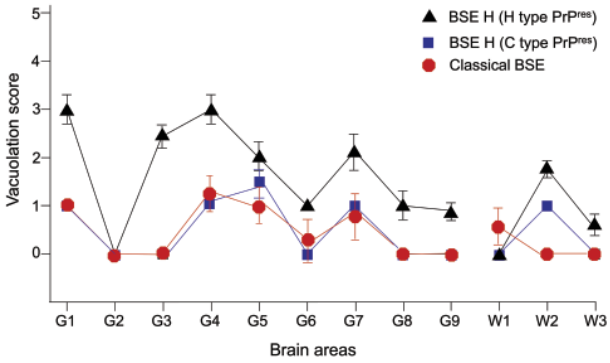

Figure 6. Vacuolar lesion profiles in brains from *Tg110* mice inoculated with BSE-H (isolate 02-2695, first passage) showing either H-type PrP^{res} phenotype (black triangles, n = 6 animals) or C-like PrP^{res} phenotype (blue squares, n = 3 animals). Lesion profile in brains from *Tg110* inoculated with BSE-C (first passage) is also included for comparison (red circles, n = 6 animals). Lesion scoring was undertaken for 9 areas of gray matter (G) and white matter (W) in mouse brains: dorsal medulla (G1), cerebellar cortex (G2), superior colliculus (G3), hypothalamus (G4) medial thalamus (G5), hippocampus (G6), septum (G7), medial cerebral cortex at the level of the thalamus (G8) and at the level of the septum (G9), cerebellum (W1), mesencephalic tegmentum (W2), and pyramidal tract (W3). Error bars indicate SE. BSE, bovine spongiform encephalopathy; BSE-H, unglycosylated PrP^{res} that is higher than BSE-C; PrP^{res}, protease-resistant prion protein; H-type, high-type Western blot profile of PrP^{res}; C-type, classical-type Western blot profile of PrP^{res}; C-like, classical BSE–like; BSE-C, classical BSE.

infectivity of this isolate consistent with the reduction of survival time observed on subpassages, approaching that for BSE-C or BSE-H isolates of presumably higher titer (i.e., producing no substantial reduction of survival time on subpassage). These results are also consistent with another comparative study of BSE-H and BSE-C transmissions in a different bovine PrP mouse line (*27*). These data suggest that atypical BSE-H and BSE-C agents have similar transmission features into *Tg110* mice.

Although all BSE-H–inoculated mice showed homogeneous survival times, a phenotypic divergence was observed in a few animals infected with 2 of the BSE-H isolates. Surprisingly, these few mice showed phenotypic features clearly distinct from those in most of the BSE-H–infected mice but similar to those of BSE-C propagated onto the same mice, according to various criteria. First, a PrP^{res} profile indistinguishable from that produced by BSE-C agent in these mice but clearly distinct from that of BSE-H in cattle, in terms of 1) apparent molecular mass of PrP^{res}, 2) PrP^{res} glycosylation pattern, 3) immunoreactivity with 12B2 mAb, and 4) pattern of labeling with Saf84 antibody. Second, the vacuolation profile essentially overlapped that in mice infected with BSE-C, with slight differences only in the mesencephalic tegmentum area. Third, the spatial distribution of PrP^{res} in the brain was clearly similar to that of mice infected with BSE-C. Fourth, PrP^{Sc} was consistently detected in the spleen, similar to mice infected with BSE-C. These similarities with BSE-C were fully retained after a second passage by using brain homogenate from mice with C-like features, whereas a BSE-H strain phenotype was maintained in mice inoculated with mouse brains homogenates containing H-type PrP^{res}.

However, C-like features emerged in only 2 of the 5 isolates tested. Because only a low proportion of the mice inoculated with these 2 isolates exhibited these novel features (3/12 and 2/10, respectively), the lack of such observation in the other 3 isolates, and in 2 other independent studies of 3 BSE-H isolates in different bovine transgenic mouse lines (*27*), could be due to the low number of inoculated mice (6 per isolate), which could be statistically insufficient for such an event. No variability was ever observed in the PrP^{res} profiles of >100 *Tg110* mice inoculated with 4 different BSE-C isolates (*29*,*36*) (Figure 1). However, a divergent evolution of the BSE agent has been reported after trans-species transmission in both wild-type (*11*) and human PrP transgenic mice (*12*,*39*,*40*).

Although further studies are required to clarify the mechanisms associated with the emergence of distinct phenotypes among individual mice, several factors would be expected to influence the probability of detecting such a variant through mouse bioassay. These factors are 1) amount or regions of cattle brain tissue taken for inoculum preparation, 2) physicochemical treatment during inoculum preparation (e.g., temperature, homogenization buffer), 3) the precise site of mouse inoculation, 4) the infectious titer of the inoculum, and 5) others unknown mouse factor affecting prion propagation and disease evolution. Because samples used in this study were prepared from the same region (brainstem) following the same precise protocol and under identical conditions, differences in inoculum preparation and conditions are unlikely. However, the possibility that the observations might be influenced by the precise neuroanatomic origin of the inoculated bovine brainstem homogenate or by other mouse bioassay–related factors cannot be excluded.

The possible cross-contamination of the BSE-H isolates material (02-2695 and 45 from 2 laboratories in different countries) by a BSE-C infectious source was judged highly improbable for several reasons. These reasons are 1) the strict biosafety procedures followed for sample collection, preparation of the inocula, inoculation scheme, and care of mice; 2) the absence of C-type PrP^{Sc} in the BSE-H inocula used for transmissions as deduced by Western blot analysis; and 3) 2 independent transmission experiments, involving separate batches of both incriminated isolates, all produced consistent results.

Together, these observations support 2 possible hypotheses. First, a minor strain component might be

present in BSE-H isolates that could emerge on subsequent transmission in *Tg110* mice. Second, a new strain component has been generated during propagation of BSE-H agent in *Tg110*. In both instances, emergence of the new strain, either in the original cattle or during propagation in *Tg110* mice, could be promoted by specific propagation conditions or by physicochemical treatment of the inoculum. In this regard, acquisition of novel properties by a sporadic cattle transmissible spongiform encephalopathy agent by a physicochemical treatment, such as that applied to carcass-derived products, has been invoked as a possible origin for the BSE epidemic (*7*).

Contrary to BSE-H, the atypical BSE-L agent retained unique and distinct phenotypic features, compared with BSE-C agent, on transmission to both bovine and human PrP transgenic mice (*26–28*). This agent, however, acquired phenotypic traits intriguingly similar to those of the BSE agent during trans-species transmission in either transgenic mice expressing ovine PrP (*28*) or inbred mouse lines. On the basis of these observations, the BSE-C agent already has

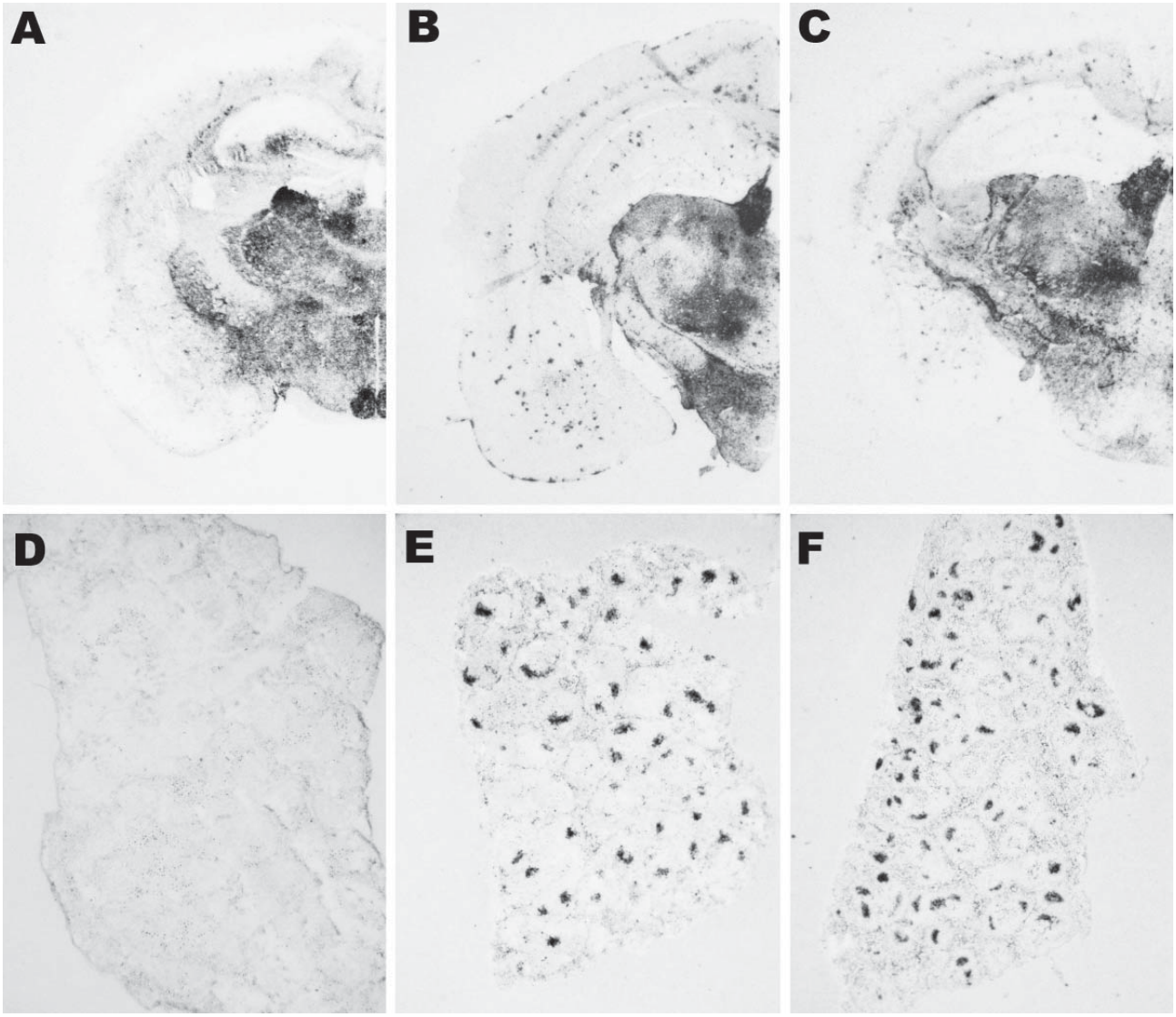

Figure 7. Abnormal isoform of host-encoded prion protein (PrP^{Sc}) deposition patterns in brain and spleen from *Tg110* mice infected with BSE-H. A–C) Paraffin-embedded tissue (PET) blots of representative coronal sections at the level of the hippocampus from *Tg110* mice infected with atypical BSE-H (isolate 02-2695, first passage) showing either high-type (A) or classical-type protease-resistant prion protein (PrP^{res}) phenotype (B). PET blot from *Tg110* mice infected with BSE-C (C) is included for comparison. D–F) PET blots of representative spleen sections: *Tg110* mice infected with atypical BSE-H (isolate 02-2695) showing high-type PrP^{res} in the brain were consistently scored as negative for PrP^{Sc} detection (D), whereas clear PrP^{Sc} deposits were always detected in BSE-H infected mice exhibiting BSE-C–like features (E), as in mice infected with BSE-C (F). Original magnification levels: panels A–C, ×20; panels D–F, ×6.BSE, bovine spongiform encephalopathy; BSE-H, unglycosylated PrP^{res} that is higher than BSE-C; BSE-C, classical BSE.

been speculated to have originated from atypical BSE-L after conversion in an intermediate host such as a sheep. However, the capacity of these BSE-L–derived agents to retain BSE phenotypic traits after reinoculation to bovine PrP transgenic mice is a key question, remaining to be demonstrated, to show whether the observed convergence truly reflects a permanent strain shift of the BSE-L agent rather than a phenotypic convergence in an experimental model.

In contrast, our results suggest that prion strain divergence might occur on propagation of atypical BSE-H in a homologous bovine PrP context and that this strain divergence could result from a permanent strain shift of the BSE-H agent toward a C-like agent that is stable in subsequent passages. These findings emphasize the potential capacity of prion diversification during propagation, even in the absence of any species barrier, and represent an experimental demonstration of the capability of an atypical, presumably sporadic, bovine prion to acquire C-like properties during propagation in a homologous bovine PrP context.

Results in transgenic mouse models cannot be directly extrapolated to the natural host. However, our observations are consistent with the view that the BSE agent could have originated from a cattle prion, such as BSE-H, and provide new insights into the nature of the events that could have led to the appearance of this agent.

This study was supported by grants from the European Union (CT2004-50657 and CT2004-023183) and from UK Food Standards Agency (M03043).

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Increasing Incidence of Invasive *Haemophilus influenzae* Disease in Adults, Utah, USA

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Since the introduction of the *Haemophilus influenzae* type b vaccine, the incidence of invasive *H. influenzae* type b disease among children has fallen dramatically, but the effect on invasive *H. influenzae* disease among adults may be more complex. In this population-based study we examined the epidemiology and outcomes of invasive disease caused by typeable and nontypeable *H. influenzae* among Utah adults during 1998–2008. The overall incidence increased over the study period from 0.14/100,000 person-years in 1998 to 1.61/100,000 person-years in 2008. The average incidence in persons ≥65 years old was 2.74/100,000 person-years, accounting for 51% of cases and 67% of deaths. The incidence was highest for nontypeable *H. influenzae* (0.23/100,000 person-years), followed by *H. influenzae* type f (0.14/100,000 person-years). The case-fatality rate was 22%. The incidence of invasive *H. influenzae* infection in Utah adults appears to be increasing. Invasive *H. influenzae* disproportionately affected the elderly and was associated with a high mortality rate.

Before the introduction of the *Haemophilus influenzae* type b (Hib) conjugate vaccine, Hib was the most common cause of bacterial meningitis in children <5 years of age (*1*,*2*). Hib caused >80% of invasive *H. influenzae* disease among children (*3*). Since the introduction of the Hib conjugate vaccine, the incidence of invasive Hib disease in children has decreased by 99% (*1*,*2*). Several studies have reported a decrease in the incidence of invasive Hib in adults following widespread use of vaccine in children (*4*,*5*).

However, although the decrease in the overall incidence of invasive disease caused by Hib for children and adults has been a remarkable public health success, changes in the epidemiology of invasive disease caused by other *H. influenzae* have been observed. The incidence of *H. influenzae* invasive disease has shifted toward adults. In a study conducted in the Atlanta, Georgia, USA, metropolitan area before Hib vaccine introduction, adults comprised 24% of all invasive *H. influenzae* cases (*6*). A more recent population-based report from Illinois showed adults accounting for 77% of invasive cases (*7*). Other encapsulated strains, including *H. influenzae* type a (Hia), have emerged as notable causes of invasive disease among children in Utah, Alaska, and the Navajo nation (*8*–*10*). Given the changing epidemiology observed elsewhere, we sought to describe the epidemiology of invasive *H. influenzae* infections among Utah adults during 1998 through 2008.

Methods

Study Design and Population

We identified all cases of laboratory-confirmed invasive *H. influenzae* among Utah residents ≥18 years of age reported to the Utah Department of Health (UDoH) from 1998 through 2008. Invasive *H. influenzae* is an immediately reportable condition in Utah. We defined invasive *H. influenzae* according to the Council of State and Territorial Epidemiologists definition: a clinically compatible case that is confirmed by isolation of *H.*

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DOI: http://dx.doi.org/10.3201/eid1709.101991

influenzae from a normally sterile site such as blood, cerebrospinal fluid, joint fluid, pleural fluid, or pericardial fluid (*11*). The UDoH enhanced their passive surveillance program in 2005 by sending out reminders to major laboratories in Utah to report invasive *H. influenzae* cases. As part of these enhancement efforts, 1 large laboratory also began automated case reporting to the UDoH in 2005. Because these changes in surveillance could introduce ascertainment bias for the final 4 years of this analysis, we conducted a parallel retrospective cohort analysis of laboratory-confirmed invasive *H. influenzae* cases among Utah residents within the Intermountain Healthcare (IH) system during 1999–2008 (on the basis of availability of complete laboratory data). IH is a vertically integrated hospital system that provides care to roughly 50% of Utah adults (Scott Lloyd, pers. comm.). The estimated market share by age group is recalculated at regular intervals. Over this period, IH made no major changes to its microbiology practices that would alter the recovery of *H. influenza* isolates.

Serotyping

H. influenzae isolated from sterile sites was identified by using standard culture techniques. Laboratories are requested to submit sterile site isolates of *H. influenzae* to the Utah Public Health Laboratory in Salt Lake City, Utah, for definitive serotyping by using monovalent antisera for *H. influenzae* types a–f. Isolates from reported *H. influenzae* cases not sent to the Utah Public Health Laboratory for serotyping were classified as not typed. All other isolates were classified as Hia–f or as nontypeable.

Demographic and Outcome Data

As part of its surveillance program, the UDoH collected demographic information including county of residence, race/ethnicity, age, gender, hospitalization, and death. Starting in 2005, the UDoH began to collect expanded clinical information including the presence of chronic medical conditions and clinical signs. These clinical data were collected at the local health department level. It should be noted that not all local health departments conducted such data collection, and collection methods varied across local health departments (e.g., chart review, patient interview, hospital information personnel interview).

Statistical Analysis

To calculate the incidence rate, we used the estimated annual Utah population among persons ≥18 years of age on the basis of the United States 2000 census (*12*). To derive an incidence for the IH cohort, we multiplied the estimated annual population by IH estimated market share of adult healthcare in Utah for a given year. Market share data were only available for 2000, 2002, and 2005–2008. For intervening years, we extrapolated using available data for the nearest years.

Categorical variables were evaluated by using standard χ^2 test for independence or Fisher exact test as appropriate. To evaluate associations between variables, we used ordinary logistic regression and exact logistic regression as appropriate. Poisson regression method with a log-linear model was performed to test for trend variation in the annual case rates for the IHC and UDoH datasets. We used SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) for all statistical analyses.

Human Subjects Protection

We obtained approval for this study from the Institutional Review Boards of the University of Utah and IH; de-identified case information was provided by the UDoH through a data sharing agreement. Informed consent was waived as all data were de-identified.

Results

We identified 121 cases of invasive *H. influenzae* in Utah adults reported to the UDoH during 1998–2008 (Table). The incidence of *H. influenzae* invasive disease was 0.66 cases per 100,000 person-years averaged over the 11 years. Incidence increased over the study period from 0.14 cases per 100,000 person-years in 1998 to a peak in 2008 of 1.61 cases per 100,000 person-years ($p = 0.0023$, by linear test for trend) (Figure 1). We observed a marked seasonality with winter predominance: 41.3% of cases occurring in winter, 26.4% in spring, 21.4% in fall, and 10.7% in summer. We observed no significant difference in distribution between sexes, with 64 cases in women and 57 cases in men ($p = 0.65$).

The incidence rates estimated by using the IH data showed a similar trend (Figure 1). We identified 94 cases of invasive *H. influenzae*, with an estimated average annual incidence of 1.05/100,000 person-years. The incidence increased from 0.54/100,000 person-years in 1999 to 1.27/100,000 person-years in 2008 ($p = 0.002$, by linear test for trend). Although the estimated rates differed, the trend in increasing incidence was similar for both data sources.

The incidence of invasive disease was markedly higher among persons ≥65 years of age; the average incidence in this age group was 2.74 cases per 100,000 person-years and increased to 6.14 cases per 100,000 in 2008. The average incidence for persons 18–34, 35–49, and 50–64 years of age were 0.25, 0.26, and 0.88 per 100,000 person-years, respectively. Compared with persons 18–34 years of age, patients ≥65 years of age had a 12-fold greater risk for invasive disease (risk ratio 12.5, 95% confidence interval [CI] 7.29–21.32; $p<0.0001$). The incidence of invasive disease increased significantly over the study period among those ≥65 years of age (Figure 2).

Table. Characteristics, by serotype, of 121 cases of invasive *Haemophilus influenzae* in adult patients, Utah, USA, 1998–2008

Characteristic	No. (%) samples								
	Type a, n = 15	Type b, n = 9	Type c, n = 1	Type d, n = 3	Type e, n = 5	Type f, n = 25	Not typeable, n = 43	Not typed, n = 20	Total
Patient age, y									
18–34	3 (20)	0	1	0	0	1 (4)	9 (20.9)	3 (15)	17 (14)
35–49	3 (20)	3 (33.3)	0	0	0	1 (4)	5 (11.6)	1 (5)	13 (10.7)
50–64	4 (26.7)	2 (22.2)	0	1	1	6 (24)	12 (27.9)	3 (15)	29 (23.9)
≥65	5 (33.3)	4 (44.4)	0	2	4	17 (68)	17 (39.5)	13 (65)	62 (51.2)
Patient sex, F	8 (53.3)	4 (44.4)	1	0	3	17 (68)	21 (48.8)	10 (50)	64 (52.8)
Sample source									
Cerebrospinal fluid	1 (6.7)	4 (44.4)	1	0	0	4 (16)	5 (11.6)	0	15 (12.3)
Blood	14 (93.3)	5 (55.6)	0	3	5	21 (84)	35 (81.3)	18 (90)	101 (83.5)
Other	0	0	0	0	0	0	3 (6.9)	2 (10)	5 (4.1)
Patient death	4 (26.7)	2 (22.2)	0	1	0	4 (16)	10 (23.2)	6 (30)	27 (22.3)

Serotype Distribution

Of the 121 patients with invasive disease, 101 (83%) isolates were serotyped. Figure 3 depicts the annual number of cases by serotype and isolates that did not undergo serotype testing. Nontypeable strains accounted for 43 cases (43%). Hib accounted for 9 cases (9%) and non-b encapsulated strains accounted for 49 cases (49%). Serotypes f and a accounted for 25 (25%) and 15 cases (15%), respectively.

The increases in invasive disease were largely because of nontypeable strains and Hif. The average incidence over 11 years of invasive disease caused by nontypeable strains was 0.23/ 100,000 person-years and rose from 0 in 1998 and 1999 to an average of 0.72/100,000 person-years during 2007 and 2008. During 1998–2008, the average incidence of disease caused by Hif was 0.14/100,000 person-years and rose to an average of 0.48/100,000 person-years during 2007 and 2008. The number of cases of Hia remained relatively steady, with an average annual incidence of 0.08/100,000 person-years.

Clinical Disease

Most (51%) invasive disease occurred in persons ≥65 years of age. Bacteremia accounted for 83% of all cases, meningitis for 12.4%, and other sterile sites for the remaining 4.6%. Nontypeable strains, Hib, and Hif accounted for 13 of the 15 cases of meningitis (Table). Infection with Hib was significantly more likely to be associated with meningitis than nontypeable strains (odds ratio [OR] 6.1, 95% CI 1.3–29.0). While invasive disease was more likely to occur in the elderly, a greater proportion of disease among patients 18–49 years of age was due to meningitis compared with patients ≥50 years of age (25% vs. 9%; p = 0.05).

Data on underlying illness was available for 69 of the 121 case-patients. Forty-six (67%) of 69 patients had chronic medical conditions; 33% had ≥2. Twenty-four patients had diabetes mellitus; 15 were immunocompromised; 14 had chronic lung disease; 11 had a malignancy; 3 had asthma; 3 each had chronic obstructive pulmonary disease, congestive heart failure, chronic liver disease/cirrhosis, and chronic kidney disease; and 1 had a history of intravenous drug use. Forty-six patients had a diagnosis of pneumonia at the time of *H. influenzae* isolation. Patients with pneumonia were significantly more likely to have ≥1 chronic illness (OR 4.48, 95% CI 1.48–13.58, p = 0.008) and significantly less likely to have meningitis (OR 0.048, 95% CI 0–0.358, p = 0.0016).

The overall case-fatality rate was 22%. Two thirds of deaths were among those ≥65 years of age. Persons ≥65 years of age had a case-fatality rate of 29% compared to 15% for those <65 years of age (p = 0.08). The case-fatality rate for patients with bacteremia (23%) was not significantly higher than for patients with meningitis (13%). The case-fatality rate did not differ significantly by serotype. Outcome did not differ significantly by the

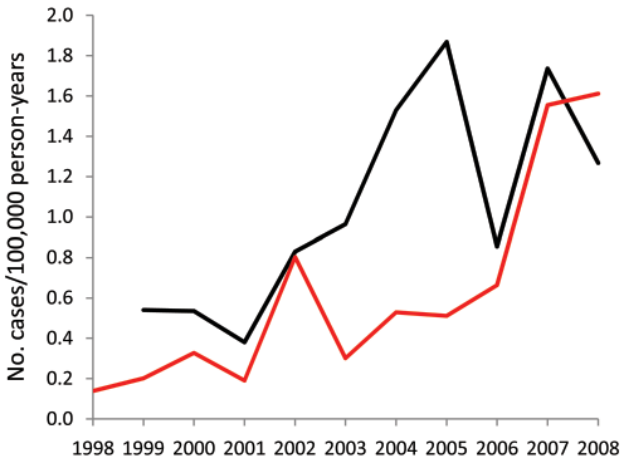

Figure 1. Comparison of annual incidence of invasive *Haemophilus influenzae* disease derived from the Utah Department of Health and Intermountain Healthcare databases, Utah, USA, 1998–2008. Black line, Intermountain Healthcare; red line, Utah Department of Health.

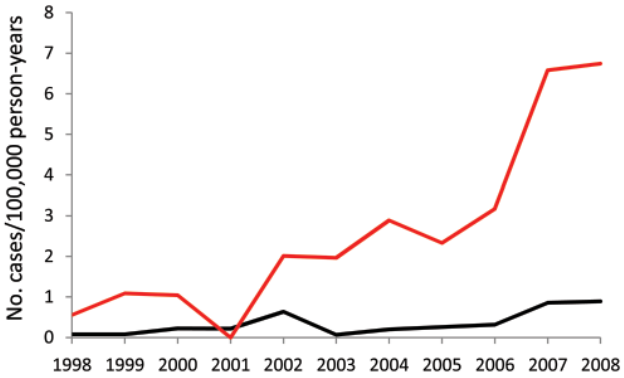

Figure 2. Annual incidence of invasive *Haemophilus influenzae* disease in adults, by age, Utah, USA, 1998–2008. Red line, age ≥65 y; black line, age 18–64 y.

presence or absence of concurrent conditions (17% vs. 26% mortality rate, respectively), but data were only available for 69 patients.

Discussion

Despite the virtual elimination of Hib in areas with widespread use of Hib vaccine, invasive disease caused by *H. influenzae* remains a major source of illness and death. The incidence of invasive *H. influenzae* in Utah adults appears to be increasing; most of the increase in *H. influenzae* disease incidence was attributable to an increase in nontypeable and Hif strains. We found that invasive *H. influenzae* disease was most common in persons ≥65 years of age and was associated with a high mortality rate.

The overall incidence of invasive *H. influenzae* disease in adults in our population, 0.66 cases per 100,000 person-years, was similar to rates observed in Illinois (1.0/100,000 person-years in 1994) (*7*) and slightly lower than rates among Alaskans during 1991–1996 (1.4/100,000 person-years) (*5*). We found a significant increase in the incidence of invasive *H. influenzae* over the study period by using 2 surveillance methods. Several studies in addition to ours suggest an increase in invasive disease caused by to non-type b *H. influenzae* among adults (*5*,*7*,*13*). The reasons for this change are unclear, and might reflect changes in the organisms, changes in the number of persons at high risk, or perhaps waning of cross-immunity induced by exposure to Hib.

As the prevalence of Hib has decreased, other encapsulated serotypes seem to have emerged as major causes of invasive disease, including Hif in Illinois and Hia in Brazil, Manitoba, and Northwestern Ontario (*7*,*10*,*14*,*15*). Similar to findings in Illinois, Hif was the second most common serotype in our analysis (25%), and cases of invasive Hif contributed substantially to the increase of invasive *H. influenzae* cases we observed in 2007–2008. Although Hia was the third most common isolate among adults in our study, it accounted for only 15% of all cases, a rate that is much lower than rates in Manitoba (29%) (*15*), Northwestern Ontario (42%) (*10*), and in children in Utah (*8*).

Nontypeable *H. influenzae* has been the most common isolate in virtually all published series among adults. In our study, it accounted for 43% of cases; similar rates were reported in other studies (*3*,*7*,*15*–*17*). The emerging role of invasive disease because of nontypeable strains is intriguing because this organism has traditionally been considered a relatively noninvasive bacteria predominantly associated with community-acquired pneumonia, chronic obstructive pulmonary disease exacerbations, and otitis media (*18*). Whether invasive strains of nontypeable *H. influenzae* have distinct genotypic and phenotypic characteristics compared with noninvasive isolates remains largely unknown. Candidate virulence factors include the adhesin genes *hmw* and *hia* and IS*1016*, an insertion element that may confer Hib-like, encapsulated properties to nontypeable strains. However, to date no single set of virulence determinants has been conclusively associated with invasive nontypeable strains (*19*–*21*). Better understanding of the factors that confer invasive capabilities might lead to improved strategies for vaccine development.

Similar to the findings in Spain, Illinois, and Alaska, rates of disease in our study were highest among older patients (*3*,*5*,*7*). The incidence increased in persons ≥65 years of age, and 29% of infections in this age group were fatal, emphasizing the disproportionate effect. Invasive *H. influenzae* was highly associated with concurrent illness. Among those for whom clinical data were available, 67% of our patients had an underlying condition, as has been observed in other studies (*3*,*7*). However, in those with and without concurrent illness, invasive *H. influenzae* showed a high overall case-fatality rate (22%). Other studies have reported that invasive *H. influenzae* is associated with high mortality rates, ranging from 13% to 29% (*3*,*4*,*6*,*7*). During

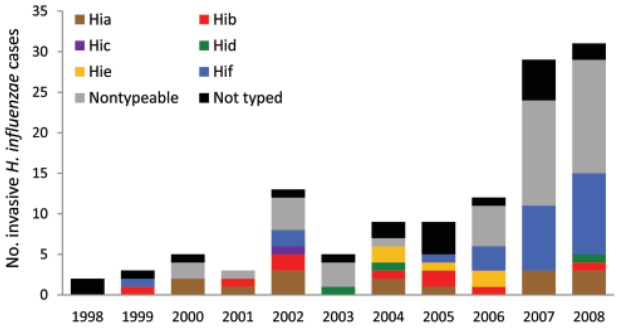

Figure 3. No. cases of invasive *Haemophilus influenzae* (Hi) disease in adults, by serotype (a–f), Utah, USA, 1998–2008.

2005–2008, the CDC Active Bacterial Core surveillance reported case-fatality rates of 13%–17% for invasive *H. influenzae* (*22*). Among adults ≥65 years of age, the incidence of invasive *H. influenzae*, the case-fatality rate and the number of attributable deaths exceeds that for invasive meningococcal disease in the most vulnerable age group, those 11–19 years of age (*23*). However, no vaccine is available for invasive disease caused by encapsulated non-type b and nontypeable *H. influenzae*. Efforts to prevent invasive *H. influenzae*, particularly among the elderly, should become a public health priority.

As with all surveillance studies, our study is subject to several limitations. The UDoH relies on passive reporting of *H. influenzae* from laboratories throughout the state, making underreporting likely. However, a study of invasive *H. influenzae* in Spain among children <5 years of age demonstrated that passive surveillance had a sensitivity of 88% (*24*). Comparison of estimated rates from the IH laboratory database and UDoH surveillance data suggests that the sensitivity of reporting among adults was 65%. Changes in reporting behavior and changes to the passive surveillance program in 2005 are potential sources of bias. Several studies have demonstrated the inaccuracy of serotyping compared with molecular diagnostic techniques (*25*,*26*). Although serotyping is still an accepted standard, had we performed capsular gene analysis, it might have changed the serotype distribution. Additionally, the small number of identified cases places limitations on analyses of serotype distribution and clinical characteristics. Finally, we had clinical information on concurrent conditions for only 57% of the cohort, and clinical information was not collected in a systematic manner across the state.

These limitations notwithstanding, we found that the incidence of invasive *H. influenzae* in Utah adults appears to be increasing, and this increase is caused mostly by the rising incidence of nontypeable *H. influenzae* and Hif. Invasive *H. influenzae*, including disease caused by nontypeable strains, has a high mortality rate. Persons ≥65 years of age are most affected by the disease and have the highest death rates. These data have implications for targeted adult *H. influenzae* vaccine development.

Acknowledgments

We thank IH and all Utah hospital microbiology laboratories for their continued efforts in *H. influenzae* reporting, and Melissa Stevens-Dimond for her support in data collection.

A.T.P. is supported by grants from the National Institute of Allergy and Infectious Diseases (U01AI082184-01) and the Centers for Disease Control and Prevention (U18-IP000303-01).

Dr Rubach just completed an Internal Medicine–Pediatrics combined residency at the University of Utah and began a fellowship in infectious diseases within the Department of Internal Medicine at Duke University in July 2011. His research interests include molecular diagnostics with field applications in nonindustrialized countries.

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Central Venous Catheter–associated *Nocardia* Bacteremia in Cancer Patients

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Central venous catheters, often needed by cancer patients, can be the source of *Nocardia* bacteremia. We evaluated the clinical characteristics and outcomes of 17 cancer patients with *Nocardia* bacteremia. For 10 patients, the bacteremia was associated with the catheter; for the other 7, it was a disseminated infection. *N. nova* complex was the leading cause of bacteremia. *Nocardia* promoted heavy biofilm formation on the surface of central venous catheter segments tested in an in vitro biofilm model. Trimethoprim- and minocycline-based lock solutions had potent in vitro activity against biofilm growth. Patients with *Nocardia* central venous catheter–associated bloodstream infections responded well to catheter removal and antimicrobial drug therapy, whereas those with disseminated bacteremia had poor prognoses.

Nocardiae are partially acid-fast, aerobic, gram-positive, branching filamentous bacteria that are found ubiquitously in soil, fresh water, and marine water (*1*,*2*). *Nocardia* spp. cause serious pulmonary infections (with occasional brain abscesses) in immunocompromised patients, primarily those with cell-mediated immunity abnormalities (*2*,*3*). Nocardiosis most commonly occurs after the organism has been introduced into the respiratory tract, but it may be acquired through direct inoculation into the skin (*4*). However, *Nocardia* bacteremia is rarely reported, even for severely immunocompromised patients with underlying malignancies (*5*,*6*).

Our first objective was to identify the clinical characteristics of *Nocardia* bacteremia and compare the clinical profiles and outcomes for patients with *Nocardia* bacteremia associated with central venous catheters (CVCs), also called central line–associated bloodstream infections (CLABSIs), with those of patients with disseminated *Nocardia* bacteremia. Our second objective was to determine whether *Nocardia* bacteria could form a biofilm on CVCs in a laboratory model and whether biofilm growth could be prevented with the use of antimicrobial lock solutions.

Patients and Methods

Clinical Characteristics

By searching the microbiology laboratory database at The University of Texas MD Anderson Cancer Center (Houston, TX, USA) from January 1998 through March 2010, we retrospectively identified 134 episodes of nocardiosis of any sources. *Nocardia* bacteremia was reported for 17 patients; 5 of these cases have been reported by Torres et al. (*7*). In addition, 2 of these 5 patients had catheter-related bloodstream infections (CRBSIs), reported by Kontoyiannis et al. (*8*). Pertinent data from patients' medical records were abstracted, including demographic characteristics, underlying malignancies, hematopoietic stem cell transplantation, graft-versus-host disease, clinical presentation, laboratory test and imaging study results, concomitant infections, antimicrobial therapy types and durations, hospital and intensive care unit stays, 72-hour and 7-day responses, and patient outcomes at 3-month follow-up.

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DOI: http://dx.doi.org/10.3201/eid1709.101810

Definitions

Neutropenia was defined as an absolute neutrophil count <500 cells/mm^3 and lymphopenia as an absolute lymphocyte count <1,000 cells/mm^3. Patients with at least 1 set of positive blood cultures for *Nocardia* bacteria were considered to have *Nocardia* bacteremia. CLABSIs were diagnosed according to Centers for Disease Control and Prevention (Atlanta, GA, USA) guidelines: a recognized pathogen cultured from ≥1 blood cultures (not including organisms considered common skin contaminants) and no apparent source of the bloodstream infection except the CVC whereby the CVC has been indwelling for >48 hours (*9*). Furthermore, CLABSIs were considered definite CRBSIs if at least 1 of the Infectious Disease Society of America definition criteria were also fulfilled: semiquantitative (>15 CFUs/catheter segment) or quantitative (>10^3 CFUs/catheter segment) catheter culture in which the same organism is isolated from the catheter segment and peripheral blood, or differential quantitative blood culture with simultaneous quantitative blood cultures from the CVC and peripheral blood with a ratio ≥3:1 (*10*). Disseminated (or secondary) *Nocardia* bacteremia was defined as the recovery of *Nocardia* bacteria from blood cultures and a non–catheter-related site (e.g., expectorated sputum, endotracheal aspiration, bronchoalveolar lavage, pleural effusion, lung tissue, or skin or brain biopsy samples) in the setting of clinical and radiographic evidence of organ involvement (pneumonia and skin lesions).

Nocardia spp. were identified on the basis of the appearance of colonies on routine media; species were identified by using a battery of biochemical tests and, after 2001, by 16S rDNA sequencing (*11*,*12*). Blood cultures and catheter tip samples were held for 7 days to ensure that no cases of *Nocardia* bacteremia were missed.

A broth microdilution MIC method had been used to perform susceptibility testing of *Nocardia* spp. according Clinical and Laboratory Standards Institute guidelines (*13*). Antimicrobial drug response had been defined as resolution or improvement of clinical manifestations and radiographic changes and negative microbiological findings.

Biofilm Formation

We used a modified Kuhn model of biofilm catheter colonization (*14*) to test *N. nova* complex and *N. puris* strains (that caused CLABSI in this study) for biofilm formation. Sterile polyurethane and silicone CVC segments were placed in 24-well tissue culture plates containing human donor plasma and incubated with shaking for 24 h at 37°C. The plasma was then replaced with 1 mL of 5.5 × 10^5 cells/mL inoculum of *Nocardia* strains. The *Nocardia* inoculum was grown in tryptic soy broth containing 10% fetal bovine serum and incubated with shaking for 24 h at 37°C. Organisms were tested in triplicate. The inoculated broth was removed, and CVC segments were washed with 1 mL of 0.9% sterile saline by shaking at 100 rpm for 30 min at 37°C. The CVC segments were transferred into a tube containing 5 mL of sterile 0.9% saline and sonicated for 15 min to disrupt any biofilm. The resulting solution was then cultured and quantified by making serial dilutions in 0.9% sterile saline and spreading them on trypticase soy agar plates with 5% sheep blood. All plates were inverted and incubated for 48 h at 37°C. The experiment was repeated 2 times.

Antimicrobial Lock Solutions

To determine whether antimicrobial lock solutions prevented the biofilm growth of *Nocardia* organisms, we used the silicone disk biofilm colonization model, as described (*14*). After washing the silicone disks with 0.9% sterile saline by shaking them for 30 min at 37°C, we transferred the disks into new 24-well tissue culture plates containing Mueller-Hinton broth (control) or the drug solution to be tested. Drug solutions included 10 mg/mL trimethoprim/sulfamethoxazole and 100 U heparin; a triple combination of 10 mg/mL trimethoprim, 30 mg/mL EDTA, and 25% ethanol; and 3 mg/mL minocycline, 30 mg EDTA, and 25% ethanol. Triple combinations of minocycline, EDTA, and 25% ethanol lock solutions were used because previous data showed that such combinations effectively eradicate bacterial organisms embedded in biofilm (*15*). After 2 h of incubation at 37°C, the disks were placed in 5 mL of 0.9% saline and sonicated for 15 min. Finally, they were vortexed for 5 seconds, and 100 μL of liquid from each disk was serially diluted and spread on trypticase soy agar plates with 5% sheep blood for quantitative culture. Plates were then inverted and incubated for 48–72 h at 37°C, and colony growth was quantified. The experiment was repeated 2 times.

Electronic Microscopy and Confocal Scanning

To verify the quantitative results, we used scanning electron microscopy to examine biofilm formation of *N. nova* complex on silicone CVC surfaces. We used silicone CVCs tested in the in vitro colonization model outlined above**.** Catheters were fixed with 2% glutaraldehyde, followed by osmium tetraoxide, tannic acid, and uranyl acetate as described (*16*). A series of ethanol dehydration steps followed, and the prepared samples were sputter coated with Au-Pd (60:40) and viewed with a Philips model XL3C scanning electron microscope (Philips Research, Eindhoven, the Netherlands). Confocal scanning laser microscopy was performed with a Leica TCSNT confocal microscope (Leica, Heidelberg, Germany). Objectives used for confocal laser microscope imaging were 100×1.4 N.A. Oil Plan Apo and 63×0.7 N.A. Plan Fluotar.

Statistical Analyses

To compare the characteristics of *Nocardia* CLABSI and disseminated *Nocardia* bacteremia patients, we used SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA). We determined differences between categorical variable frequencies by using the χ^2 or Fisher exact tests and compared continuous variables by using the Wilcoxon rank-sum test. All tests were 2-sided and had a maximum significance level of 0.05.

Results

The demographic characteristics and outcomes of the 17 patients in the study are shown in Table 1. All patients had had CVCs inserted before examination and collection of blood for culture. Ten (59%) patients had CLABSIs (Table 2); the remaining 7 (41%) had disseminated (secondary) *Nocardia* bacteremia for which the respiratory tract (pneumonia) was the primary source. Concomitant infections, most commonly with cytomegalovirus, were noted for 10 (59%) patients. Hematologic malignancies were more common in patients with disseminated *Nocardia* bacteremia than with *Nocardia* CLABSIs (100% vs. 50%; p = 0.044). *N. nova* complex was the most commonly identified causative species for nocardemia (6 patients). Other causative species were *N. asteroides* complex (5 patients), *N. veterana* (2), *N. brasiliensis* (1), and *N. puris* (1). We found no significant differences in species distribution among patients with CLABSIs and disseminated bacteremia. Furthermore, by looking at the temporal distribution of cases of *Nocardia* bacteremia and CLABSI nocardemia, we noticed no cluster that would suggest we were dealing with an outbreak.

Patients with CLABSIs had shorter hospital stays than did those with disseminated bacteremia (median 5 days vs. 24 days, respectively; p = 0.01) and were more likely to experience a response to therapy within 72 hours (90% vs. 0%; p<0.001) or 7 days (90% vs. 29%; p = 0.035). Mortality rate at 3-month follow-up was also lower for patients with CLABSI (10% vs. 43%; p = 0.25).

All *Nocardia* isolates were susceptible to trimethoprim/sulfamethoxazole, amikacin, and linezolid. Susceptibility rates to ceftriaxone, clarithromycin, imipenem, minocycline, tobramycin, and amoxicillin and clavulanate were 91%, 87%, 85%, 55%, 42%, and 22%, respectively. All *N. nova* isolates were susceptible to clarithromycin. No resistance to minocycline was found, but 45% of isolates had intermediate susceptibility MICs. *N. nova* complex isolates showed resistance to amoxicillin and clavulanate and to gentamicin in 80% and 60% of cases, respectively, whereas 91% of all *Nocardia* species were resistant to ciprofloxacin.

All patients underwent CVC removal. The most commonly used drug was trimethoprim/sulfamethoxazole (14 patients), used alone or in combination with beta-lactams (8 patients with carbapenems and 2 with

Table 1. Characteristics and outcomes of 17 cancer patients with *Nocardia* bacteremia, The University of Texas MD Anderson Cancer Center, January 1998–March 2010*

Characteristic	Disseminated bacteremia (n = 7), no. (%) patients	CLABSI (n = 10), no. (%) patients	p value
Male sex	3 (43)	4 (40)	>0.99
Cancer type			0.044
Hematologic	7 (100)	5 (50)	
Solid tumor	0	5 (50)	
Concomitant infection	7 (100)	5 (50)	0.044
Cytomegalovirus	4 (57)	1 (10)	0.1
Invasive fungus	3 (42)	1 (10)	0.25
Nocardia type†			0.74
N. nova complex	3 (43)	3 (30)	
N. asteroides complex	1 (14)	4 (40)	
N. veterana	1 (17)	1 (11)	
N. brasiliensis	1 (17)	0	
N. puris	0	1 (11)	
Antimicrobial drug treatment outcome			
Response to therapy	5 (71)	9 (90)	0.54
Response within 72 h	0	9 (90)	<0.001
Response within 7 d	2 (29)	9 (90)	0.035
Death within 3 mo	3 (43)	1 (10)	0.25
Nocardia bacteremia breakthrough despite trimethoprim/sulfamethoxazole prophylaxis	1	2	>0.99

*CLABSI, central line–associated bloodstream infection. Patient median age (range) for those with disseminated infection 56 y (32–73 y), for those with CLABSI 44 y (11–77 y); p = 0.31. Median hospital stay (range) for those with disseminated infection 24 d (8–53 d), for those with CLABSI 5 d (2–18 d); p = 0.01. Median antimicrobial drug treatment duration (range) for those with disseminated infection 50 d (21–105 d), for those with CLABSI 82 d (14–120 d); p = 0.56.
†Species were not determined for 2 isolates.

Table 2. Diagnostic and microbiologic profile for 10 cases of *Nocardia* CLABSI, The University of Texas MD Anderson Cancer Center, January 1998–March 2010*

CLABSI case no.	Diagnostic criteria	CRBSI
1	Differential quantitative blood culture (CVC ≥1,000 CFU/mL; peri = 3 CFU/mL) and quantitative catheter tip culture (10^3 CFU/tip)	Definite
2	Positive peripheral blood culture with positive semi-quantitative catheter tip culture (>15 CFU/tip)	Definite
3	Differential quantitative blood culture (CVC ≥1,000 CFUs/mL; peri = 1 CFU/mL)	Definite
4	CVC blood culture positive with positive semiquantitative catheter tip culture (>15 CFU/tip)	Definite
5	Differential quantitative blood culture (CVC ≥1,000 CFU/mL; peri = 1 CFU/mL)	Definite
6	Positive peripheral and CVC blood cultures with positive quantitative catheter tip culture (4,000 CFU/tip)	Definite
7	Differential quantitative blood culture (CVC ≥1,000 CFU/mL; peri = 20 CFUs/mL) and positive quantitative catheter tip culture (4,000 CFU/tip)	Definite
8	Positive peripheral and CVC blood culture but negative catheter tip culture	Probable
9	Positive peripheral and CVC blood culture but negative catheter tip culture	Probable
10	Positive CVC and peripheral blood culture but negative catheter tip culture	Probable

*CLABSI, central line–associated bloodstream infection; CRBSI, catheter-related bloodstream infection, defined according to Infectious Disease Society of America guidelines (*10*); CVC, central venous catheter; peri, peripheral. CVC values indicate blood for culture collected from CVC; peri values indicate blood for culture collected from peripheral vessel.

ceftriaxone), minocycline (5 patients), or amikacin (4 patients). A positive response occurred in 14 patients, and breakthrough *Nocardia* bacteremia occurred in 3 patients receiving trimethoprim/sulfamethoxazole prophylaxis (2 with CLABSIs and 1 with disseminated bacteremia).

Nocardia isolates adhered extensively to polyurethane and silicone CVCs and formed extensive biofilms (Figure 1). Quantitative biofilm cultures isolated ranged from 4.0×10^5 to 1.1×10^7 CFUs of tested *Nocardia* cells from biofilm matrices that adhered to polyurethane and silicone CVC surfaces. Furthermore, trimethoprim/sulfamethoxazole with heparin, minocycline, and EDTA in 25% ethanol, and trimethoprim and EDTA in 25% ethanol significantly decreased the biofilm biomasses on treated vs. control plates after a 2-hour exposure ($p = 0.003$), resulting in complete eradication of *Nocardia* spp. in biofilm on silicone disks (Figure 2).

Electron and confocal scanning laser microscopic studies of the CVC tip in a patient with an *N. nova* CLABSI showed *Nocardia* spp. adhering to the surface and colonies of multilayered clusters embedded in biofilm matrix (Figure 3). Scanning electron microscopy studies of *N. nova* complex (disseminated *Nocardia* bacteremia) showed a heavy biofilm matrix covering filamentous clusters (Figure 4, panel A). *N. nova* complex (definite CLABSI) formed branching networks of filamentous bacteria encompassed in an intense heavy biofilm matrix (Figure 4, panel B).

Discussion

We have documented that *Nocardia* spp. that cause clinical CLABSI also form heavy biofilm on the surfaces of polyurethane and silicone CVCs; that trimethoprim- and minocycline-based lock solutions eradicate adherent catheter-related *Nocardia* spp. in the biofilm matrix (thereby demonstrating Koch's postulate as it relates to *Nocardia* CLABSI); and that *Nocardia* bacteremia in cancer patients can occur as CLABSI in 59% of cases. *Nocardia* CLABSI responds well to antimicrobial agents and CVC removal

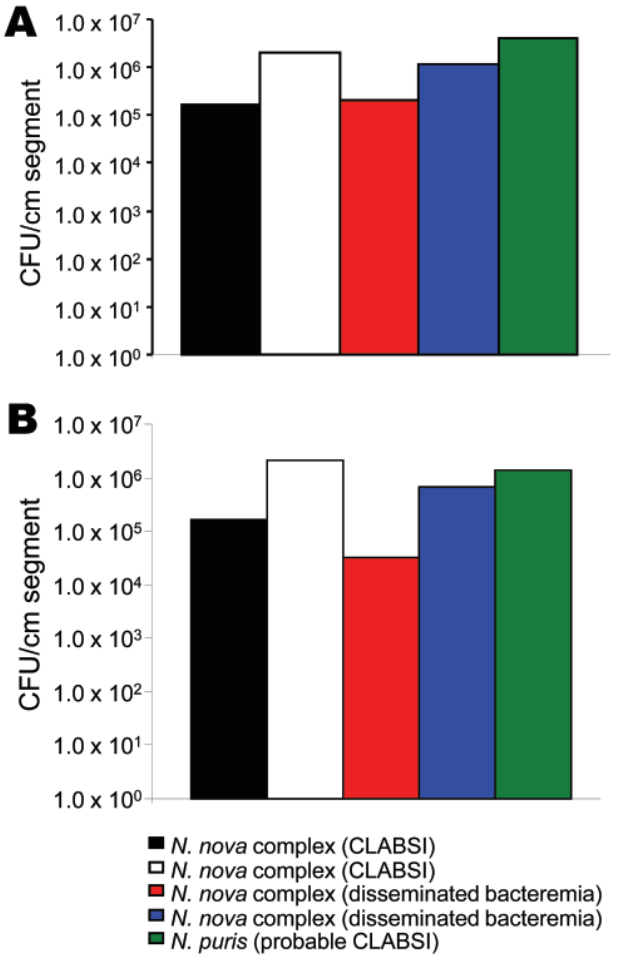

Figure 1. *Nocardia nova* and *N. puris* quantitative biofilm formation, as assessed by biofilm colonization model. *Nocardia* spp. isolates adhered to polyurethane (A) and silicone (B) central venous catheter segments with extensive biofilms. CLABSI, central line–associated bloodstream infection.

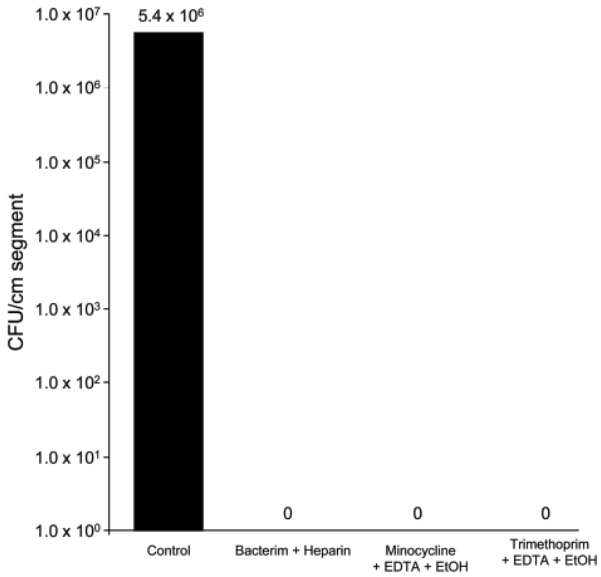

Figure 2. Antibiofilm agents inhibition of biomass of *Nocardia nova* complex biofilms. *N. nova* complex biofilms were grown for 24 h on silicone disks, placed in 24-well tissue culture plates, and exposed to trimethoprim/sulfamethoxazole (Bacterim) and heparin; trimethoprim, EDTA, and ethanol (EtOH); minocycline, EDTA, and ethanol; or Mueller-Hinton broth medium (control) for 2 h. Minocycline and trimethoprim-based lock solutions completely inhibited the *N. nova* complex biofilm biomass compared with controls (p = 0.003).

and is associated with a short hospital stay, whereas disseminated *Nocardia* bacteremia is associated with a poor prognosis.

Identification of *Nocardia* is more rapid, precise, and accurate with PCR and 16S rDNA sequencing than with standard phenotypic techniques (*17–19*). *N. asteroides* complex, which includes *N. farcinica*, *N. nova*, and *N. asteroides* sensu stricto, is the most commonly identified causative species in cancer patients (*7*). In our study, *N. nova* complex was responsible for 35% of *Nocardia* bacteremia episodes. *N. nova* is differentiated from other members of *N. asteroides* complex by DNA homologic characteristics; it is more appropriate to refer to *N. nova* as *N. nova* complex because it comprises 4 distinct species (*12*,*17*,*20*). Furthermore, the incidence of *N. nova* complex bacteremia may have been underestimated in our study because the species was not identified for 5 cases of infection with *N. asteroides* complex. *N. nova* complex strains may have higher tendencies toward CVC adherence, biofilm formation, and hematogenous spread.

Nocardia bacteremia is rare, even in severely immunocompromised patients with underlying malignancies (*11*). A 1998 review of the medical literature found only 36 cases of *Nocardia* bacteremia worldwide over 52 years (*5*). All *Nocardia* bacteremia patients in our study had indwelling CVCs before diagnosis and subsequently had catheters removed. Cancer patients depend immensely on vascular access devices, and CVCs may be the source and focus of *Nocardia* bacteremia, given the ubiquitous presence of these organisms in the environment, their possible acquisition through the skin, and their ability to adhere to CVCs through biofilm formation on the surface of catheters as shown in our study (Figure 1) (*7*,*21*,*22*).

Nocardia CLABSI cases, in contrast to disseminated *Nocardia* bacteremia cases, were associated with a favorable outcome. Catheter-related *Nocardia* bacteremia might be less likely to invade remote anatomical structures

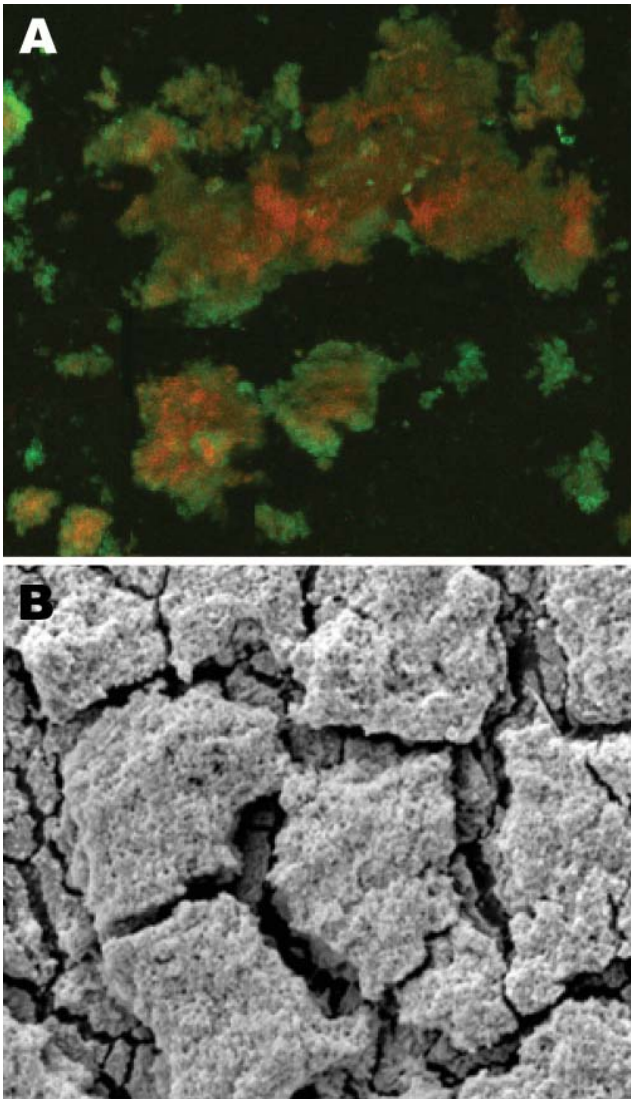

Figure 3. A) Confocal scanning laser microscopy image of central venous catheter tip in a patient with *Nocardia nova* complex central line–associated bloodstream infection. Bright green objects are viable biofilm bacteria, and orange-red objects are dead bacteria. Original magnification ×25. B) Scanning electron microscopy image of central venous catheter tip reveals biofilm surface structure. Original magnification ×5,000.

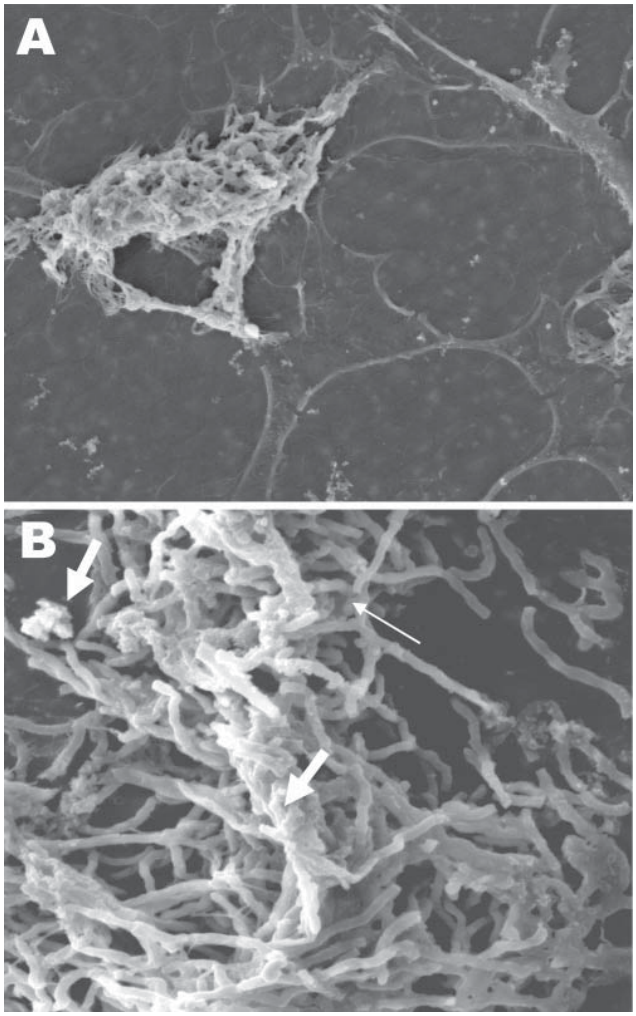

Figure 4. Scanning electron microscopy of *Nocardia* spp. biofilm on silicone central venous catheters. A) *N. nova* complex (disseminated *Nocardia* bacteremia) on the surface, showing heavy biofilm matrix covering filamentous cells. B) *N. nova* complex (definite central line–associated bloodstream infection) showing network of filamentous (thin arrow), partially covered with opaque biofilm matrix (thick arrows). Original magnifications ×2,500.

and form deep-seated infections. Disseminated *Nocardia* bacteremia, on the other hand, was associated with higher rates of admission to intensive care units, more frequent occurrence with hematologic malignancy, longer hospital stays, and lower antimicrobial therapy response rates. Factors that could have contributed to the poor outcome include involvement of the lungs and other organs; severity of the underlying disease (hematologic malignancy); and a higher rate of concomitant infections, most commonly with cytomegalovirus and invasive fungi (Table 1).

Although trimethoprim/sulfamethoxazole was uniformly active against *Nocardia* spp. and the most commonly used drug for *Nocardia* bacteremia, breakthrough *Nocardia* bacteremia occurred in 3 patients despite receipt of trimethoprim/sulfamethoxazole prophylaxis. Given that *Nocardia* spp. were shown in our model to form an antimicrobial drug–resistant multilayered biofilm matrix, in which they embed themselves, it is not surprising that 2 of the 3 cases of breakthrough *Nocardia* bacteremia that occurred during trimethoprim/sulfamethoxazole prophylaxis were CLABSIs. CVC biofilm colonization through formation of an antimicrobial drug–resistant matrix is the main factor in the pathogenesis of CRBSIs. The biofilm enables *Nocardia* spp. to protect themselves from the relatively low serum concentrations of antimicrobial drugs given orally and, hence, create a foothold from which they can invade the bloodstream through the surface of the CVC intravascular segment. Prophylactic antimicrobial drugs given orally are ineffective at breaking down the matrix and eradicating the bacteria, which poses a therapeutic and prophylactic challenge for cancer patients with a CVC. Furthermore, trimethoprim/sulfamethoxazole has been ineffective when used alone, especially against disseminated forms of nocardiosis (*23*).

Intraluminal antimicrobial lock therapy has been proposed for the prevention and treatment of CLABSIs (*24–27*). For antimicrobial lock therapy, the catheter lumen is filled with 2–4 mL of antimicrobial solution at a concentration 100- to 1,000-fold higher than the MIC of the drug or its usual target systemic concentration; to eradicate the organisms embedded in the intraluminal biofilm, the solution is then allowed to dwell (lock) while the catheter is not in use (*27*). We found antimicrobial catheter lock solutions with active agents such as trimethoprim/sulfamethoxazole or minocycline to be effective against *Nocardia* biofilm. Use of these solutions might be a valid way to prevent *Nocardia* CLABSIs and salvage the CVC, particularly in cancer patients with CLABSI, for whom removal of the CVC might not be possible because of severe thrombocytopenia or lack of other vascular access.

Although no complete correlation exists between in vitro susceptibility and clinical outcome, antimicrobial drug susceptibility tests should be performed for *Nocardia* isolates in immunocompromised patients to guide therapy. Drug susceptibility of *Nocardia* spp. varies among species (*22*).

In agreement with previous results, our findings also confirmed in vitro activity of trimethoprim/sulfamethoxazole, amikacin, and linezolid against *Nocardia* spp. (*17*,*28–31*). However, in our study, *N. nova* complex was characterized by its susceptibility to clarithromycin and resistance to amoxicillin and clavulanate. This distinctive characteristic of *N. nova* complex is associated with the presence of membrane-bound penicillinase inducible by clavulinic acid (*17*,*27*). *N. veterana* had an antimicrobial drug susceptibility profile similar to that of *N. nova*

complex. No minocycline resistance was observed, but 45% of isolates had intermediate susceptibility MICs, as has been reported (*32–34*).

CVC removal, along with the use of a combination of antimicrobial agents guided by antimicrobial drug susceptibility, should be the cornerstone of treatment of *Nocardia* bacteremia, particularly CLABSIs. We recommend using a combination of amikacin, carbapenems, and trimethoprim/sulfamethoxazole until the *Nocardia* isolate and its antimicrobial drug susceptibility can be determined (*17*,*32*,*33)*.

In conclusion, *Nocardia* bacteria promote heavy biofilm formation in CVCs, and trimethoprim and minocycline in combination with anticoagulants as lock solutions have potent activity against *Nocardia* biofilm formation. In this study, *N. nova* complex isolates were the leading cause of *Nocardia* bacteremia. Isolation of *Nocardia* bacteria from the blood should always prompt consideration of *Nocardia* CLABSIs in cancer patients with indwelling CVCs, especially in the absence of signs and symptoms of pneumonia or disseminated infection.

Dr Al Akhrass recently completed an infectious disease fellowship at The University of Texas MD Anderson Cancer Center and is board certified in internal medicine. His research interests include catheter-related infections and biomarkers and infection.

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Endemic Scrub Typhus–like Illness, Chile

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We report a case of scrub typhus in a 54-year-old man who was bitten by several terrestrial leeches during a trip to Chiloé Island in southern Chile in 2006. A molecular sample, identified as related to *Orientia tsutsugamushi* based on the sequence of the16S rRNA gene, was obtained from a biopsy specimen of the eschar on the patient's leg. Serologic analysis showed immunoglobulin G conversion against *O. tsutsugamushi* whole cell antigen. This case and its associated molecular analyses suggest that an *Orientia*-like agent is present in the Western Hemisphere that can produce scrub typhus–like illness. The molecular analysis suggests that the infectious agent is closely related, although not identical, to members of the *Orientia* sp. from Asia.

The primary hosts for *Rickettsia* species are arthropods that can also act as disease vectors for humans and other vertebrates. Ticks are vectors for most rickettsioses caused by spotted fever group rickettsiae. Alternative vectors for rickettsiae are well known, including fleas as vectors for murine typhus (*R. typhi*) and flea-borne spotted fever (*R. felis*), mites as vectors of rickettsialpox (*R. akari*), and scrub typhus (*Orientia tsutsugamushi*), and lice as vectors for epidemic typhus (*R. prowazekii*) (*1*).

In Chile, the last outbreak of epidemic typhus began in 1933 and continued through 1939 (*2*). In the following years, effective control and sanitary measures were developed and implemented. No new cases of rickettsial disease have been reported in this country since 1976 (*3*). Scrub typhus, caused by *O. tsutsugamushi*, which is usually transmitted by trombiculid mites in Asia, northern Australia, and the western Pacific region has never been described in Chile (*4*). Although sporadic cases of scrub typhus have been reported well outside the traditionally endemic regions (*5*,*6*), no reports are known of scrub typhus being acquired in the Western Hemisphere (*4*). In addition, no human case of rickettsial spotted fever has been documented in Chile, although there is evidence of rickettsial infections in dogs (*7*) and of the presence of *R. felis* in cats and cat fleas (*8*). We report a case of scrub typhus–like illness in Chile.

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DOI: http://dx.doi.org/10.3201/eid1709.100960

Materials and Methods

The patient was a previously healthy 54-year-old man who recalled having been bitten by terrestrial leeches on several occasions, but not by ticks. He was hospitalized after symptoms including a high-grade fever developed. During treatment, a black eschar with an erythematous halo on the left leg was found. A biopsy sample from the leg eschar was submitted to the laboratory for histopathologic analysis and subjected to microscopy. A routine blood chemistry panel was analyzed. In addition, an ELISA to detect *O. tsutsugamushi*–specific immunoglobulin G was performed with acute-phase and convalescent-phase serum samples (*9*).

The same skin biopsy samples of the eschar and rash were submitted for molecular biology analysis. DNA was extracted from the skin biopsy samples by using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The prokaryotic 16S rRNA gene was amplified and sequenced by using described primers (*10*). The PCR products were purified with the GFX DNA gel band purification kit (GE Healthcare, Piscataway, NJ, USA) and sequenced by using

the BigDye Terminator version 3.1 Cycle Sequencing Kit and a 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Sequences obtained from the leg and arm samples were assembled by using the Sequencher DNA Software (Gene Codes Corporation, Ann Arbor, MI, USA) and were judged to be identical. The sequences were initially compared with other 16S rRNA sequences in GenBank by using the National Center for Biotechnology Information BLAST network service software (*11*). The sequence of the biopsy sample (referred to as the Chiloé Island sample) has been deposited in GenBank under accession no. HM155110. Sequence differences between the Chiloé Island sample and isolates of *O. tsutsugamushi* were determined after aligning 16S rRNA sequences, using ClustalX in MEGA4 (*12*).

The patient had been involved in ecological studies at a university field camp at the southern end of Chiloé Island, in southern Chile. The region is rainy and has abundant natural vegetation and evergreen forests. During January 2006, the patient spent 3 weeks on a field study (sleeping in a log cabin) with daily forest incursions. He recalled having been bitten by terrestrial leeches on several occasions but not by ticks.

A week after returning to the capital, Santiago, and 6 days before his admission to the hospital, a high-grade fever, headache, myalgias, and scanty dry cough developed. Four days later, a rash appeared in the abdominal region that progressed to his face and limbs. At admission, the patient had an axillary temperature of 39ºC, pulse 101 beats/min, blood pressure 110/75 mm Hg, and bilateral conjunctival suffusion. He had an extensive rash on his face, trunk, and limbs but not on the palms and soles, with a microvesicular center in some of the lesions (Figure 1, panel A). A black eschar with an erythematous halo on the left leg was found. He recalled having been bitten by a leech ≈3 weeks before (Figure 1, panel B).

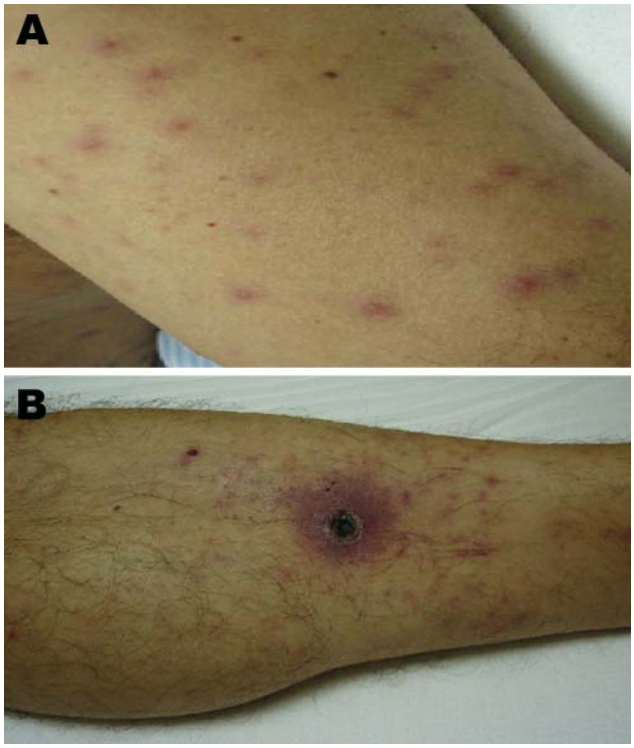

Figure 1. Evidence of acute infection of the skin and subcutaneous tissue in patient admitted for treatment of scrub typhus-like symptoms in Chile. A) Rash on admission, left arm. B) Necrotizing eschar with erythematous halo over the left leg.

Results

Dermis and subcutaneous fat showed a necrotizing leukocytoclastic vasculitis, perivascular infiltrates with lymphocytes and macrophages, and extravasation of erythrocytes (Figure 2, panel A). Gram, Giemsa, and Warthin-Starry silver stains did not show any microorganisms. A tissue sample recovered from a paraffin-embedded sample for electron microscopy, showed round and oval rickettsia-like microorganisms, maximum diameter 0.2–0.5 μm, inside the cytoplasm endothelial cells (Figure 2, panel B).

Blood tests showed a leukocyte count of 9,200 cells/mm^3, with 28% immature forms and a slight elevation of hepatic aminotransferase levels (aspartate aminotransferase 198 U/L [reference range 10–40 U/L], alanine aminotransferase 256 U/L [reference range 10–55 U/L], and alkaline phosphatase 338 U/L [reference range 45–115 U/L]) with normal bilirubin level. Blood cultures (2 sets) were negative as were serologic test results for measles, varicella, leptospirosis, and HIV. A spotted fever rickettsiosis was suspected and doxycycline (100 mg 2×/d) was started on the day after admission. ELISA to detect *O. tsutsugamushi* specific immunoglobulin G on the acute-phase sample had no reactivity (titer <100) to the Karp, Kato, Gilliam combined whole-cell ELISA antigen, whereas the convalescent-phase serum sample, collected 4 months after hospitalization, had a titer of 400, showing that seroconversion had occurred between the 2 time points. These same serum samples were examined for reactivity to ELISA antigens from rickettsial spotted fever group and rickettsial typhus group. The serum samples were nonreactive to both groups of antigens (titer <100). Skin punch biopsy specimens (4 mm) from the skin lesions (eschar and rash) were taken on the second day after admission. After the second day of antimicrobial drug therapy, the patient's general condition markedly improved and the fever subsided. On the following days the skin lesions began to fade. The patient was discharged in good health condition on the fifth day of hospitalization.

DNA from the biopsy sample was used for a molecular analysis in order to identify the infectious agent. Analysis found that the sequence of the 16S rRNA gene obtained from the skin biopsy specimen showed ≈97% sequence similarity with isolates of *O. tsutsugamushi* (39–44 nt differences in a 1,265-bp alignment of the 16S rRNA gene). Recently, a case of scrub typhus was reported from Dubai outside the normal range of the disease (*5*). A similar level of difference (42-nt differences) was seen when the Chiloé Island sample was compared with the *O. chuto* sp. nov. sample from Dubai.

Sequence differences among various isolates of *O. tsutsugamushi* ranged from 1 to18 nt, and *O. chuto* sp. nov. differed from the *O. tsutsugamushi* samples by 23 to 31 nt differences. Comparisons were also made with 16S rRNA sequences from 2 leech-associated forms placed within the genus *Rickettsia* (*13,14*), and with taxa from genus *Neorickettsia*, a group of obligate intracellular forms placed within the Ehrlichiaceae that have been isolated from trematodes. When compared with 2 isolates that represent presumptive members of the genus *Rickettsia* that are endosymbionts of leeches in Japan, sequence similarity to the Chiloé Island sample averaged 91.9% (97-nt differences). As expected, if no special selective effect resulted from being associated with leeches as a vector, the degree of sequence change is equivalent to that found when the 16S rRNA gene sequence of the Chiloé Island sample is compared with other members of *Rickettsia* not associated with leeches (sequence similarity 91.6%).

Isolates of *O. tsutsugamushi* averaged 91.8% sequence similarity to other members of *Rickettsia*, and the *O. chuto* sp. nov. sample averaged 92.2% similarity to non-leech *Rickettsia* spp. The 16S rRNA gene sequence showed even greater divergence when the Chiloé Island sample was compared with 3 *Neorickettsia* spp., with sequence similarity averaging 83.4% (average diversity 215 nt). Isolates of *O. tsutsugamushi* averaged 82.7% sequence similarity to *Neorickettsia*, and the *O. chuto* sp. nov. sample averaged 82.2% similarity. Taken together, these results are consistent with 16S rRNA gene sequence of the Chiloé Island sample being representative of an *Orientia* spp.–like form.

The phylogenetic relationship of the Chiloé Island sample with other isolates of *O. tsutsugamushi* was inferred by using the neighbor-joining method (*15*), and is shown in Figure 3. The evolutionary distances were computed using the maximum composite–likelihood method (*16*) and are in units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). A total of 1,256 positions were identified in the final dataset. Phylogenetic analyses were conducted in MEGA4 (*12*). Sequences of the 16S rRNA gene sequences from the 3 taxa of *Neorickettsia*

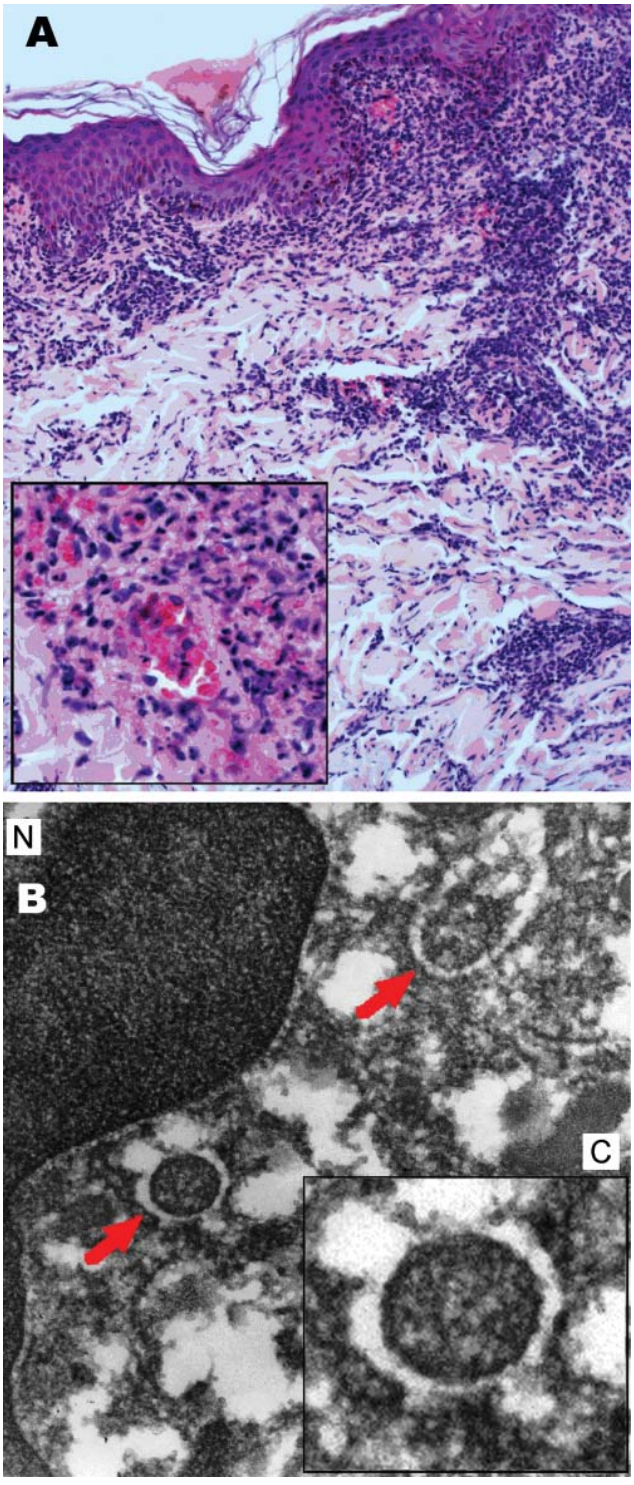

Figure 2. Results of biopsy analysis of tissue sample from eschar on the left leg of patient admitted for treatment of scrub typhus–like symptoms, Chile. A) Leukocytoclastic vasculitis. Hematoxylin and eosin stained; original magnification ×200, inset ×400. B) Endothelial cell, showing nucleus (N) within the cytoplasm (C, inset). Arrows show similar round and oval organisms, electron-dense, surrounded by electron-lucent halo of rickettsial type microorganisms. Electron microscopy; original magnification ×15,000, inset ×20,000.

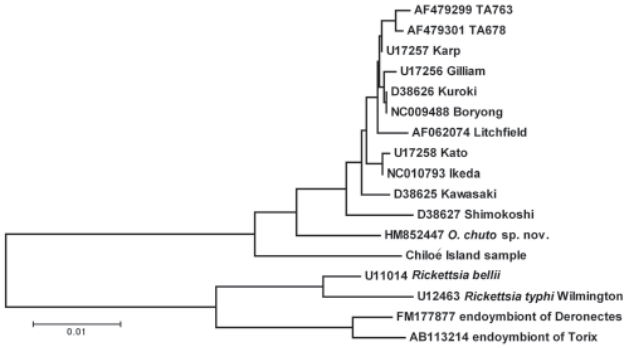

Figure 3. Evolutionary relationships of Chiloé Island sample compared with other isolates of *Orientia tsutsugamushi,* with *O. chuto* sp. nov. and with taxa from *Rickettsia*, determined by the method of neighbor joining (*15*). The tree is drawn to scale; scale bar indicates nucleotide substitutions per site. Numbers on branches represent percentage of 1,000 bootstrap replicates that include the enclosed clade. Entries on the tree are identified by GenBank accession number and isolate name.

were used to root the tree. The Chiloé Island sample is well differentiated from sequences from *O. tsutsugamushi* and *O. chuto* sp. nov., being separated in 100% of bootstrap replicates of the analysis, but clearly significantly closer to the samples classified within *Orientia*, compared with other rickettsiae in the sequence databases.

Two other PCR assays were performed that were specific for sequences of *O. tsutsugamushi* and the genus *Rickettsia* by using primers that targeted a portion of the *groEL* gene because of its higher power of differentiation between closely related taxa. However, no appropriate size amplicons were produced. Moreover, results of a quantitative real-time PCR assay for *O. tsutsugamushi* (*17*) and 2 PCR assays (47 kDa/*HtrA* gene and *groEL* gene) used with the DNA preparation extracted from acute-phase serum sample (2 days after admission, 1 day after antimicrobial drug treatment) were negative (data not shown).

In the study of the new form, *O. chuto* sp. nov., from Dubai, the 47-kDa/*HtrA* gene was amplified. The degree of sequence divergence compared with isolates of *O. tsutsugamushi* was substantial, averaging >17% *(5)*. Given that the Chiloé Island sample shows almost twice as much divergence from *O. tsutsugamushi* for the 16S rRNA sequence compared with *O. chuto* sp. nov., it is not unreasonable that substitutions in the PCR primer sites of the Chilean sample exist, explaining the negative results that were found in our study.

Discussion

We describe a case of rickettsiosis acquired in Chiloé Island, where the local population is mostly of the Huilliche ethnic background. One of the ancient local legends refers to a disease developing in persons who penetrate the jungle, with the development of high fever and red spots all over the body. However, no scientific medical report had confirmed this finding.

Even though the existence of scrub typhus has never been recorded in Chile, its vector, the trombiculid mite (Acari: Trombiculidae), has been recently described in wetlands from a distant region of southern Chile, although not on Chiloé Island (*18*). Our patient recalled specifically having been bitten by a leech in the site where an eschar later developed. Terrestrial leeches are common on Chiloé Island vegetation. These include members mainly from the family *Mesobdellidae*, including the species *Mesobdella gematta* and *Nesophilaemon skottsbergi* (*19*). The leeches live among trees, ferns, bushes, and fallen leaves. All are sanguivorous parasites of vertebrate animals, and local persons are frequently exposed to leech bites on the island. Rickettsiae have been reported in leeches in Japan (*13,14*). In those studies, the glossiphoniid leech species harbored bacteria of the genus *Rickettsia*, as assessed by electron microscopy and PCR analysis.

The results of analysis of the 16S rRNA gene sequence suggest that the sample reported represents a previously unreported, divergent form (species) of *Orientia* spp.–like bacteria. The degree of sequence differentiation from isolates of *Orientia* spp. previously studied in Asia and the Middle East indicates that the Chiloé Island sample is not simply a transplanted form from Asia that happened to be discovered in Chile, but rather it represents a long divergent lineage and may be indicative that other *Orientia* spp.–like pathogens are to be found outside southern and eastern Asia or northern Australia. The difficulty of obtaining PCR amplification of additional sequences, such as the GroEL and 47-kD protein genes, would be consistent with the identification of a new lineage divergent from Asian forms of *O. tsutsugamushi*. Moreover, the reactivities of the serum samples to *O. tsutsugamushi* Karp, Kato, Gilliam ELISA antigens (titer 400) suggest that the cross-reactivity of assay antigens to those of the new sample may exist but be limited, again consistent with a divergent lineage.

Future steps following the case presented will involve investigating whether Chiloé Island's leeches carry rickettsiae and whether these rickettsiae, according to additional DNA sequence analysis, are closely related to members of *O. tsutsugamushi* or if they represent a new lineage within or closely related to the known forms of *Orientia*. If no related rickettsiae are identified from leeches, an alternative possibility is that trombiculid mites are present on Chiloé Island and that these are the vectors of the pathogen. However, the observation that the eschar developed at the site of leech attachment would appear to argue against an alternative vector. Nevertheless, chiggers,

the proven vector free-living stage in the mite life cycle that feeds on the vertebrate hosts, are small and easily overlooked. Thus, a mite cannot be positively excluded as the vector in this case. Whether other sporadic cases of human rickettsial illness may have occurred in that area should also be the subject of future investigation.

This study was supported by the Global Emerging Infections Surveillance and Response System, a Division of the Armed Forces Health Surveillance Center (work unit no. 0000188M.0931.001.A0074); and National Institutes of Health, National Eye Institute (grant no. EY090703).

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Geographic Distribution of Endemic Fungal Infections among Older Persons, United States[1]

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To investigate the epidemiology and geographic distribution of histoplasmosis, coccidioidomycosis, and blastomycosis in older persons in the United States, we evaluated a random 5% sample of national Medicare data from 1999 through 2008. We calculated national, regional, and state-based incidence rates and determined 90-day postdiagnosis mortality rates. We identified 776 cases (357 histoplasmosis, 345 coccidioidomycosis, 74 blastomycosis). Patient mean age was 75.7 years; 55% were male. Histoplasmosis and blastomycosis incidence was highest in the Midwest (6.1 and 1.0 cases/100,000 person-years, respectively); coccidioidomycosis incidence rate was highest in the West (15.2). On the basis of available data, for 86 (11.1%) cases, there was no patient exposure to a traditional disease-endemic area. Knowledge of areas where endemic mycosis incidence is increased may affect diagnostic or prevention measures for older adults at risk.

Fungal infections have become an increasing problem for older persons in the United States (*1*–*4*). Compared with years past, older adults today are more likely to be considered for transplantation, receive aggressive regimens of chemotherapy, or take immunosuppressive drugs for rheumatologic or autoimmune diseases. In addition, increasing longevity has enabled older adults to travel and participate in outdoor activities where they might be exposed to opportunistic fungal organisms that they did not encounter in their youth or for which primary immunity has waned. Major opportunistic infections in older adults include the endemic mycoses histoplasmosis, blastomycosis, and coccidioidomycosis. These infections are acquired through inhalation of spores in the environment and are often associated with outdoor activities and geographic exposures (*2*,*5*–*8*). Increasing age and decreasing cell-mediated immunity as a result of transplantation, chemotherapy, or other immunosuppressive medications (e.g., tumor necrosis factor–α inhibitors) are the main predisposing factors (*1*,*2*,*9*).

Few data describing the incidence and geographic distribution of endemic mycoses in older persons in the United States are available. For infections such as histoplasmosis or blastomycosis, much of the information regarding geographic distribution of infection was described decades ago for younger adults (*10*,*11*). The most frequently cited study that describes areas of endemicity for histoplasmosis in the United States was published in 1969 by Edwards et al. (*10*). The study identified histoplasmosis endemicity on the basis of histoplasma skin testing, a diagnostic method of unknown sensitivity and probably poor specificity. The study population was military recruits; no older persons were included. In a recent report, Chu et al. described hospitalizations for endemic mycoses in adults and children by using 2002 Nationwide Inpatient Sample Data (*12*). Hospitalization rates for adults were described per region, but incidence rates, specifically for older patients, were not available. Moreover, outpatient cases were not captured; thus, rates were underestimated. Additional contemporary data regarding endemic mycoses among older persons in the United States are needed and would be helpful for identifying disease patterns and the geographic distribution of infection and for targeting areas

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DOI: http://dx.doi.org/10.3201/eid1709.101987

[1]This research was presented in part at the 74th American College of Rheumatology Annual Meeting, November 7–11, 2010, Atlanta, Georgia, USA.

for focused disease prevention. We describe the geographic distribution of endemic mycoses.

Methods

We conducted a retrospective cohort study by using 1999–2008 claims data for a random 5% national sample of Medicare beneficiaries. Inclusion criteria for entry into the cohort were as follows: 1) age ≥65 years at start of follow-up; 2) having full Medicare coverage (parts A and B, not in a Medicare Advantage plan) for at least 13 consecutive months; 3) living in one of the 50 US states or Washington DC; and 4) not having claims for any endemic mycosis during a 12-month period before the start of follow-up (to avoid misclassifying prevalent cases as incident cases). Cohort follow-up began on the earliest date of meeting all eligibility criteria and ended on the earliest of the date of death, loss of full coverage, or diagnosis of endemic mycosis.

Clinical Data and Definitions

Patient data included demographics, concurrent medical conditions, and diagnosis of endemic mycosis. Endemic mycoses were identified by using codes from the International Classification of Diseases, 9th Revision (ICD-9) (histoplasmosis 115.x, coccidioidomycosis 114.x, blastomycosis 116.x). An incident case of an endemic mycosis required 1 inpatient claim (primary or secondary hospital discharge diagnosis) or at least 2 outpatient claims at least 7 days apart but within 90 days. Two outpatient claims were used to improve definition specificity (*13*). For a subpopulation of the cohort (beneficiaries with Medicare/Medicaid Part D data from 2006 through 2007), outpatient information about antifungal drugs was available. From these data, we developed a more specific case definition and compared results with our primary definition. The more specific case definition was an ICD-9 code for the mycosis plus receipt of a prescription for fluconazole, itraconazole, or voriconazole within 60 days of the diagnosis.

Concurrent medical conditions were identified by use of primary or secondary discharge diagnoses or outpatient visit ICD-9 codes; they were defined as 1 physician claim within 6 months before the case date. Diagnosis codes used for specific conditions are as follows: chronic obstructive pulmonary disease (COPD), 491, 492, 493.2, 496; chronic kidney disease, 585; diabetes mellitus, 250; solid tumor malignancy, 140–199 (excluding 173); hematologic malignancy, 200–208; and neutropenia, 288.0. Mortality rates were determined for 90 days after diagnosis.

Statistical Analyses

Descriptive statistics were calculated for demographic and clinical characteristics. National, regional, and state-based incidence rates (no. cases/100,000 person-years) were determined for the endemic mycoses. State-specific crude incidence rates for the endemic mycoses were calculated as the number of cases divided by the number of person-years of observation among eligible participants residing in each state. Nationwide incidence was obtained by computing the number of total cases of each endemic mycosis divided by the number of person-years of observation among eligible participants in our sample. Geographic regions were specified as South, Midwest, Northeast, and West on the basis of 2010 Census definitions. The geographic distribution of endemic mycoses was determined by indicating incidences by state on a US map. The primary geographic distribution analysis included all patients in the cohort identified as having an endemic mycosis. A secondary sensitivity analysis, excluding those patients with a change in primary residence in the claims data during the study period, was also performed to determine whether persons who had recently moved affected incidence rates. Finally, cases that occurred outside of traditional endemic areas were identified.

We defined endemicity on the basis of previously published studies describing geographic distribution (*8*,*10*–*12*,*14*,*15*). Histoplasmosis-endemic states were North Dakota, South Dakota, Nebraska, Kansas, Oklahoma, Texas, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Wisconsin, Illinois, Mississippi, Alabama, Kentucky, Tennessee, Indiana, Michigan, Ohio, West Virginia, Pennsylvania, New York, Georgia, North Carolina, and South Carolina. Blastomycosis-endemic states were histoplasmosis-endemic states plus Vermont. Coccidioidomycosis-endemic states were California, Utah, New Mexico, Arizona, Texas, Nevada, and Colorado. Statistical analyses were performed by using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Patient Characteristics

The 5% random Medicare sample comprised 1,913,247 beneficiaries who were eligible for the analysis (Table 1). Among these patients, 775 cases of endemic mycoses were identified (357 histoplasmosis, 345 coccidioidomycosis, 74 blastomycosis), of which 244 (31.5%) were diagnosed by outpatient visit codes only (Table 1). Patient mean age was 75.7 years; 55% of patients were male. Concurrent medical conditions among case-patients with any of the 3 mycoses mentioned above included COPD (34.8%, 95% confidence interval [CI] 28.4%–39.8%), diabetes mellitus (22%, 95% CI 19.9%–27.0%) solid malignancy (16.5%, 95% CI 11.9%–27.0%), and rheumatoid arthritis (5.2%, 95% CI 0–6.1%.) The frequency of underlying solid malignancy was higher among patients with blastomycosis (27%) than among patients with histoplasmosis (18.8%) or coccidioidomycosis (11.9%). In contrast, COPD

Table 1. Characteristics of Medicare beneficiaries with mycoses, United States, 1999–2008

Characteristic	No. (%) patients			
	Histoplasmosis, n = 357	Coccidioidomycosis, n = 345	Blastomycosis, n = 74	Total, n = 1,913,247*
Male sex	180 (50.4)	200 (58.1)	42 (56.5)	807,204 (42.2)
White race	342 (95.8)	62 (89.9)	61 (82.4)	1,679,198 (87.8)
Region				
Midwest	169 (47.3)	63 (18.3)	29 (39.2)	494,139 (25.8)
North	24 (6.72)	14 (4.0)	2 (2.7)	375,987 (19.7)
South	145 (40.6)	25 (7.25)	41 (55.4)	733,676 (38.4)
West	19 (5.3)	243 (70.4)	2 (2.7)	309,525 (16.2)
Rural location†	137 (38.4)	52 (15.1)	34 (46.6)	502,973 (26.3)
Concurrent medical conditions				
Chronic obstructive pulmonary disease	142 (39.8)	107 (31.0)	21 (28.4)	102,936 (5.4)
Diabetes mellitus	71 (19.9)	80 (23.2)	20 (27.0)	204,726 (10.7)
Solid malignancy	67 (18.8)	41 (11.9)	20 (27.0)	128,766 (6.7)
Hematologic malignancy‡	12 (3.4)	<11	<11	13,393 (0.7)
Rheumatoid arthritis	19 (5.3)	21 (6.1)	0	21,046 (1.1)
Chronic kidney disease‡	20 (5.6)	25 (7)	<11	13,393 (0.7)
Neutropenia‡	<11	<11	<11	3,826 (0.2)
90-day mortality§	35 (9.2)	32 (9.3)	<11	

*Random national sample of 5% of Medicare beneficiaries with claims during 1999–2008; selected for cohort were those who were age ≥65 years at start of follow-up, had full Medicare coverage (parts A and B, not in a Medicare Advantage plan) for at least 13 consecutive months; lived in the 50 US states or Washington, DC; and did not have claims for any endemic mycosis during a 12-month period before the start of follow-up. Mean age of those with mycoses was 75.7 years.
†Rural residential status defined by linkage of 9-digit ZIP codes to the rural-urban commuting area code for the corresponding census block.
‡Per Centers for Medicare and Medicaid Services data use guidelines, cell sizes <11 cannot be shown.
§Those who died within 90 days after mycosis diagnosis.

was more common among patients with histoplasmosis (39.8%) than among patients with coccidioidomycosis (31%) or blastomycosis (28.4%). Mortality rate at 90 days postdiagnosis was 9.5% and was similar for all endemic mycoses.

Incidence Rates

In the United States, the highest incidence rate was for histoplasmosis (3.3) (Figure 1), followed by coccidioidomycosis (3.2) (Figure 2) and blastomycosis (0.7) (Figure 3; Table 2). A geographic distribution was evident (Figure 1). Incidence rates for histoplasmosis were highest for the Midwest (6.1), especially Indiana (13.0) and Arkansas (12.0). Incidence rate for coccidioidomycosis was highest in the West (15.2), especially in Arizona (90.5) and California (10.1). Incidence rate for blastomycosis incidence was greatest in the Midwest (1.0), especially Mississippi (6.4) and Wisconsin (5.7).

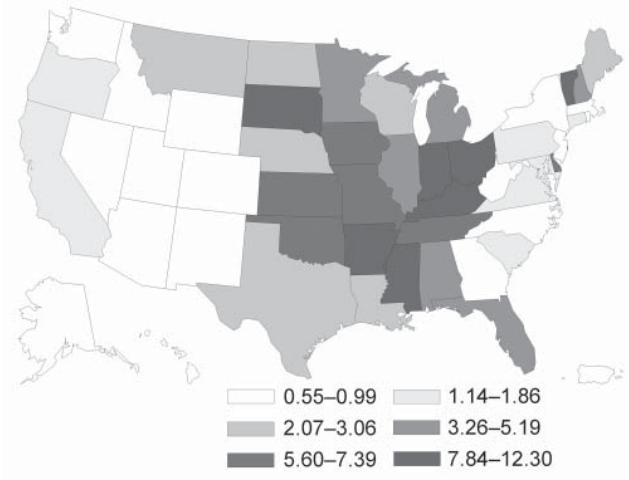

Figure 1. Geographic distribution of histoplasmosis in persons ≥65 years of age, United States, 1999–2008. Values are no. cases/100,000 person-years.

Cases of all 3 mycoses were identified in patients living outside traditional mycosis-endemic areas. To better assess these cases from non–mycosis-endemic areas, we identified whether patients had a medical claim in a traditional mycosis-endemic area during the study period (suggestive of travel to such an area) or previously resided in a mycosis-endemic area (based on residence while enrolled in Medicare). Of 357 histoplasmosis case-patients, 42 (11.8%) had no exposure to a mycosis-endemic area on the basis of available claims data. This finding was similar for blastomycosis (<11/74) and coccidioidomycosis (37/345, 10.7%) cases.

Alternative Case Definition

During 2006–2007, cases for 17 outpatients were identified by ICD-9 code only; Medicare Part D drug data were available. Among these patients, 10 (59%) had received a prescription for an antifungal drug in the 2 months after diagnosis (2/8 with histoplasmosis, 5/6 with coccidioidomycosis, 3/3 with blastomycosis).

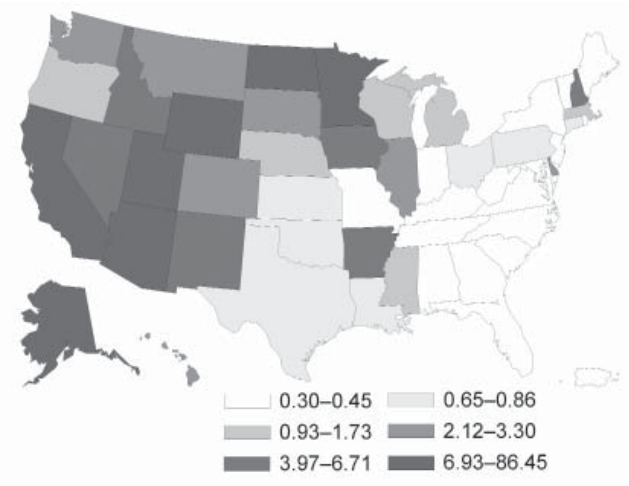

Figure 2. Geographic distribution of coccidioidomycosis in persons ≥65 years of age, United States, 1999–2008. Values are no. cases/100,000 person-years.

Discussion

This retrospective cohort study defined geographic distribution of endemic mycoses in older persons in the United States enrolled in Medicare and may help improve diagnostic or prevention measures for those at risk. These endemic mycoses were geographically distributed, but not all occurred in a traditionally mycosis-endemic area. Histoplasmosis was most common, although the highest state-based incidence rates were seen for coccidioidomycosis. As older persons in the United States continue to travel and participate in outdoor activities, exposure to these pathogens may increase. Moreover, increasing age and decreasing cell-mediated immunity as a result of transplantation, chemotherapy, or other immunosuppressive medications increase the risk for endemic mycoses (*1*,*9*). Overall, most cases occurred in patients without known immunocompromising conditions.

Studies estimating US incidence of histoplasmosis, coccidioidomycosis, or blastomycosis are limited, especially among older Americans. Our data suggest that the geographic distribution of these mycoses in older persons in the United States enrolled in Medicare is consistent with prior descriptions for younger patients (*10*,*12*); however, ≈10% of cases were identified in patients with primary residencies outside of mycosis-endemic regions. Our findings of increased incidence of histoplasmosis in the Southeast and Midwest were similar to prevalence estimates with use of skin testing among US Navy recruits (*12*). Our study expands on that early research by identifying cases of histoplasmosis, not antigen sensitivity, in an older population not described previously. Chu et al. reported a similar distribution of infection among children and adults with use of a similar case-finding method but evaluated only hospitalization data, potentially underestimating cases (*12*). Approximately 30% of our cases were identified only by outpatient physician claims.

Other studies have evaluated endemic mycoses in older adults but have not evaluated US geographic distribution (*2*,*4*). Leake et al. reported that coccidioidomycosis was more likely to develop in elderly persons who had recently moved to Arizona (*2*). Blair et al. compared clinical manifestations of coccidioidomycosis among older and younger patients and determined that immunosuppression, independent of age, was a predictor of widespread coccidioidomycosis (*4*). We used a sensitivity analysis and compared the complete cohort and a cohort that did not include patients who moved during the study but found that those who had recently moved did not affect regional incidence rates (data not shown). Of note, ≈10% of patients with an endemic mycosis had not lived or received medical services (based on available claims data) in a traditionally mycosis-endemic area, underscoring the need to consider these infections even in non–mycosis-endemic areas.

In our study population, concurrent conditions were common and, for the most part, similar in frequency among the endemic mycoses. COPD was the most common underlying disease for each endemic mycosis. Chu et al. found that immunosuppression, defined as hematologic or immunologic deficiency or transplantation, was more common in cases of histoplasmosis, when compared with the other endemic mycoses (*12*). Although we did not define immunosuppression as reported by Chu et al., solid malignancy was more frequent in cases of blastomycosis. In most cases, patients were without known immunocompromising conditions. Overall mortality rate

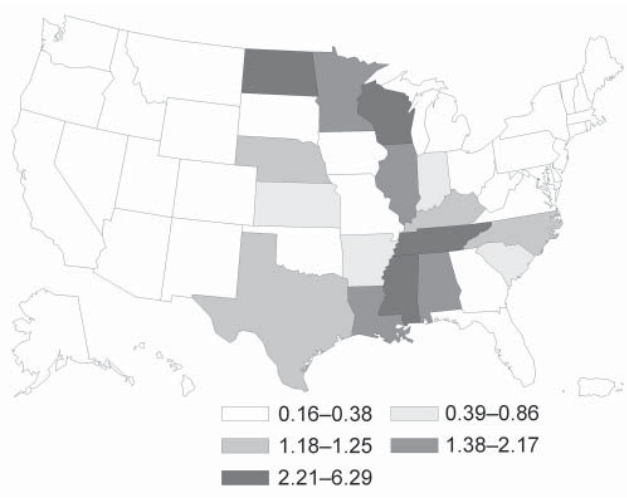

Figure 3. Geographic distribution of blastomycosis in persons ≥65 years of age, United States, 1999–2008. Values are no. cases/100,000 person-years.

Table 2. Incidence of endemic mycoses among cohort of Medicare beneficiaries, by region, United States, 1999–2008*

Location	No. cases/100,000 person-years (95% confidence interval)		
	Histoplasmosis, n = 357	Coccidioidomycosis, n = 345	Blastomycosis, n = 74
Midwest	6.1 (5.3–7.1)	2.0 (1.5–2.6)	1.1 (0.7–1.5)
Northeast	1.1 (0.7–1.7)	0.5 (0.3–0.9)	0.05 (0.01–0.30)
South	3.5 (3.0–4.1)	0.6 (0.4–0.9)	1.0 (0.8–1.4)
West	1.1 (0.7–1.7)	15.2 (13.4–17.2)	0.1 (0.03–0.50)
All of United States	3.4 (3.0–3.7)	3.2 (2.9–3.6)	0.7 (0.6–0.9)

*Random national sample of 5% of Medicare beneficiaries with claims during 1999–2008; selected for cohort were those who were age ≥65 years at start of follow-up, had full Medicare coverage (parts A and B, not in a Medicare Advantage plan) for at least 13 consecutive months; lived in the 50 US states or Washington, DC; and did not have claims for any endemic mycosis during a 12-month period before the start of follow-up. Mean age of those with mycoses was 75.7 years.

for patients with endemic mycoses was low and similar to that seen by Chu et al. (*12*).

The use of Medicare 5% sample data enables national representative estimates of disease occurrence in older Americans, but several limitations deserve mention. The results described from 5% sample Medicare data may not be representative of the entire older American population and may not be valid for other populations outside the United States or for those with other insurance plans. There may be some degree of ascertainment bias because recognition of cases may vary by geographic region. The validity of our identification of presumed cases of endemic mycoses by using ICD-9 codes in claims data are uncertain. Few published data are available that evaluate the positive predictive value of codes for endemic mycoses, compared with other case ascertainment methods, but positive predictive values for opportunistic mycoses approach 70% (*16*–*18*). Our validation, with use of 2006–2007 Medicare Part D drug data for outpatients, suggests that our primary definition is reasonably specific for defining cases of coccidioidomycosis or blastomycosis.

In conclusion, among this cohort of Medicare beneficiaries, histoplasmosis was the most common endemic mycosis. Geographic distribution among older persons in the United States for histoplasmosis, coccidioidomycosis, and blastomycosis is evident, although ≈10% of cases were identified for patients without evidence of claims or residence in traditionally mycosis-endemic areas. Knowledge of areas of increased incidence may improve diagnostic or prevention measures in older adults at risk for endemic mycoses, including those receiving immunosuppressive medications or with new environmental exposures.

This study was supported by the Agency for Healthcare Research and Quality (R01HS018517). E.D. received research support from Amgen.

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Seroepidemiologic Study of Pandemic (H1N1) 2009 during Outbreak in Boarding School, England

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We conducted a seroepidemiologic study during an outbreak of pandemic (H1N1) 2009 in a boarding school in England. Overall, 353 (17%) of students and staff completed a questionnaire and provided a serum sample. The attack rate was 40.5% and 34.1% for self-reported acute respiratory infection (ARI). Staff were less likely to be seropositive than students 13–15 years of age (staff 20–49 years, adjusted odds ratio [AOR] 0.30; ≥50 years AOR 0.20). Teachers were more likely to be seropositive than other staff (AOR 7.47, 95% confidence interval [CI] 2.31–24.2). Of seropositive persons, 44.6% (95% CI 36.2%–53.3%) did not report ARI. Conversely, of 141 with ARI and 63 with influenza-like illness, 45.8% (95% CI 37.0%–54.0%) and 30.2% (95% CI 19.2%–43.0%) had negative test results, respectively. A weak association was found between seropositivity and a prophylactic dose of antiviral agents (AOR 0.55, 95% CI 0.30–0.99); prophylactic antiviral agents lowered the odds of ARI by 50%.

In April 2009, an influenza A subtype H1N1 virus was isolated from persons in Mexico and the United States (*1*). This virus was responsible for the first influenza pandemic of the 21st century. The first cases of pandemic influenza A (H1N1) 2009 virus infection in the United Kingdom were reported on April 27, 2009, in a married couple who returned to Scotland after visiting Mexico (*2*). Several school outbreaks were reported soon after the virus was introduced into the United Kingdom (*3*,*4*), and influenza transmission in school settings was suggested as one of the primary drivers of the spread (*5*). In the United Kingdom, boarding schools have long been recognized as a good indicator population for the onset of seasonal influenza, leading to the establishment of Medical Officers of Schools Association surveillance scheme (*6*). In many schools, a high percentage of students and staff receive a seasonal influenza vaccine each year.

On May 27, 2009, a case of pandemic (H1N1) 2009 virus infection was confirmed in a student at a large boarding school in southeastern England from a respiratory sample submitted through the Medical Officers of Schools Association scheme. Public health authorities subsequently established that an ongoing outbreak of influenza-like illness (ILI) had occurred in this school in the 2 weeks before identification of the index case. Thirteen persons with onset of symptoms on or before that of the index case-patient also had positive test results, and health officials hypothesized that many unconfirmed clinical cases were also caused by infection with the emergent strain. This was the first recognized outbreak of the pandemic strain in a boarding school in the United Kingdom. In accordance with the Health Protection Agency's (HPA) guidance at the time, postexposure antiviral prophylaxis was offered to all staff and students, and any person exhibiting symptoms of ILI was offered testing and prescribed a treatment dose of antiviral drugs. This outbreak and the public health control measures have been reported (*7*).

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DOI: http://dx.doi.org/10.3201/eid1709.100761

Influenza viruses are readily transmitted among residents in enclosed institutional settings (*8*). Challenge studies have suggested that one third of persons infected by influenza may be asymptomatic (*9*). In population studies, the proportion of asymptomatic influenza infections has been estimated at 50% (*10*), but whether similar proportions exist for pandemic (H1N1) 2009 is uncertain. Evidence exists regarding the effect of previous seasonal influenza vaccination on the acquisition of pandemic (H1N1) 2009 (*11–15*). This outbreak, with apparent transmission to many students before it was reported, provided opportunities to quantify rates of asymptomatic infection in a closed setting and study the association between exposure to the 2008–09 seasonal influenza vaccine and the use of antiviral agents with pandemic influenza (H1N1) 2009. We conducted a seroepidemiologic study in a boarding school population to describe the clinical spectrum of disease caused by the 2009 pandemic strain and to quantify the proportions of symptomatic and asymptomatic infections.

Methods

The study population was the 1,307 students and 825 staff attending and working at the boarding school, and all were invited to participate in the study. However, because the investigation occurred during an examination period, not all students and staff were present, and the exact number staying at the school during this period is unknown. All students were boarders; some staff members lived on the school grounds, and others lived outside.

Most of the outbreak cases occurred in May 2009. Study participants were asked to complete an online questionnaire and provide a single serum sample. Samples were collected from June 11 through June 26, the last day of term. Collection of data from the online questionnaire also began on June 11 and continued until October 15.

Serologic testing by hemagglutination inhibition (HI) was carried out as previously described (*16–18*) at the Centre for Infections, HPA, London, using egg-grown NIBRG122 (reverse genetics derivative of A/Engl/195/2009). Serum specimens were pretreated with receptor-destroying enzyme II (Denka Seiken Co., Ltd, Tokyo, Japan), 1:4 (vol/vol), at 37°C for 19 h, followed by heat inactivation at 56°C for 1 h. The assay was performed by mixing 25 μL of virus suspension (containing 4 hemagglutinating units) with an equal volume of receptor-destroying enzyme II–treated serum, followed by 1 h incubation at room temperature, after which 25 μL of 0.5% (vol/vol) turkey erythrocytes was added to each well. Serum specimens were tested in a 2-fold serial dilution series with an initial dilution of 1:8 and ending at 1:1,024. Titers were expressed as a reciprocal of the highest serum dilution that fully prevented hemagglutination. Serum specimens with no reactivity in the first dilution (<8; considered negative) were assigned a titer of 4; serum specimens that showed titers ≥1,024 were assigned a numerical value of 1,024 for statistical analysis. Serologic samples were excluded from statistical analyses if a person had reported illness within 14 days of sample collection because previous data suggested that 14–21 days is required for a measurable immune response (*18*).

The online questionnaire collected data on demographic characteristics: sex; age (age groups, years: 13–15, 16–18 [students]; 20–49, ≥50 [staff]); symptoms; severity (self-described as mild, moderate, severe); self-reported use of antiviral drugs for treatment or prophylaxis; and self-reported 2008–09 seasonal influenza vaccination. Results from these questionnaires were subsequently linked to the serology results. Questionnaires were excluded if the person reported being away from the school during the outbreak or if symptom onset occurred after June 10, 2009.

The outcomes of interest were seropositivity and clinical cases of acute respiratory infection (ARI). Seropositivity was defined as having an HI titer ≥32, i.e., a titer 4× the minimum detection limit. Similar definitions have been used in population-based serosurveys in other countries (*19*,*20*) and have been shown to be specific in identifying recent infection in children (*21*). For sensitivity analysis, we refitted the final logistic regression model (below) using an alternative cutoff value of 1:8, the minimum detection limit (*22*).

A clinical case of ARI was defined as a person reporting any one of the following respiratory symptoms; runny/blocked nose, sore throat, or cough. Those reporting ARI were further subcategorized into a more specific case definition, i.e., cases of ILI, defined as a person reporting ≥1 of the symptoms listed above and fever. Exposures of interest were the use of antiviral drugs, prescribed for prophylaxis or treatment, and seasonal trivalent influenza vaccine in the previous year (2008–09).

We estimated the proportion of asymptomatic cases by determining the proportion of the population with positive serologic test results but no symptoms of ARI. We also estimated the attack rate for those with ARI, ILI, and positive serologic test results and their distribution according to demographic variables.

Logistic regression models were constructed to estimate the independent association of antiviral drugs and seasonal influenza vaccine and the odds of being seropositive or having an ARI. Age was included in the model as a covariate; other linear predictors were included if model fit was significantly improved (likelihood-ratio [L-R] test $p<0.05$). Interaction between age group and antiviral agents for prophylaxis; and seasonal influenza vaccine was tested to determine whether these associations between predictors and seropositive status and ARI differed according to age group, and therefore according to student and staff categories. If interaction was observed (i.e., the

model was improved by including the interaction term), students and staff would be reported separately. A further model was also fitted for staff to investigate whether staff role and sex were associated with a seropositive status or ARI. Data analysis was carried out by using Stata version 11 (StataCorp LP, College Station, TX, USA).

Informed consent was sought from all students and their parents or guardians if students were <16 years old. Because this was a field epidemiology study conducted during an emerging pandemic and involved a novel virus with unknown clinical effects, HPA did not require formal ethical approval since any information gained was essential in illuminating the effects of the infection and indicating possible control measures.

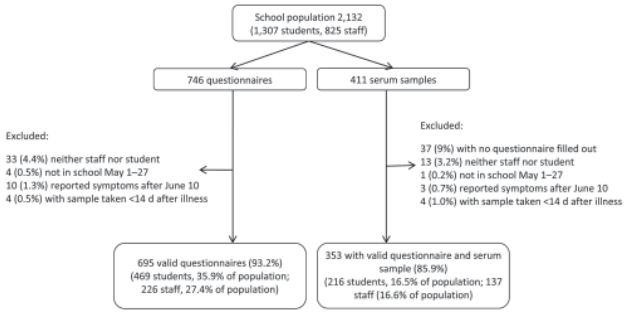

Figure. Number and proportion of boarding school staff and student populations who completed a questionnaire and had matched serologic test results, England, 2009.

Results

Sample Population

In total, 746 questionnaires were completed online, of which 695 (93.2%) met the inclusion criteria (Figure). This represented 35.9% of the 1,307 students and 27.4% of 825 staff who usually reside at the school. In total, 411 persons gave a serum sample and 353 (85.9%) were matched to a valid questionnaire (Figure). Of persons with a questionnaire and matched serologic test result, 216 were students and 137 staff, which accounts for 16.5% of the registered student population and 16.6% of the registered staff population; these 353 persons composed our final cohort (Figure).

Representativeness of Study Populations

The distribution of the study population by age, sex (staff only; all students were male), occupation (staff only), self-reported illness, history of seasonal influenza vaccine in the previous year, and the use of antiviral agents is shown in the online Appendix Table (www.cdc.gov/EID/content/17/9/100761-appT.htm). To determine whether our sample was representative of the total school population, we compared the final study population (questionnaire and matched serologic results) to those who completed only the questionnaire and to the whole school population. For staff, the proportion of men and women in the final study population was similar to the proportion that answered the questionnaire only (51.8% vs. 48.2% and 48.2% vs. 51.8%, respectively; χ^2 p = 0.58). The proportions of students in age groups 13–15 years and 16–18 years were similar for those completing a questionnaire only (46.7% vs. 53.3%) compared to those in the matched study sample (41.7% vs. 58.3%; χ^2 p = 0.25) (online Appendix Table), and similar to the school's student population. Teaching staff made up 31.6% of those of the final study population and 38.6% of the questionnaire-only population (χ^2 p = 0.52). For persons reporting ARI, differences were significant between those who completed a questionnaire only and those who also gave a blood sample for students (37.3% vs. 48.3%; χ^2 p = 0.020) and staff (10.2% vs. 30.8%; χ^2 p<0.001). This selection bias could have led to an overestimation of the infection attack rate.

Symptoms by Self-reported Illness and Serologic Test Results

For ARI, the attack rate was estimated at 35.9%, (237/661, 95% confidence interval [CI] 32.2%–39.6%) or 16.6% (110/661, 95% CI 13.9%–19.7%) by using the definition for ILI (online Appendix Table). Of those who reported ARI and ILI, serologic test results were negative for 64/141 (45.4%, 95% CI 37.0%–54.0%) and 19/63 (30.2%, 95% CI 19.2%–43.0%) persons, respectively.

We found 143 seropositive persons, which gives an attack rate for infection of 40.5% (95% CI 35.3%–45.8%; Table 1). Of these 143 persons, 4 students did not answer the question relating to their illness status, and of the remaining 139 persons for whom illness history was available, 62 (44.6%, 95% CI 36.2%–53.3%) did not report ARI.

In crude analyses, persons who reported ARI were more likely to be seropositive (62/199, 54.2%; crude odds ratio [OR] 2.66, 95% CI 1.70–4.16) than were those without illness (77/141, 29.9%). The odds of having serologic evidence of infection increased when the illness reported met the case definition for ILI (crude OR 4.44, 95% CI 2.45–8.02) (Table 1).

We also found an association between severity of reported illness and seropositivity. Overall, those reporting moderate or severe illness were more likely to be seropositive than those reporting mild illness (crude OR 2.21, 95% CI 1.10–4.41). We found no association between seropositivity and duration of illness (Table 1).

Association between Self-reported Illness, Infection, and Interventions

Overall, fewer students reported antiviral drug use than did staff (59.4% vs. 80.0%, p<0.001). Most of those taking a treatment dose were students (16/20, 80%) (Table 2). All students completed their prophylactic course of antiviral agents vs. 91% of staff. More students reported having had the 2008–09 trivalent seasonal influenza

Table 1. Association of demographic characteristics, clinical illness, and interventions with study participants' positive serologic test results during outbreak of pandemic (H1N1) 2009 at a boarding school, England*

Variable	No. participants	No. (%) with positive serologic test result	Odds ratio (95% CI)
Total	353	143 (40.5)	
Demographics			
Category			
Students	216	123 (56.9)	7.74 (4.48–13.35)
Staff	137	20 (14.6)	1
Age group, y			
13–15	90	46 (51.1)	1
16–18	126	77 (61.1)	1.50 (0.87–2.60)
20–49	71	12 (16.9)	0.19 (0.09–0.41)
≥50	66	8 (12.1)	0.13 (0.06–0.31)
Sex, staff only†			
F	71	5 (7.0)	1
M	66	15 (22.7)	3.88 (1.32–11.39)
Role, staff only‡			
Nonteaching	93	6 (6.5)	1
Teaching	43	14 (32.6)	7.00 (2.46–19.90)
Clinical illness			
ARI			
No	199	62 (31.2)	1
Yes	141	77 (54.6)	2.66 (1.70–4.16)
ILI			
No	277	95 (34.3)	1
Yes	63	44 (69.8)	4.44 (2.45–8.02)
Severity, n = 153§			
Mild	81	38 (46.9)	1
Moderate and severe	59	39 (66.1)	2.21 (1.10–4.41)
Duration, d, n = 153§			
1–2	14	6 (42.9)	1
3–6	52	27 (51.9)	1.44 (0.44–4.73)
7–10	18	12 (66.7)	2.67 (0.63–11.28)
>10	23	14 (60.9)	2.07 (0.54–8.00)
Interventions			
Took antiviral drugs			
No	96	48 (50)	1
Yes	207	68 (32.9)	0.49 (0.30–0.80)
Use of antiviral drugs: PEP vs. treatment dose			
No antiviral drugs	96	48 (50.0)	
PEP dose only	187	56 (30.0)	0.43 (0.26–0.71)
Treatment dose	20	12 (60)	1.5 (0.56–4.00)
Completion of PEP course of antiviral drugs¶			
No antiviral drugs	96	48 (50.0)	1
Completed	25	9 (36.0)	0.56 (0.23–1.40)
Not completed	159	45 (28.3)	0.39 (0.23–0.67)
Seasonal influenza vaccine			
No	105	29 (27.6)	1
Yes	230	106 (46.1)	2.24 (1.36–3.69)

*Categories in which the response was missing or unknown are shown in the online Appendix Table (www.cdc.gov/EID/content/17/9/100761-appT.htm).
CI, confidence interval; ARI, acute respiratory infection; ILI, influenza-like illness; PEP, postexposure prophylaxis.
†All students were male.
‡1 staff member was not included in the analysis because his occupation was unknown.
§Of those that self-reported ARI.
¶Excluding those who reported taking the treatment dose of antiviral drugs.

Table 2. Association of demographic characteristics and interventions with study participants' reports of ARI during outbreak of pandemic (H1N1) 2009 at a boarding school, England*

Variable	No. participants	No. (%) with ARI	Odds ratio (95% CI)
Total	695	237 (34.1)	
Demographics			
Category			
Students	469	187 (39.9)	2.53 (1.75–3.65)
Staff	226	50 (22.1)	1
Age group, y			
13–15	219	75 (34.2)	1
16–18	250	112 (44.8)	1.55 (1.06–2.28)
20–49	111	32 (28.8)	0.70 (0.42–1.15)
≥50	87	18 (20.7)	0.48 (0.26–0.86)
Sex, staff only†			
F	109	24 (22.0)	1
M	117	26 (22.2)	0.98 (0.52–1.83)
Role, staff only			
Nonteaching	135	26 (19.3)	1
Teaching	85	23 (27.1)	1.48 (0.78–2.82)
Interventions			
Took antiviral drugs			
No	198	81 (40.9)	1
Yes	393	110 (28.0)	0.55 (0.38–0.79)
Use of antiviral drugs: PEP vs. treatment dose			
No antiviral drugs	198	81 (40.9)	1
Yes, PEP	352	78 (22.2)	0.40 (0.27–0.59)
Yes, treatment dose	41	32 (78.1)	4.87 (2.20–10.77)
Completion of PEP course of antiviral drugs†			
No antiviral drugs	198	81 (40.9)	1
Not completed	51	14 (27.5)	0.44 (0.17–1.15)
Completed	294	62 (21.1)	0.38 (0.22–0.65)
Seasonal Influenza vaccine			
No	216	66 (30.6)	1
Yes	425	161 (37.9)	1.35 (0.95–1.92)

*Categories in which the response was missing or unknown are shown in the online Appendix Table (www.cdc.gov/EID/content/17/9/100761-appT.htm).
ARI, acute respiratory infection; CI, confidence interval; PEP, postexposure prophylaxis.
†Excluding those who reported taking the treatment dose of antiviral drugs.

vaccine than staff (81.1% vs. 50.0%; p<0.0001 in the matched sample).

In logistic regression models for ARI and serologic status (Table 3), including age group, significantly improved the fit of the models (both L-R tests p<0.001 compared models, including only antiviral drug use and vaccination status). No evidence of effect modification was found between age group and antiviral drugs or vaccination status for either outcome (L-R test p = 0.87 and p = 0.77, respectively). Therefore, stratified models were not fitted for staff and students.

Staff in the age groups 20–49 years and ≥50 years (adjusted ORs [AORs] 0.30 [95% CI 0.12–0.73] and 0.20 [95% CI 0.08–0.53], respectively) were less likely to have positive serologic test results than students 13–15 years of age (Table 3). This effect was not observed when ARI was used as the outcome. Weak evidence suggests that those 16–18 years of age were more likely to be seropositive and have ARI than those 13–15 years of age (AOR 1.85 [95% CI 0.95–3.60] and 1.57 [95% CI 0.98–2.53], respectively). Although odds of seropositivity did not increase significantly with receipt of 2008–09 seasonal influenza vaccine (p = 0.10), the point estimate was >1.

Likewise, the point estimate of the AOR for the association between taking a prophylactic dose of antiviral drugs and seropositivity was <1 (p = 0.045). In a similar model, with ARI as the outcome of interest, having received a prophylactic dose significantly reduced the odds of ARI (AOR 0.41, 95% CI 0.27–0.61).

For the staff-only models (Table 4), staff role improved the model that predicted serologic results and ARI (L-R test p <0.001 and 0.01, respectively). After staff role was taken into account, including sex as a factor did not improve the accuracy of either model and was therefore not included. For the multivariable logistic regression model, which included only staff, age groups, staff role, exposure to prophylactic dose of antiviral agents, and having received the 2008–09 seasonal influenza vaccine were considered. Teachers were more likely be seropositive than other staff members (AOR 7.47, 95% CI 2.31–24.18), and no

Table 3. Multivariable analysis of all study participants in relation to having ARI or serology-confirmed infection during outbreak of pandemic (H1N1) 2009, England*

Variable	AOR (95% CI) for ARI in questionnaire sample	AOR (95% CI) for positive test result in matched sample
Age group, y		
13–15	1	1
16–18	1.57 (0.98–2.53)	1.85 (0.95–3.60)
20–49	1.00 (0.53–1.89)	0.30 (0.12–0.73)
≥50	0.66 (0.32–1.34)	0.20 (0.08–0.53)
Took antiviral drugs for PEP†		
No	1	
Yes	0.41 (0.27–0.61)	0.55 (0.30–0.99)
Seasonal Influenza vaccine		
No	1	1
Yes	1.01 (0.63–1.62)	1.81 (0.91–3.59)

*For ARI, n = 654 who completed questionnaires; for serology-confirmed infection, n = 333 who completed questionnaires and had a matched serology sample. ARI, acute respiratory infection; AOR, adjusted odds ratio; CI, confidence interval; PEP, postexposure prophylaxis.
†Persons who reported taking treatment dose of antiviral agents were excluded.

association was found between seropositivity outcome and age, taking the prophylactic dose of antiviral drugs, or receiving the influenza vaccine (Table 4).

When the final logistic models were refitted by using the minimum detection limit (≥1:8) to define seropositive status, this change made little difference to the point estimates of the ORs for antiviral drug use, age group, or seasonal vaccine. For the staff-only model, using a cutoff value ≥1:8 changed the point estimates for taking antiviral agents and age groups (≥50 vs. 20–49 years) to >1; however, neither linear predictor was significantly associated with the outcome with either cutoff value.

Discussion

This study describes the seroprevalence of infection with the pandemic (H1N1) 2009 virus in an enclosed institutional environment and provides evidence of widespread infection among both students and staff before the outbreak became evident to public health authorities. Attack rates for infection were estimated as 40.5% by serologic testing and as 34.1% by clinical illness (ARI). An estimated 44.7% serology-positive persons did not report symptoms of ARI, which agreed with previous findings (*10*,*23*). No significant association was found between seropositivity and prophylaxis with antiviral drugs, although some evidence showed that it reduced the odds of ARI. The point estimate of the AOR indicated nonsignificant increased odds of infection (indicated by serologic results) for persons who had received the 2008–09 seasonal influenza vaccine, although it did not increase the odds for ARI.

Our study has some limitations, however. The uncertainty regarding the associated illness of pandemic (H1N1) 2009 at the time the study was initiated made the selection of a random sample not feasible. A pragmatic approach was therefore chosen to offer the entire school population the opportunity of being included in the study, resulting in 17% of the registered school population providing a serum sample. Because the study was conducted during an examination period, and some students and staff were absent, the size of the population from which the study sample was drawn is not known. However, because this was definitely less than the registered population, our response rate estimation was conservative. The distribution of our study population was not significantly different from the school's population in age, school year, and sex (among teachers). However, the subsample of persons who provided a serum sample likely were not representative of persons who answered the questionnaire. For example, persons who provided a serum sample were more likely to have reported an ARI than persons who responded to the questionnaire only. This resulted in the overestimation of attack rates. Selection bias in the serology study subsample is also evident in the ORs for the 2008–09 seasonal influenza vaccine, for which the ORs were different, according to whether a serologic or clinical outcome was used, because the effect would be expected to be in the same direction. In addition, vaccination status was self-reported and could not be validated against official records, and we did not collect dates that antiviral drugs were used from each person.

We used a cutoff value of an HI titer ≥32 to indicate recent seroconversion. A previous study (*18*) has indicated that cross-reactive antibody to pandemic (H1N1) 2009 virus was prevalent in England's population before the pandemic and that seroprevalence was strongly associated with age. In addition, a high proportion of children at the school

Table 4. Multivariable analysis of staff only in relation to having ARI or serology-confirmed infection during outbreak of pandemic (H1N1) 2009, England*

Variable	AOR (95% CI) for ARI in questionnaire sample	AOR (95% CI) for positive test result in matched sample
Age group, y		
20–49	1	1
≥50	0.71 (0.32–1.57)	0.91 (0.26–3.14)
Role		
Nonteaching	1	1
Teaching	1.18 (0.56–2.52)	7.47 (2.31–24.18)
Took antiviral drugs for PEP†		
No	1	1
Yes	0.34 (0.15–0.77)	0.66 (0.18–2.39)
Seasonal influenza vaccine		1
No	1	0.76 (0.35–1.67)
Yes	2.36 (0.69–8.11)	2.36 (0.69–8.11)

*n = 226. ARI, acute respiratory infection; AOR, adjusted odds ratio; CI, confidence interval; PEP, postexposure prophylaxis.
†Persons who reported taking the treatment dose of antiviral drugs were excluded.

had been vaccinated and were therefore unlikely to be representative of children in England. Conflicting evidence exists regarding the effect of prior trivalent influenza vaccination on cross-reactive titers for pandemic (H1N1) 2009 virus in persons <55 years of age (*21*,*24*). Therefore, some misclassification of cases (persons who seroconverted as a result of exposure to pandemic [H1N1] 2009 virus) likely occurred, leading to possible overestimation of the proportion of asymptomatic patients. However, the proportion of misclassified seropositive persons is likely small, particularly among children. Ideally, paired samples (collected before and after the outbreak) would have been able to measure seroconversion; however, this opportunity was not available.

These results highlight the fact that, depending on the virulence and transmissibility of an emerging influenza pandemic virus, extensive transmission may occur in a closed setting and thus by implication in the community over and above the observed clinical disease. This finding has notable implications for predicting the future course of a pandemic because the subsequent pool of those susceptible after initial transmission will diminish (*18*). Current policy in closed settings in the United Kingdom is to isolate or place symptomatic persons in cohorts after diagnosis to minimize the risk for onward transmission. If a substantial proportion of mildly symptomatic or even possibly asymptomatic persons were able to transmit infection, current policy would be of limited value. First, infection may be widespread within an institution long before it becomes apparent to public health authorities; second, a large number of persons may be infected but asymptomatic or mildly symptomatic when the first case is diagnosed. Although conclusions can be drawn from this study that rapid transmission of influenza occurred in this environment and that infection may not always produce symptoms, the evidence of transmission of the influenza virus by asymptomatic persons remains scant (*25*).

Our findings indicate that the use of prophylactic antiviral agents lowers the odds of an ARI by ≈50% but has no effect on reducing the odds of serologic infection. Several interpretations of these findings are possible; for example, while prophylactic antiviral agents might not reduce the risk for infection, they could protect from clinical disease. Published evidence from the occurrence of seasonal influenza has indicated that timely administration of prophylactic antiviral agents to close contacts of infected persons reduces the risk for disease (*26*). However, our results should be viewed with caution. First, the serology sample was a much smaller subset of the questionnaire survey respondents, and the results for a serologic outcome indicate a lack of power. Thus, the effectiveness of antiviral prophylaxis would be underestimated. Also, the low specificity of the case definition for ARIs could lead to an overestimation of the effect on clinical disease.

The association between self-reported illness and severity of illness with an increasing likelihood of seropositivity suggests that even in facilities with a limited diagnostic capacity, simple definitions of ILI may be a more specific indicator of the true presence of infection in communities in which a proven outbreak is under way. Fever was an essential part of the clinical case criteria for testing in the United Kingdom, in contrast to the United States, where the clinical criteria were either a "respiratory illness" (recent onset of ≥2 of the following: rhinorrhea/nasal congestion, sore throat, cough, fever or feverishness) or an ILI (fever >37.8°C [100°F], plus cough or sore throat).

This study has demonstrated evidence of widespread infection with pandemic (H1N1) 2009 virus in a closed setting, with a substantial proportion of asymptomatic persons. Although the study highlights the difficulties of obtaining a large representative sample from a boarding school population during a pandemic influenza outbreak, it also illustrates the value of such rapid field epidemiologic investigations in understanding an emergent threat. This was particularly relevant during the emergence of pandemic (H1N1) 2009 virus at a time when its pathogenicity was uncertain and the benefit of using antiviral agents for postexposure prophylaxis was unclear. The results of this seroepidemiologic study in an outbreak setting during the pandemic of novel pandemic (H1N1) 2009 highlight the need for health authorities to agree on protocols for similar investigations during future pandemics.

Acknowledgments

We acknowledge the support of colleagues in this investigation: Peter Hawtin and the team at the HPA Southampton Laboratory, Josh Bruce, Anne Maduma-Butshe, and Gail Fairbairn.

Ms Johnson was a senior epidemiological scientist at the HPA South East Regional Unit, London, at the time of this study. Her responsibilities included routine outbreak investigations and surveillance of communicable disease. Her research interests focus on intervention epidemiology and the structural determinants of the distribution of communicable diseases.

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Leptospirosis as Frequent Cause of Acute Febrile Illness in Southern Sri Lanka

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To determine the proportion of fevers caused by leptospirosis, we obtained serum specimens and epidemiologic and clinical data from patients in Galle, Sri Lanka, March–October 2007. Immunoglobulin M ELISA was performed on paired serum specimens to diagnose acute (seroconversion or 4-fold titer rise) or past (titer without rise) leptospirosis and seroprevalence (acute). We compared (individually) the diagnostic yield of acute-phase specimens and clinical impression with paired specimens for acute leptospirosis. Of 889 patients with paired specimens, 120 had acute leptosoirosis and 241 had past leptospirosis. The sensitivity and specificity of acute-phase serum specimens were 17.5% (95% confidence interval [CI] 11.2%–25.5%) and 69.2% (95% CI 65.5%–72.7%), respectively, and of clinical impression 22.9% (95% CI 15.4%–32.0%) and 91.7% (95% CI 89.2%–93.8%), respectively. For identifying acute leptospirosis, clinical impression is insensitive, and immunoglobulin M results are more insensitive and costly. Rapid, pathogen-based tests for early diagnosis are needed.

Leptospirosis is an endemic zoonosis in the tropics, where a favorable climate enables the pathogenic spirochete *Leptospira interrogans* to survive in the environment (*1*). Furthermore, many tropical residents have repeated direct and indirect exposures to infected animals that excrete leptospires in their urine (*2*).

Sri Lanka, with a rapidly growing population of ≈20 million, has a reported annual incidence of leptospirosis of 5.4 cases/100,000 persons, the sixth highest incidence worldwide (*3*). Approximately 28% of Sri Lanka's workforce is employed in agriculture, and reported cases of leptospirosis fluctuate with rainfall and farming cycles. Historically, ≈200 cases per million population per year have been reported from the southern and north–central regions, where the disease is hyperendemic (*3*). However, incidence rates are imprecise estimates because leptospirosis is easily confused with undifferentiated fever of other causes (*1*), and few cases are laboratory confirmed (*4*). In the past 2 decades, clinical cases have been increasingly reported (*5*), including >7,000 cases in 2008 (*6*).

To determine the prevalence of acute and past leptospirosis in southern Sri Lanka, assess tools for acute diagnosis, and identify associated features, we collected epidemiologic and clinical data and paired serum specimens from a prospective cohort of children and adults with undifferentiated fever. The institutional review boards of the University of Ruhuna, Johns Hopkins University, and Duke University Medical Center approved this study.

Materials and Methods

Study Participants

We recruited patients in the emergency department, acute care clinics, and adult and pediatric wards of the Karapitiya Teaching Hospital in Galle, the largest (1,300-bed) hospital in southern Sri Lanka, during March–October 2007. We enrolled consecutive febrile (38°C, tympanic) patients ≥2 years old without antecedent (≤7 days) trauma or hospitalization who sought treatment during clinic hours (8:00 AM–4:00 PM Monday–Friday, and 8:00 AM–2:00 PM Saturday). Study doctors verified patient eligibility and willingness to return for follow-up and obtained written informed consent from patients (≥18 years of age) or

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DOI: 10.3201/eid1709.100915

parents (of those <18 years of age) and assent from those 12–17 years of age.

Study personnel recorded structured epidemiologic and clinical data, including duration of illness and the clinical provider's presumptive diagnosis, on a standardized form. Study doctors then obtained blood for on-site clinician-requested testing and subsequent off-site research-related testing. Patients returned for clinical and serologic follow-up 2–4 weeks later or were visited at home if they were unable to return and could be located. Blood was centrifuged, and serum specimens were frozen on site at −80°C, shipped on dry ice, and thawed only when separated into aliquots and when tested.

Serologic Testing

We tested paired serum specimens for the presence of specific *Leptospira* immunoglobulin (Ig) M by ELISA (Institut Viron Serion GmgH, Warburg, Germany), according to the manufacturer's instructions. Briefly, rheumatoid factor (RF)-absorbent was first diluted 1:4 in buffer. Serum specimens from patients and controls were then diluted (1:100) in RF-absorbent buffer to accomplish removal of IgM RF, transferred to antigen-coated microtest wells, and incubated at 37°C for 60 min. After wells were washed with phosphate-buffered saline, antihuman IgM (conjugated to alkaline phosphatase and *p*-nitrophenylphosphate) was added. After incubation of the wells for 20 min, sodium hydroxide was added to each well to stop the reaction, and the absorbance at 405 nm was measured.

The ELISA provided qualitative results—positive, negative, and equivocal (borderline positive/negative). Using a standard curve and evaluation table provided with the kit, we obtained the optical density measurements, which were adjusted for plate-to-plate variation with a correction factor and gave quantitative results that correlated with titers (*7*).

Case Definitions

Acute leptospirosis was defined as definitive seroconversion (negative acute-phase serum specimen to positive convalescent-phase serum specimen) or the equivalent of a 4-fold rise in IgM titer. We excluded from analyses of acute leptospirosis specimens with equivocal IgM test results or those lacking a convalescent-phase sample.

Past leptospirosis was defined as stable or decreasing IgM titers. We excluded from analyses of past leptospirosis specimens with equivocal IgM test results or those lacking a convalescent-phase sample. IgM seroprevalence was defined as the prevalence of leptospirosis by *Leptospira* IgM in acute-phase serum specimens, independent of whether a convalescent-phase specimen was obtained or its result.

Statistical Analysis

Proportions were compared by the χ^2 test or Fisher exact test and continuous variables by Student *t* test or the rank sum test if distribution was not normal. Confidence intervals (CIs) for risk ratios were calculated by exact methods. We assessed IgM in the acute-phase sample for seroprevalence and clinical impression was compared with results of paired-serum specimen testing for acute leptospirosis. We specifically correlated epidemiologic features, duration of illness, and symptoms and signs with serologic test results. Analyses were performed with Stata IC 11.0 (StataCorp LP, College Station, TX, USA).

Results

Patient Characteristics

Paired serum specimens were available from 889 (82.4%) of 1,079 patients consecutively enrolled. Among those, a diagnosis of acute leptospirosis could be confirmed or refuted for 773 (87.0%) of 889, because serologic results were inconclusive for 116. The likelihood of a participant's returning for convalescent-phase serum sampling and clinical follow-up did not differ by age ($p = 0.10$). Female patients were slightly more likely to return for follow-up (85.8 vs. 80.6%; $p = 0.03$). Most (90.2%) patients lived in rural areas and were more likely to return for follow-up than were those who lived in urban areas (83.5 vs. 71.4%; $p = 0.002$). The proportion with secondary education was similar in the 2 groups (21.7 vs. 19.6%; $p = 0.51$), as was reported duration of fever and of illness ($p = 0.15$ and $p = 0.13$, respectively).

Of the 773 patients with conclusive serologic results, the median age was 30.1 years (interquartile range [IQR] 19–47 years). More patients were male (60.6%) than female, and the median age did not differ by sex ($p = 0.78$). The median reported duration of fever and of illness was 3 days (IQR 2–5 days and 2–7 days, respectively). Many (37.6%) reported taking an antimicrobial drug before seeking treatment. The median interval between acute-phase and convalescent-phase follow-up was 21 days (IQR 15–33 days).

Diagnosis of Acute Leptospirosis

Acute leptospirosis was confirmed for 120 patients (Figure 1): by seroconversion for 96 patients and by a 4-fold rise in titer for 24 patients (in 21 acute-phase specimens with positive results and in 3 acute-phase specimens with equivocal results); acute leptospirosis was excluded for 653 patients. Data on presumptive clinical diagnosis were available for 714 patients, including for 109 of 120 with acute leptospirosis. Of these patients, 25 received a correct diagnosis of acute leptospirosis, and 84 received an incorrect diagnosis of another disease. The sensitivity

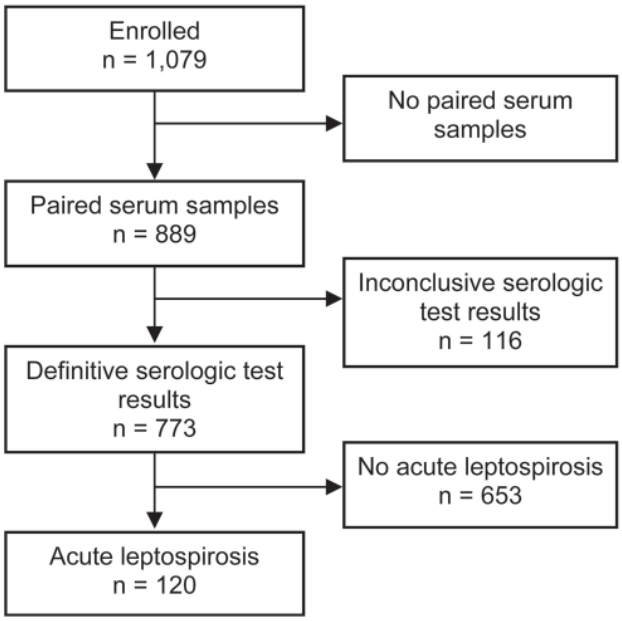

Figure 1. Flowchart indicating selection of study participants with a diagnosis of acute leptospirosis, southern Sri Lanka, 2007.

and specificity of clinical impression were 22.9% (95% CI 15.4%–32.0%) and 91.7% (95% CI 89.2%–93.8%), respectively. Finally, 279 patients were seropositive at enrollment, including 201 with past leptospirosis, 40 with possible recent leptospirosis (second specimen equivocal), 21 with acute leptospirosis, and 57 without paired serum specimens. Therefore, if acute-phase IgM had been used to diagnose acute leptospirosis instead of paired serum specimens, only 21 of 120 acute infections would have been identified (sensitivity 17.5%, 95% CI 11.2%–25.5%), and 201 of 653 patients without acute leptospirosis would have been given an erroneous diagnosis (specificity 69.2%, 95% CI 65.5%–72.7%). Thus, IgM seropositivity at the acute-phase visit correlated poorly with acute leptospirosis.

Demographic Features of Patients with Acute or Past Leptospirosis

The demographic characteristics of patients are listed in Table 1. Those with acute or past leptospirosis were older (median 32 years vs. 27 years; p = 0.01) than those without acute or past leptospirosis. Reporting exposure to paddy fields (relative risk [RR] 1.9, 95% CI 1.6–2.1; p<0.0001) and working as a farmer (RR 1.9, 95% CI 1.6–2.3; p = 0.0001) were strongly associated with acute and past leptospirosis. Reporting no fresh water exposure and boiling drinking water were protective against leptospirosis. Patients enrolled during March–June were less likely to have acute leptospirosis than those enrolled during July–October (Figure 2). Children <10 years of age were much less likely to have acute or past leptospirosis; in contrast, leptospirosis was more common in older adolescents and young adults (Figure 3).

The median duration of illness at hospital visit for those with acute leptospirosis diagnosed by seroconversion was 3 days (IQR 2–5 days), and for those with acute leptospirosis diagnosed by a 4-fold rise in titer, 4 days (IQR 3–5 days; p = 0.09). The median interval between serum sampling was 22 days (IQR 15–31 days). The follow-up time was slightly longer for those with leptospirosis diagnosed by 4-fold change in titer than for those with diagnosis by seroconversion (median 26 vs. 20 days; p = 0.08). The median age was 33.6 years (IQR 18.7–45.6 years), and more patients were male (69.2%) than female (p = 0.07).

Clinical Features of Acute Leptospirosis

Clinical features associated with acute leptospirosis are listed in Table 2. Headache was the most frequent (≈80%) symptom reported; lethargy, muscle pain, and joint pain were also reported by >50% of patients. Lethargy and cough

Table 1. Demographic characteristics of febrile patients with acute or past leptospirosis versus those who had neither acute nor past leptospirosis, southern Sri Lanka, 2007*

Demographic characteristic	% With acute or past leptospirosis, n = 361	% With neither acute nor past leptospirosis, n = 412	p value
Median age, y (IQR)	32 (20–46)	27 (16–47)	0.02
Male sex	60	64	0.14
Residence			
Urban	8	9	0.88
Rural	92	91	
Type of work			<0.0005
Home	27	25	
Laborer	26	21	
Farmer	6	1	
Merchant	2	4	
Student	20	25	
Other	20†	24	
Animal exposures			
Dog	57†	54	0.43
Rodent	27	30	0.35
Cow	7	4†	0.13
Swim/bathe/wade			
None	66	82	<0.0005
River	14	11	
Paddy field	19	4	
Other	2‡	3	
Water source			0.001
Tap	31	33	
Boiled	6	14	
Well	63	52	
Other	0.3	1	

*IQR, interquartile range.
†Does not add to 100% due to rounding.
‡Adds to >100% due to multiple exposures.

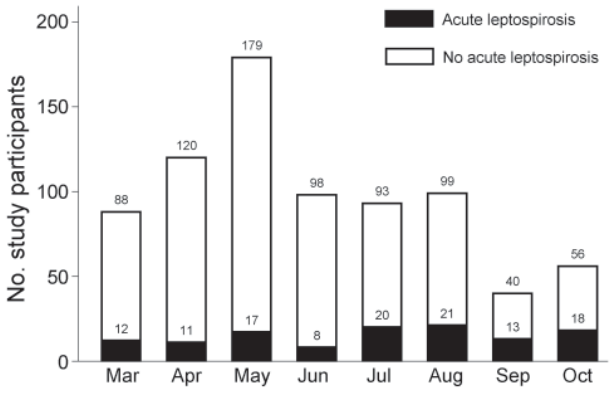

Figure 2. Leptospirosis cases by month among study patients enrolled with fever, southern Sri Lanka, 2007

were reported less often in patients with acute leptospirosis, and oliguria, dysuria, and muscle and joint pain were reported more often. Patients with acute leptospirosis were more likely to have conjunctival suffusion (RR 2.4, 95% CI 1.7–3.4; p<0.0001) and less likely to have pharyngeal exudates. Abdominal tenderness and hepatomegaly were slightly more frequent in those with acute leptospirosis. Jaundice, splenomegaly, arthritis, rash, and meningismus were uncommon in both groups. Patients with acute leptospirosis had similar leukocyte counts to patients who did not, and slightly lower hemoglobin concentrations and platelet counts and lower absolute lymphocyte counts. A greater proportion of patients with acute leptospirosis were admitted to the hospital (84.2% vs. 70.1%) than were others with fever (p = 0.002), but they did not have a longer stay (median 4 days, IQR 3–6 days; p = 0.83). At the convalescent-phase follow-up visit, patients with acute leptospirosis reported a longer total duration of fever than others (5 days [IQR 3–7 days] vs. 4 days [IQR 3–6 days]; p = 0.008). No one with confirmed acute leptospirosis died, but most (11 of 12) deaths occurred before follow-up. Among those who died, the acute-phase serum specimen was IgM-negative for 8 patients, IgM-positive for 2, and results were equivocal for 1.

Discussion

We found that leptospirosis was a common, but often clinically unsuspected, cause of fever among unselected patients seeking care in southern Sri Lanka. Farming and rice paddy work were associated with increased risk for leptospirosis, as was exhibiting acute febrile illness during the harvesting season (July–October). In our setting, testing acute-phase serum specimens alone for IgM was less sensitive and specific for diagnosing acute leptospirosis than was diagnosis by observation of clinical features.

Isolation of *Leptospira* spp. confirms acute infection, but requires special media that must be incubated for up to 13 weeks, and has low sensitivity (*1*). Therefore, the diagnostic standard for acute leptospirosis is a definitive rise in titer between paired serum specimens (*1*). Historically, these results have been obtained by the microscopic agglutination test (MAT). Serum specimens are first reacted with live antigen suspensions of different leptospiral serovars. After incubation, the serum-antigen mixtures are examined and titers determined. For paired serum specimens, the highest dilution of serum at which 50% agglutination occurs must be determined, a laborious and inherently subjective task (*1*).

Sensitivity is compromised if all locally relevant serovars are not represented, and live cultures of all serovars tested must be maintained whether live or formalin-killed antigens are used. Subculturing many *Leptospira* spp. weekly is hazardous for personnel, and laboratory-acquired infections occur (*1*). Reading a MAT requires a dark-field microscope, which is unavailable in most laboratories, including Karapitya Teaching Hospital. Furthermore, a MAT detects both IgM and IgG and lacks sensitivity and specificity when early acute-phase serum specimens alone are tested rather than paired specimens (*1*). Patients with fulminant illness may die before seroconversion occurs. A MAT may also be less sensitive than an IgM ELISA, even for convalescent-phase specimens. Relative to isolation of *Leptospira* spp., the reported sensitivities of MATs for acute, late acute-phase, and convalescent-phase serum specimens were 30%, 63%, and 76%, respectively, and of IgM ELISA, 52%, 89%, and 93%, respectively, in 1 study in Barbados (*8*). In another study, results of MAT and IgM ELISA for a single early acute-phase specimen were comparable (49%) (*9*).

To overcome the practical pitfalls of MATs, we chose to test paired serum specimens by IgM ELISA, which requires only an inexpensive plate reader, is relatively easy to perform, and provides objective, reproducible results as demonstrated by a parallel comparison of results of

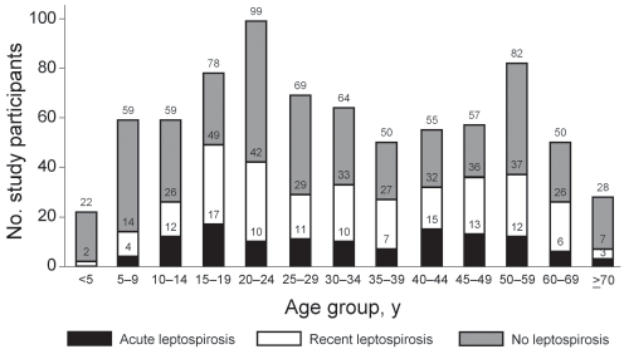

Figure 3. Age distribution of study patients enrolled with fever, southern Sri Lanka, 2007

Table 2. Clinical characteristics of febrile patients with acute leptospirosis versus those without acute leptospirosis, southern Sri Lanka, 2007*

Clinical characteristic	With acute leptospirosis, n = 120	Without acute leptospirosis, n = 653	p value
Symptom			
Headache	81	78	0.63
Sore throat	28	29	0.96
Cough	44	60	<0.005
Dyspnea	15	18	0.48
Joint pain	56	43	<0.01
Muscle pain	62	46	<0.005
Lethargy	58	70	<0.01
Abdominal pain	22	18	0.41
Emesis	45	37	0.10
Diarrhea	13	12	0.80
Dysuria	20	13	<0.05
Oliguria	17	8	<0.005
Sign			
Mean temperature, °C (SD)	38.6 (0.6)	38.5 (0.6)	0.17
Median heart rate, beats/min (IQR)	80 (72–100)	84 (76–96)	0.85
Mean body mass index, kg/m^2 (SD)	20.7 (4.8)	19.6 (5.1)	<0.05
Conjunctival suffusion	29	12	<0.0005
Pharyngeal exudate	8	15	<0.05
Lymphadenopathy	24	23	0.73
Jaundice	2	2	0.73
Lung crackles	10	14	0.27
Tender abdomen	13	9	0.21
Hepatomegaly	8	5	0.31
Laboratory parameter, median (IQR)			
Leukocytes, cells/μL	7,800 (5,700–10,500)	7,900 (5,600–11,300)	0.52
Absolute neutrophil count, cells/μL	5,530 (3,854–8,424)	5,313 (3,344–7,952)	0.49
Absolute lymphocyte count, cells/μL	1,638 (1,210–2,574)	2,140 (1,541–2,856)	<0.005
Hemoglobin, g/dL	12.3 (11.6–13.5)	12.6 (11.7–13.8)	<0.05
Platelets, × 1,000/μL	200 (164–256)	231 (190–289)	<0.0005

*Values are % patients in that category (with vs. without leptospirosis), except as indicated. IQR, interquartile range.

multiple commercial assays (*9*). Furthermore, results of performing IgM ELISA on paired serum specimens from patients from various geographic regions have compared favorably (sensitivity 86.5%, specificity 97.0%) with MAT results (*10*). These data suggest that at least 13.5% of febrile illnesses in our cohort were acute leptospirosis. We assayed paired specimens for IgM instead of IgG, because the kinetics of IgG are more variable (*11*,*12*). IgM generally appears within 1 week of symptoms and persists for months to years after infection (*13*) with titers higher than those of IgG throughout (*11*,*14*). Additionally, in some patients for whom leptospirosis is confirmed by culture and MAT, IgG never develops (*11*,*13*).

We chose a commercially available IgM ELISA that has performed comparably to others in detecting serovars likely present in southern Sri Lanka (*9*). IgM is inherently cross-reactive, and thus serovars themselves are not detected. In a recent study from central Sri Lanka, the predominant serovars were Mednensis and Hardjo, but others included Australis, Ballum, Canicola, Celledoni, Cynopteri, Pomona, and Robinsoni (*15*). Previously, identification of the serovar Icterhemorrhagiae in Sri Lanka led to control of the rodent vector (*16*). The assay we used has reliably reacted with serovars Icterohemorrhagiae, Canicola, Grippotyphosa, Bataviae, Pomona, Tarassovi, Copenhageni, Bratislava, Hebdomadis, Sejroe, Australis, Panama, Pyrogenes, Patoc, Hardjo, and Cynopteri (*7*,*17*).

Notably, detection of acute-phase IgM did not predict which patients had acute leptospirosis, despite its widespread use as an acute diagnostic test. Retrospective studies suggest sensitivities and specificities of 36%–53% for single acute-phase IgM and 90%–99% for MAT on paired serum specimens, respectively (*9*,*10*). The varied sensitivity likely reflects different case definitions and control groups, timing of acute-phase specimen collection (up to 42 days after onset), geography and serovar distribution, platforms and protocols (e.g., ELISA ± use of RF absorbent, indirect hemagglutination, and dot-ELISA and IgM dipsticks), and convenience sampling. Notably, ELISA of single (acute-phase) serum specimens has performed as well or better than MAT or indirect hemagglutination of single serum specimens, so those strategies are not advised (*10*).

The most widely recognized problem with using acute-phase IgM to identify acute leptospirosis is that

many persons in disease-endemic areas are expected to have preexisting antibodies. Some have advocated higher cut-offs to discriminate between acute infection and preexisting antibodies (*1*), because patients may be harmed as much by incorrectly attributing fever to leptospirosis as by falsely excluding it. However, data to support this approach are lacking, and misclassification could occur both early in acute infection (impaired sensitivity because antibody is not yet present) and later (impaired specificity because antibody is persistent). In our rigorous comparison of single vs. paired serum specimens, we found acute-phase IgM had especially poor sensitivity (17.5%), since patients sought treatment early (≈3 days), and more acute infections were identified by seroconversion than by a definitive rise in titer. The median duration of illness in those diagnosed by rise in titer versus seroconversion tended to be longer (4 days [IQR 3–5 days] vs. 3 days [IQR 2–5 days], respectively; p = 0.09). Requiring a higher cutoff titer would further impair sensitivity. Hence, acute-phase IgM testing alone has multiple limitations for diagnosis of acute leptospirosis, regardless of the cut-off.

Only a few studies have evaluated the use of serologic testing for identifying leptospirosis in febrile cohorts. In Laos, 372 febrile patients were evaluated with ELISA (Panbio Ltd., Brisbane, Queensland, Australia) and immunochromatographic testing (ICT), which was compared with the MAT; acute leptospirosis (single titer ≥400 or 4-fold rise in titer) was identified in 23 (12.4%) of 186 patients (*18*). The sensitivity of ELISA and ICT was relatively high (60.9% and 47.3%, respectively), which could be explained by a long duration of fever (median 9 days). The sensitivity of ELISA for acute-phase versus convalescent-phase serum specimens was comparable (60.9% and 65.2%, respectively), but convalescent-phase serum specimens were obtained 4.5 days after acute-phase serum specimens. The specificity of both assays was similarly poor (65.6% for ELISA and 75.5% for ICT). In Thailand, Cohen et al. identified acute leptospirosis in 67 (9.5%) of febrile subjects using 2 rapid assays, a dipstick, and latex slide agglutination test (*19*). Patients sought treatment after a mean of 3.4 days of fever and returned 22 days later. Compared with MAT on paired serum specimens, the sensitivity of testing acute-phase serum with the dipstick and latex slide agglutination tests was 22% and 13%, respectively, which is similar to our findings.

Strengths of our study include the rigorous, prospective design, uniquely large sample size, inclusion of an unstudied population believed to be at high risk, an unusually high rate of follow-up, and clinical correlation. To minimize selection bias, we used standardized criteria to sequentially enroll a large cohort (≈900 patients) with thorough follow-up to enable assessment of acute-phase IgM testing versus clinical impression and relevant epidemiologic and clinical features. Those patients from whom paired serum specimens were not available differed only slightly from the included population. We excluded the few with equivocal results to avoid possible misclassification with resultant potential failure to identify significant predictive clinical features. By rigorously distinguishing acute from recent leptospirosis, we were able to confirm that myalgias and arthralgia were frequent symptoms and that the presence of conjunctivitis or conjunctival suffusion is diagnostically helpful. Leptospirosis in this cohort was relatively mild, as evidenced by stable vital signs, the absence of jaundice, and complete blood counts within nearly normal ranges. The sparse laboratory data reflect standard clinical practice in which automated testing is largely unavailable in the public sector, expensive in the private sector, and thus infrequently obtained.

Our results might have differed if we had used a different diagnostic standard, but culture, for example, is insensitive, labor intensive, unlikely to be available soon at Karapitya Teaching Hospital and many similar hospitals, and too slow to guide clinical management. We may have misclassified the number of days with fever, since temperatures are infrequently taken at home or in the hospital; however, duration of fever correlated well with that of symptoms, and no systematic bias would be expected, since etiologic diagnoses were not known when patients sought treatment at the hospital. Our estimate of leptospirosis may be low if a wider array of serovars is circulating in southern Sri Lanka than were detected by the ELISA used; however, no other available commercial assay would have been expected to be more sensitive.

We conclude that leptospirosis causes substantive illness in southern Sri Lanka. Furthermore, we found that testing acute-phase serum specimens for IgM has multiple limitations for the diagnosis of acute leptospirosis, because a positive result more often denoted past infection than an acute infection, and results were negative early in infection. Clinical impression is comparatively better without added cost (*20*). Paired serum specimens can provide rigorous diagnosis, but patients and clinicians need rapid diagnosis to guide clinical management. A few antigen-based or nucleic acid–based rapid tests have been described, but prospective clinical validations are limited (*17*,*21*–*25*). Rapid, pathogen-based tests for early diagnosis need to be developed.

Acknowledgments

We thank the members of the microbiology laboratory at the Medical Faculty, University of Ruhuna, and the clinical staff at Karapitiya Teaching Hospital for their assistance.

Patient enrollment was supported by the Hubert-Yeargan Center for Global Health and the Duke University Medical Center Chancellor's Tsunami Relief Fund. Laboratory testing and M.E.R.

were supported by a Johns Hopkins Center for Global Health Junior Faculty Grant, a Clinician Scientist Career Development Award from Johns Hopkins School of Medicine, and the National Institute of Allergy and Infectious Dieseases, National Institutes of Health (K23AIO83931).

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Inpatient Capacity at Children's Hospitals during Pandemic (H1N1) 2009 Outbreak, United States

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Release date: August 23, 2011; Expiration date: August 23, 2012

Learning Objectives

Upon completion of this activity, participants will be able to:

- Compare the 2009 H1N1 influenza pandemic with past influenza pandemics
- Evaluate the occupancy of children's hospitals in the United States during the 2009 H1N1 influenza pandemic
- Analyze the relative effects of the 2009 H1N1 influenza pandemic on emergency departments and inpatient services
- Distinguish the number of additional admissions required in 2009 to push the children's hospital system to full capacity.

Editor

Karen L. Foster, Technical Writer/Editor, *Emerging Infectious Diseases. Disclosure: Karen L. Foster has disclosed no relevant financial relationships.*

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Disclosures: ***Matthew Hall, PhD; Evan S. Fieldston, MD, MBA, MSHP; Paul D. Hain, MD; Thomas V. Brogan, MD;*** *and* ***Michael B. Mundorff, MBA, MHSA,*** *have disclosed no relevant financial relationships.* ***Marion R. Sills, MD, MPH,*** *has disclosed the following relevant financial relationships: received grants for clinical research from Agency for Healthcare Research and Quality.* ***Harold K. Simon, MD, MBA,*** *has disclosed the following relevant financial relationships: received grants for clinical research from Baxter International Inc. (rehydration clinical trial); AspenBio Pharma, Inc (appendicitis screening); National Institutes of Health (progesterone planning trial through the Pediatric Applied Research Network), all through Emory University.* ***Daniel B. Fagbuyi, MD,*** *has disclosed the following relevant financial relationships: owns stock, stock options, or bonds from Medco Health Solutions, Inc.; Orexigen Therapeutics, Inc.* ***Samir S. Shah, MD, MSCE,*** *has disclosed the following relevant financial relationships: received grants for clinical research from National Institutes of Health; Agency for Healthcare Research and Quality; Robert Wood Johnson Foundation.*

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DOI: 10.3201/eid1709.101950

Quantifying how close hospitals came to exhausting capacity during the outbreak of pandemic influenza A (H1N1) 2009 can help the health care system plan for more virulent pandemics. This ecologic analysis used emergency department (ED) and inpatient data from 34 US children's hospitals. For the 11-week pandemic (H1N1) 2009 period during fall 2009, inpatient occupancy reached 95%, which was lower than the 101% occupancy during the 2008–09 seasonal influenza period. Fewer than 1 additional admission per 10 inpatient beds would have caused hospitals to reach 100% occupancy. Using parameters based on historical precedent, we built 5 models projecting inpatient occupancy, varying the ED visit numbers and admission rate for influenza-related ED visits. The 5 scenarios projected median occupancy as high as 132% of capacity. The pandemic did not exhaust inpatient bed capacity, but a more virulent pandemic has the potential to push children's hospitals past their maximum inpatient capacity.

During March and April 2009, a novel influenza A (H1N1) virus began to spread in North America that disproportionately affected children, who constituted half of patients hospitalized for influenza-related illness (IRI) during spring 2009 (*1*–*3*). After a summertime decline, the virus returned to full activity in the fall, and children continued to have the highest rates of illness and hospitalization (*4*). As a result, pediatric providers and children's hospitals cared for large numbers of patients with pandemic (H1N1) 2009 virus (*5*,*6*). Despite the high attack rate for children, the pandemic virus was milder than prior pandemic viruses. The attack rate for pandemic (H1N1) 2009 was lower for children (17.9%) (*7*) than it was for each of the past 3 pandemics in the United States (1918, 1958, and 1968) (21%–54%) (*8*), and the hospitalization rate was lower for children by >48-fold (0.17/1,000 symptomatic children (*9*) vs. estimates as high as 8.5/1,000 symptomatic children [*10*]).

Because of relatively low virulence, pandemic (H1N1) 2009 resulted in comparatively fewer hospitalizations than feared, but it greatly affected ambulatory settings and emergency departments (EDs) (*11*–*13*). The exact effect on children's hospitals remains unknown because published studies have reported only regional data and have quantified hospital admissions rather than inpatient occupancy (*14*–*16*). Assessing use of capacity in the context of a low-virulence influenza pandemic can provide insight into how a more virulent virus might directly affect children's hospitals and indirectly affect all health care systems throughout their catchment areas. Occupancy levels above and beyond existing capacity limits would represent a true crisis that would dramatically affect the already-stretched health care–delivery system (*17*). Because children's hospitals play an integral role in coordinating health delivery (*18*), defining the limits of capacity reserve and quantifying how close these hospitals came to exhausting these limits can help the entire health care system better plan for more virulent pandemics or other disaster-type events.

With the effect of pandemic (H1N1) 2009 on children's hospitals as a collective case study, we evaluated how close to full capacity US children's hospitals functioned during the outbreak of pandemic (H1N1) 2009 and the implications for the health care systems had we not been fortunate regarding the low virulence of subtype H1N1 influenza (*19*). The objectives of this study were to 1) compare occupancy at US tertiary care children's hospitals during the pandemic period with occupancy during the 2008–09 seasonal influenza outbreak, 2) measure how close each hospital came to exhausting capacity for inpatient beds, and 3) measure the effect on capacity if pandemic (H1N1) 2009 during fall 2009 had been more severe.

Methods

Source Data

This ecologic analysis used data from the Pediatric Health Information System (PHIS), which includes ED and inpatient data from 41 free-standing nonprofit tertiary care children's hospitals in all regions of the United States. The Child Health Corporation of America (Shawnee Mission, KS, USA) and participating hospitals jointly validate data quality and reliability (*20*). This analysis comprises data from the 34 PHIS hospitals that provided codes indicating intensive care unit (ICU) and non-ICU bed designations for the study period.

Study Participants

We defined the pandemic (H1N1) 2009 period and other influenza epidemic periods using national influenza circulation data obtained from the World Health Organization collaborating laboratories and the National Respiratory and Enteric Virus Surveillance System (*21*). Using as a threshold the weeks with >20% positive test results as reported in the Morbidity and Mortality Weekly Report (Centers for Disease Control and Prevention, Atlanta, GA, USA) (*22*,*23*), we defined the period of pandemic (H1N1) 2009 as September 5–November 20, 2009. To compare inpatient resource use during this period with that during a seasonal influenza period, we used the weeks of seasonal influenza from the 2008–09 season (January 31–March 20, 2009), defined using the same 20% threshold (*23*).

Because specifically identifying patients with pandemic (H1N1) 2009 was not feasible, we used a standard list of International Classification of Diseases, 9th Revision, codes developed for measuring IRI to determine resource use of inpatient beds (*5*). This list comprises International

Classification of Diseases, 9th Revision, codes 460–496 or 510–519 as the primary or secondary discharge diagnosis and captures not only primary infections with influenza, but also secondary infections (e.g., bacterial pneumonia) and exacerbations of other conditions (e.g., asthma).

Measures

Our primary measure was midnight occupancy for non-ICU beds and for ICU beds. The numerator for occupancy comprised all children (age 0–18 y; median age 3.1 y, interquartile range [IQR] 1.0–8.1 y) occupying non-ICU and ICU beds on each day of the study period. We obtained denominator data (i.e., annual number of licensed, in-service beds) from the Child Health Corporation of America and confirmed them by an email survey to each hospital's designated PHIS contact. Step-down beds were categorized as non-ICU. If a patient spent at least 1 midnight in an ICU bed during his or her hospital stay, admission was considered an ICU admission and was not counted as a non-ICU admission. We included all hospitalized patients of any admission status (observation or inpatient) to fully quantify hospital occupancy. We excluded newborn nursery and mental health admissions and those designated beds from the analysis.

For our second objective, we defined the threshold as 100% occupancy on the basis of licensed, in-serviced beds as capacity. Although lower thresholds have been suggested as the point at which quality and safety decline (*24*,*25*), 100% represents the scenario in which a hospital has actually exhausted its typical capacity of in-service beds. In calculating non-ICU and ICU occupancy, we counted the number of patients in each bed type at the midnight at the end of the day.

For our third objective, we analyzed the 26 PHIS hospitals for which ED data were available. In our models, we varied 2 parameters and described the effect on inpatient occupancy: 1) number of ED IRI visits and 2) ED-to-hospital admission rate. For the first, we used estimates from the 2 most recent, severe prior influenza pandemics (1957 and 1968), when the estimated upper bound of the attack rate was 36% (*9*,*10*). Estimates of the attack rate for pandemic (H1N1) 2009 for April–December 2009 were 17.9%, based on 55 million cases (*7*) in a July 2009 population of 307 million (*26*). Assuming the per-case rate of ED visits remained fixed, we estimated that ED IRI visits could have been 2× what they were if the attack rate had been similar to these 2 prior pandemics.

For the second parameter, we used the ED admission rate of 14.0% observed during the 2003–04 seasonal influenza weeks (November 1, 2003–January 9, 2004), 1 of the most severe recent influenza seasons (*27*,*28*). We also modeled a 30% admission rate (the upper end of overall ED admission rate for study hospitals in 2008), which actually falls well below the rate projected from hospitalization estimates of earlier influenza pandemics and epidemics (*9*,*10*).

Analysis

To compare occupancy during the fall pandemic (H1N1) 2009 period with baseline, we calculated the number of admissions, bed-days, and the occupancy for all beds, non-ICU beds, and ICU beds for the 2009 pandemic period and for 2 comparison periods: the entire prior calendar year (2008) and the prior seasonal influenza period. We assessed the statistical significance of the difference in median occupancy between the 2009 pandemic period and the seasonal influenza comparison period using the Wilcoxon rank-sum test.

To measure how close each hospital came to exhausting capacity, we calculated how many additional non-ICU and ICU patients could have been admitted by each hospital. For each day, we counted the number of unoccupied beds of each type and modeled how many additional patients were needed to fill all available beds for each hospital. For hypothetical additional patients, we modeled patients' continued presence iteratively for each day of the study period as non-ICU and ICU patients on the basis of the characteristics of patients admitted during the fall 2009 pandemic with IRI (e.g., 20% with a 1-day length of stay, 35% with a 2-day stay, 30% with a 3-day stay). For both models, we assigned bed-days of each stay to each respective area, ICU and non-ICU. To index the total number of additional patients needed to fill the hospital to capacity across hospitals, we then calculated the number of additional patients per 10 beds (non-ICU or ICU).

To measure the effect on capacity of a more severe outbreak of pandemic (H1N1) 2009, we calculated the number of ED IRI visits and the ED-to-inpatient admission rate for ED IRI visits for the 26 PHIS hospitals for which ED data were available. We then used the same modeling methods described above to model the number of additional bed-days (non-ICU, ICU) in each scenario. We expressed findings from the 6 scenarios in 2 ways: 1) percentage of hospital days >100% and 2) as median (IQR) occupancy. In these models, we made 4 assumptions. First, we assumed that the rate of non-IRI ED admissions remained unchanged. Second, we assumed that hospitals did not react to high occupancy by rescheduling elective admissions, an assumption based on a prior analysis of the same data set (*17*). Third we assumed that the number of ICU and non-ICU beds remained fixed for each calendar year. Fourth, we assumed that the inpatient length-of-stay distribution was not shifted toward longer hospitalizations during a more virulent pandemic.

We performed all analyses with SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA) and considered p values

<0.05 statistically significant. The study protocol was approved by the Colorado Multiple Institutional Review Board with a waiver of informed consent.

Results

The 11-week period of evaluation during the fall 2009 pandemic period included a median of 2,774 (IQR 2,219–3,319) admissions and 19,283 (IQR 15,842–21,315) bed-days (Table 1). Median overall inpatient occupancy was 95% (IQR 85%–99%), whereas median overall occupancy during the 2008–09 seasonal influenza period was 101% (IQR 96%–110%) and, for the entire calendar year 2008, 91% (IQR 87%–95%). Hospitals' experiences varied considerably, with hospital-level median occupancy ranging from 57.4% to 128.0% (online Technical Appendix, www.cdc.gov/eid/content/17/9/101950-Techapp.pdf). To reach 100% occupancy during the pandemic period, for every 10 beds of each type, hospitals would have needed to admit a median of 0.2 (IQR 0.1–0.3) additional patients per day for non-ICU beds and 0.7 (IQR 0.5–0.9) per day for ICU beds (Table 2).

For the 26 hospitals for which ED and inpatient data were available, the median ED-to-hospital admission rate for IRI patients was 5.4% (IQR 3.3%–8.1%). Different hypothetical scenarios for ED IRI volume and admission rates would have differently affected the frequency of hospital days exceeding the 100% occupancy threshold for exhausting capacity reserves (Table 3). The actual experience in 2009 (scenario A) resulted in 23.3% of hospital days with ≥100% occupancy across the 26 hospitals. Had the hospitals instead experienced the IRI admit rate from the 2003–04 influenza season (14.0%) applied to the same volume of patients, 37.6% of hospital days would have been ≥100% full (scenario B). Had the admission rate been 30% for 2× the volume of patients, 85.7% of hospital days would have been ≥100% full, exhausting capacity reserves (scenario F).

Individual hospital experience varied considerably (Figure). For each hospital, the dot-plots we constructed show the distribution of occupancy data across hospitals for each of the 6 scenarios. For our worst-case scenario (scenario F), median occupancy would have been 132% (IQR 124%–145%).

Discussion

We examined the effect on children's hospitals' resources during fall 2009 when pandemic influenza A (H1N1) 2009 virus was active. We demonstrated that children's hospitals faced high levels of occupancy (median 95%) in regular inpatient care areas and ICUs, but this situation did not differ from typical levels of high occupancy commonly experienced at some hospitals. Despite the mild virulence of pandemic (H1N1) 2009 virus, children's hospitals needed only <1 additional admission per 10 inpatient beds to reach 100% occupancy. Additionally, the pandemic occurred during early fall, when viruses that cause respiratory and gastrointestinal illnesses (which typically increase occupancy at children's hospitals) were not circulating widely. Models representing an outbreak of a more virulent influenza virus based on historical comparisons demonstrate that modest increases in ED visits or ED admission rates would have resulted

Table 1. Hospital characteristics and aggregated experiences in 34 PHIS hospitals, United States, all of 2008, 2008–09 seasonal influenza period, and fall 2009 pandemic (H1N1) 2009 period*

Hospital characteristics	Calendar year 2008, 52 wk, median (IQR)	2008–09 seasonal influenza, 8 wk, median (IQR)†	Fall 2009 pandemic influenza, 11 wk, median (IQR)‡
Overall§			
Beds, no.	255 (221–316)	265 (219–319)	265 (219–319)
Admissions, no.	12,105 (9,913–14,247)	1,871 (1,489–2,197)	2,774 (2,219–3,319)
Bed-days, no.	89,117 (70,691–96,346)	15,299 (13,087–17,312)	19,283 (15,842–21,315)
Occupancy, %	91 (87–95)	101 (96–110)	95 (85–99)
Non-ICU			
Beds, no.	129 (91–179)	132 (90–181)	132 (90–181)
Admissions, no.	10,575 (8,415–13,022)	1,595 (1,346–2,030)	2,419 (1,838–2,907)
Bed-days, no.	81,735 (65,703–87,171)	13,933 (11,946–15,902)	16,703 (1,2051–18,843)
Occupancy, %	94 (91–103)	108 (100–117)	98 (92–105)
ICU¶			
Beds, no.	33 (25–48)	37 (27–51)	37 (27–51)
Admissions, no.	1,654 (1,167–22,80)	245 (189–316)	401 (296–516)
Bed-days, no.	8,299 (5,502–11,083)	1,576 (1,155–1,889)	1,873 (1,094–2,638)
Occupancy, %	72 (44–87)	80 (51–97)	65 (47–88)

*PHIS, Pediatric Health Information System; IQR, interquartile range; ICU, intensive care unit; MMWR, Morbidity and Mortality Weekly Report (Centers for Disease Control and Prevention, Atlanta, GA, USA).
†MMWR reporting weeks 4–11 (January 31–March 20, 2009).
‡MMWR reporting weeks 35–45 (September 5–November 20, 2009).
§Excluding neonatal and behavioral health patients and designated beds.
¶Excluding neonatal intensive care units.

Table 2. Additional admissions needed to fill hospitals to 100% occupancy during 11-week fall 2009 pandemic (H1N1) 2009 period, per 10 beds per day, in study of 34 PHIS hospitals, United States*

Measure	Non-ICU	ICU
Minimum	0.0	0.2
1st quartile	0.1	0.5
Median†	0.2	0.7
3rd quartile	0.3	0.9
Maximum	1.3	2.0

*PHIS, Pediatric Health Information System; ICU, intensive care unit.
†Median indicates the hospital requiring the median number of patients to fill all beds during the pandemic period.

in substantial overcrowding among the large cohort of children's hospitals in our study.

These findings are notable in the context of national disaster planning related to children. The National Commission on Children and Disasters' 2010 Report to the President and Congress recommended that additional resources provide a "formal regionalized pediatric system of care to support pediatric surge capacity" and emphasizes that children's hospitals are central to such regionalization (*18*). Our study shows that children's hospitals, the central component of this proposed regionalized system, routinely operate so close to capacity that little available reserve exists for even a modest surge of inpatients. For a hospital with 150 non-ICU beds and 50 ICU beds, an additional 3.0 non-ICU and 3.5 ICU admissions per day would have exhausted capacity. Although the 2009 influenza pandemic did not do so, surge capacity is scarce, as demonstrated by the many hospitals that are already operating at or near maximum capacity in their EDs and inpatient areas (*17*,*29*).

Federal planners have suggested that surge capacity should accommodate 500 inpatients per million population, but such capacity does not exist for children under normal circumstances; capacity for only 193 inpatients per million children is available during typical winter weekdays (*29*–*31*). Although we expressed our findings in terms of hospital occupancy rather than on a population basis, our findings are similar to those raising alarm about limited inpatient capacity in the face of a pandemic or disaster.

Pandemics extend over many weeks and affect large regions, if not the entire country. Although the hospitals may be able to handle such levels of occupancy on a short-term basis, whether they could do so for prolonged periods is unclear. Even though a health care system's capacity reserve cannot be designed on a daily basis to handle a pandemic, the frequent level of high occupancy already experienced by children's hospitals and the resulting lack of a buffer for a pandemic-associated surge should be considered by individuals and organizations involved with planning and disaster preparedness (*32*,*33*). Planning for such events at hospital and regional levels may be improved with data about current capacity reserves and how perturbations can affect that capacity.

In previous studies of large-scale epidemics, hospitals have altered standards of care—as occurred in Toronto during the 2003 outbreak of severe acute respiratory syndrome—to meet increased patient needs (*30*,*34*,*35*). During the outbreak of severe acute respiratory syndrome, restrictions on scheduled (i.e., elective) admissions were imposed in Toronto (*36*). Although we did not study scheduled admissions, our analysis suggests in a more virulent pandemic (scenario F), hospitals would have run out of space even if they had rescheduled the 15%–25% of scheduled pediatric admissions; this percentage includes the 20% of elective admissions for chemotherapy, a treatment that is not amenable to prolonged postponement (*37*).

Our findings are subject to several limitations. The 34 hospitals in this study represent a subset of the ≈250 US children's hospitals and may not be representative of these children's hospitals or of other hospitals that admit children, even though the study included children's hospitals in all regions of the country. The analysis did not consider measures that individual hospitals and regional systems might use to reduce occupancy, such as canceling scheduled admissions, which would have caused us to overestimate occupancy. On the other hand, our assumption about length of stay would have caused us to underestimate occupancy. Our analysis used midnight census; actual daytime occupancy most likely was higher (*38*), and thus true surge capacity was even lower than estimated. Finally, the modeled scenarios were based on historical comparisons, which represent a range of potential demands on the health care system.

Table 3. Predictive model of the percentage of hospital days (ICU and non-ICU) during at ≥100% hospital occupancy, using 11-week pandemic period fall 2009 pandemic period data as baseline occupancy, in study of 34 PHIS hospitals, United States*

	ED IRI admission rate		
ED IRI volume	Fall 2009 pandemic period†	2003–04 seasonal influenza period‡	Hypothetical
Admission rate, %	5.4	14.0	30
Same as during fall 2009 pandemic period, median (IQR)†	A: 23.3 (0–42.9)	B: 37.6 (11.6–58.4)	C: 63.6 (23.4–83.1)
100% increase from pandemic period, median (IQR)	D: 64.9 (16.9–81.8)	E: 72.7 (24.6–81.8)	F: 85.7 (40.2–98.7)

*ICU, intensive care unit; PHIS, Pediatric Health Information System; ED, emergency department; IRI, influenza-related illness; MMWR, Morbidity and Mortality Weekly Report (Centers for Disease Control and Prevention, Atlanta, GA, USA). Letters correspond to scenarios shown in the Figure.
†MMWR reporting weeks 35–45 (September 5–November 20, 2009).
‡MMWR reporting weeks 44–53 (November 1, 2003–January 9, 2004).

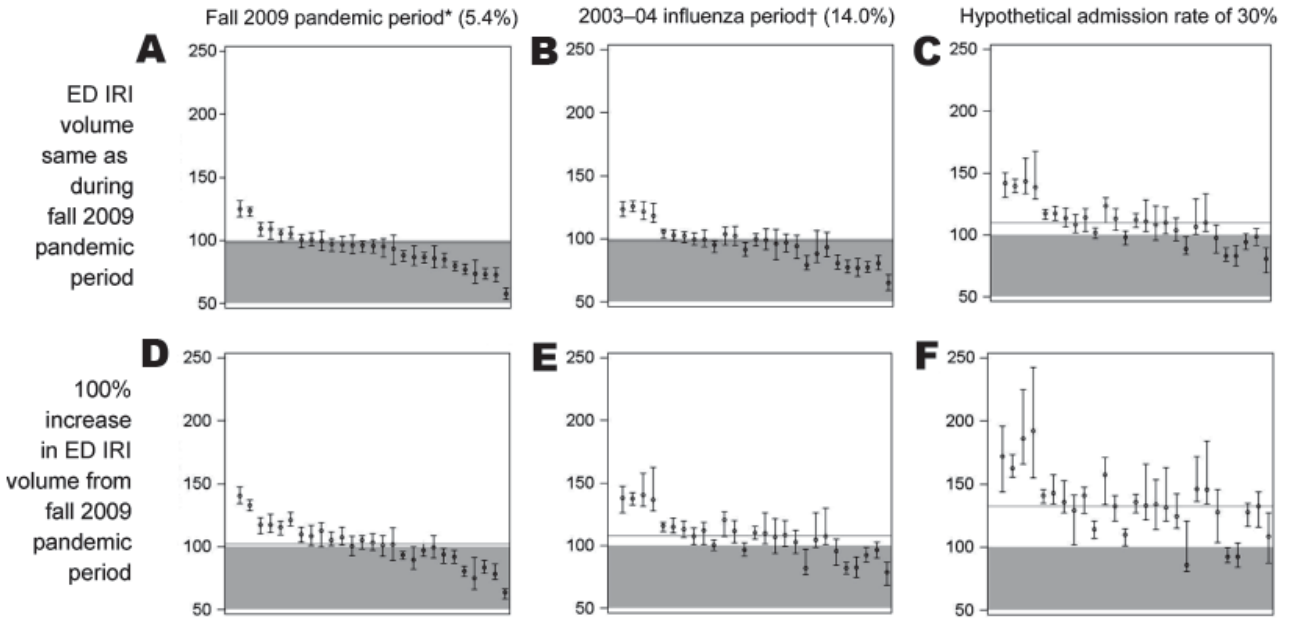

Figure. Predictive model of hospital occupancy during 11-week outbreak of pandemic influenza A (H1N1) 2009 in the United States, by ED IRI admission rate and ED IRI volume, using fall 2009 pandemic period data as baseline. Percentages given indicate hospital admission rate during period or for hypothetical scenario. Gray area indicates 100% occupancy. Each circle represents median occupancy from 1 hospital; vertical whiskers indicate interquartile range. y-axes indicate percentage occupancy; x-axes indicates individual hospitals. Median (interquartile range) occupancy across study hospitals: A) 95.9% (85.0%–100.3%); B) 97.9% (80.5%–102.7%); C) 109.7% (97.9%–117.1%); D) 102.2% (91.7%–112.4%); E) 108.0% (96.4%–114.9%); F) 132.4% (124.5%–145.5%). Letters correspond to scenarios in Table 3. *MMWR reporting weeks 35–45 (September 5–November 20, 2009). †MMWR reporting weeks 44–53 (November 1, 2003–January 9, 2004). ED, emergency department; IRI, influenza-related illness; MMWR, Morbidity and Mortality Weekly Report (Centers for Disease Control and Prevention, Atlanta, GA, USA).

For hospitals and government agencies, the results of our study should prompt review of preparedness planning and reconsideration of surge capacity. Systemwide resource limitations must be considered because ambulatory and inpatient services interrelate. The outbreak of low-virulence pandemic (H1N1) 2009 virus affected EDs disproportionately but left inpatient services relatively unaffected (*13*). Exploring the parameters of more severe epidemics might allow planners at individual hospitals, as well as regional health administrators, to consider what alterations in standards may be necessary.

M.R.S. received support from the Agency for Healthcare Research and Quality (5R03HS016418). S.S.S. received support from the National Institute of Allergy and Infectious Diseases (K01 AI73729) and the Robert Wood Johnson Foundation under its Physician Faculty Scholar Program.

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Mycobacterium chelonae-abscessus Complex Associated with Sinopulmonary Disease, Northeastern USA

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Release date: August 22, 2011; Expiration date: August 22, 2012

Learning Objectives

Upon completion of this activity, participants will be able to:

- Analyze the *M. chelonae-abscessus* complex
- Distinguish the molecular identity of "*M. franklinii*"
- Identify the most common clinical source of "*M. franklinii*"
- Evaluate the antimicrobial susceptibility of "*M. franklinii*"

Editor

Beverly Merritt, Technical Writer/Editor, *Emerging Infectious Diseases. Disclosure: Beverly Merritt has disclosed no relevant financial relationships*.

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DOI: http://dx.doi.org/10.3201/eid1709.101667

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Members of the *Mycobacterium chelonae-abscessus* complex represent *Mycobacterium* species that cause invasive infections in immunocompetent and immunocompromised hosts. We report the detection of a new pathogen that had been misidentified as *M. chelonae* with an atypical antimicrobial drug susceptibility profile. The discovery prompted a multicenter investigation of 26 patients. Almost all patients were from the northeastern United States, and most had underlying sinus or pulmonary disease. Infected patients had clinical features similar to those with *M. abscessus* infections. Taxonomically, the new pathogen shared molecular identity with members of the *M. chelonae-abscessus* complex. Multilocus DNA target sequencing, DNA-DNA hybridization, and deep multilocus sequencing (43 full-length genes) support a new taxon for these microorganisms. Because most isolates originated in Pennsylvania, we propose the name *M. franklinii* sp. nov. This investigation underscores the need for accurate identification of *Mycobacterium* spp. to detect new pathogens implicated in human disease.

Infections caused by members of the *Mycobacterium chelonae-abscessus* complex remain a serious public health problem, and their role has expanded, with growing numbers of therapeutic interventions that disrupt the competency of the human immune system. Before 2001, only 2 species, *M. chelonae* and *M. abscessus,* were recognized as members of the complex. Since that time, 4 new species have been added to the complex: *M. immunogenum*, *M. massiliense*, *M. bolletii,* and *M. salmoniphilum*. With the exception of *M. salmoniphilum*, all members of the complex have been implicated in human disease (*1–4*). Recently investigators have noted a lack of separation of *M. bolletii* and *M. massiliense* from *M. abscessus* and have proposed they be classified as a subspecies of *M. abscessus* (*5*).

Members of the *M. chelonae-abscessus* complex represent *Mycobacterium* species that cause invasive skin and soft tissue infections, pneumonia, bloodstream infections, and abscesses in immunocompetent and immunocompromised hosts (*6,7*). Definitive identification by the clinical laboratory is needed for outbreak detection and for performance of susceptibility testing for patient management. Currently, the taxonomic relationships among members of the *M. chelonae-abscessus* complex lack clarity. The species are biochemically inert and their genetic signatures by partial 16S rRNA gene sequencing are often similar, which makes identification a great challenge for clinical laboratories.

In 2007, we detected a group of clinical isolates that were misidentified as *M. chelonae* with an atypical antimicrobial drug susceptibility profile. All isolates were from Pennsylvania, and as an interim identification, we labeled these isolates as CV for *M. chelonae* variant. Our discovery prompted a large multistate investigation that involved obtaining clinical correlation, retrospective and prospective collections of isolates with similar CV characteristics, and examination of a large set of known clinical isolates and type strains from the *M. chelonae-abscessus* complex. Given the taxonomic complexities and current ambiguities within the *M. chelonae-abscessus* group, we performed a comprehensive analysis of the potentially new species, including DNA-DNA hybridization, multilocus sequencing, and deep multilocus sequencing. We describe a new pathogen, *M. franklinii* sp. nov., a proposed new member of the *M. chelonae-abscessus* complex that was isolated from 26 patients in the United States. We discuss its potential role in human disease.

Methods

Isolates

All available type strains of *M. chelonae-abscessus* complex were obtained from American Type Culture Collection (ATCC), Collection of Institut Pasteur, or Culture Collection, University of Göteborg, Sweden. Previously identified clinical isolates of *M. abscessus*, and *M. chelonae* by partial 16S rRNA gene sequencing and internal transcribed spacer (ITS) PCR were retrieved for comparative analysis. A subset of these isolates was described in a prior study (*8*). CVs were defined as isolates that were cefoxitin susceptible or showed intermediate resistance, and identified as *M. chelonae* by partial 16S rRNA gene sequencing and ITS PCR (*9*) by Associated Regional and University Pathologists Laboratories and the Hospital of the University of Pennsylvania or by failure to amplify *hsp65* by PCR restriction fragment length polymorphism analysis (*10,11*) by the University of Texas Health Science Center. Microbiologic and medical records were reviewed for clinical information for select isolates. Clinical case reviews were conducted under institutional review board approved protocols at Associated Regional and University Pathologists Laboratories, Children's Hospital of Philadelphia, University of Pennsylvania, and the University of Texas Health Science Center. Study isolates CV002 (ATCC [pending] and DSMZ 45524) and CV005 (ATCC [pending]) have been deposited into culture collections.

Multilocus Sequencing

DNA extractions, PCR, and sequencing reactions were performed as described (*8*). Amplifications and sequencing reactions were performed by using primers specific for ≈1,400 bp of the 16S rRNA 5F (5′-TTGGAGAGTTTGATCCTGGCTC-3′) and 1492R (5′-ACGGITACCTTGTTACGACTT-3′), ≈700 bp of

rpoB, ≈400 bp of *sodA* (*12*), ≈240 bp of the ITS (*13*), and ≈400 bp of *hsp65* (*11*) genes or region. Sequence alignments and phylogenetic trees were constructed by using neighbor-joining method with Kimura 2-parameter distance correction model and 1,000 bootstrap replications in MEGA4 (*14*). Only unique sequences (sequevars) were included in the trees.

Standard for Identification

Final species identifications were based on comparisons of sequences for the full 16S rRNA and the partial *rpoB* genes to GenBank references of type strains. Full-length 16S rRNA sequences were used in this study, and we used 99.5% shared identity for identification to a type strain sequence. Species identification for *rpoB* gene was based on an identity of 98.0%–100% as outlined by Adekambi et al. (*12*,*15*).

DNA-DNA Hybridization

Purified DNA of the type strains and the patient isolates CV002, CV004, CV005, CV006, and CV005 was prepared as described (*16*). CV002 and CV005 strains were labeled with [^{32}P] dCTP using the Nick Translation Kit (Invitrogen, Carlsbad, CA, USA). Labeled DNA from the CV002 was hybridized with unlabeled DNA from isolates CV002, CV004, CV005, CV006, and CV015 and with unlabeled DNA from the type strains. Labeled DNA from patient isolate CV005 was then hybridized with unlabeled DNA from isolates CV002 and CV005. The reciprocal experiment was performed because of the nearness of CV005 to the 70% cut-off designated by Wayne (*17*) and the 0% divergence obtained in the first experiment. Hybridization was performed as previously described (*18*). All reactions were performed in duplicate at 70°C. The relative binding ratio (RBR) was calculated by using the method of Brenner et al. (*19*). The percentage divergence (calculated to the nearest 0.5%) was determined by assuming that each degree of heteroduplex instability, when compared with the melting temperature of the homologous duplex, was caused by 1% unpaired bases (*19*).

Deep Multilocus Sequencing

Single-end DNA libraries of *M. bolletii* CIP 108541^{T}, *M. chelonae* ATCC 35752^{T}, *M. immunogenum* CIP 106684^{T}, *M. massiliense* CCUG 48898^{T}, and CV002 were prepared using Illumina DNA Sample Kit (Illumina, Inc., San Diego, CA, USA) according to manufacturer's recommendations. Sequencing was performed in individual flow cell lanes on the Illumina Genome Analyzer (Illumina, Inc.) at the University of Utah Huntsman Cancer Institute Core Sequencing Facility.

De novo assembly of raw Illumina sequence data was achieved by using Velvet software (*20*). Velvet was run in 2 parts, velveth and velvetg. For velveth, the hash length was set at 23 (value was selected by calculations in the software manual); default settings were used for all other parameters. In velvetg, the –cov cutoff value was set to auto (setting allows software to automate appropriate coverage cutoff), and –min_contig_lgth was set to 100; all other settings were default.

The genome of *M. abscessus* CIP 104536^{T} (*21*) was used as the source reference set of 123 genes that were identified as likely core genome components for the phylum *Actinobacter* by Ventura et al. (*22*). The set of reference genes was randomly divided into 5 similarly sized sets to facilitate analysis. SeqMan (DNASTAR Inc., Madison, WI, USA) was used to align the assembled contigs from the sequenced species against each of the sets of reference genes. DNA and inferred amino acid sequences were aligned using MEGA. Only near full-length genes were used in future comparisons. Confirming the translation of the gene was in the correct reading frame relative to the different isolates substantiated quality of each assembled gene. The DNA and amino acid sequences were concatenated for each isolate and sequences alignments and phylogenetic trees were constructed in MEGA using the neighbor-joining method. Kimura 2-parameter distance correction was used for DNA and Poisson correction model was used for amino acid trees; 1,000 bootstrap replications were used for each tree constructed.

Susceptibility Testing

We determined antimicrobial susceptibility by broth microdilution using the recommended Clinical and Laboratory Standards Institute guidelines for rapidly growing *Mycobacterium* spp. (*23*). Some isolates were not tested for all antimicrobial agents and the concentrations of antimicrobial agents that were tested varied in the panels. MICs of clarithromycin were assessed at 3 days.

Results

Identification of Isolates by Multilocus Sequencing

We obtained 6 type strains representing all members of the *M. chelonae-abscessus* complex. All type strains and all 127 archived isolates underwent multilocus sequencing. For the 127 archived clinical isolates, we identified 64 *M. abscessus*, 58 *M. chelonae*, and 5 *M. massiliense* isolates. We designated an additional 26 isolates as CV on the basis of our case definition. All 26 CV isolates underwent *rpoB* gene sequencing, and a subset (n = 11) underwent multilocus sequencing. Unique sequevars are designated among the species *M. abscessus, M. massiliense, M. bolletii, M. chelonae,* and CV isolates.

16S rRNA Gene

Alignments of 1,341 bp of the 16S rRNA gene show 8 unique sequevars from 14 variable bp positions (Figure 1). *M. chelonae* isolates had the most variability with 4 sequevars and an intraspecies variability of 0.2% (1–3 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 2 sequevars differing by 1 bp. Two *M. massiliense* sequevars were observed and differed by 1 bp with 1 sequevar identical to an *M. abscessus* sequevar. A single sequevar was found for the CV isolates, and was identical to the type strain of *M. chelonae*.

ITS Region

Alignments of 233 bp of the ITS region show 18 unique sequevars from 30 variable positions (Figure 2). *M. chelonae* isolates had the most variability with 12 sequevars and an intraspecies variability of 1.7% (1–4 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates showed 4 sequevars with an intraspecies variability of 0.9% (1–2 bp). One *M. massiliense* sequevar was observed, which was identical to 1 of the *M. abscessus* sequevars. Two sequevars were found for the CV isolates and differed by 2 bp (0.9%). The CV sequevars were most closely associated with *M. salmoniphilum* ATCC 13758^T at 98.7% (3 bp).

hsp65 Gene

Alignments of 361 bp of the *hsp65* gene show 11 unique sequevars from 41 variable positions (Figure 3). *M. chelonae* isolates had the most variability with 5 sequevars and an intraspecies variability of 1.9% (1–7 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 3 sequevars with an intraspecies variability of 1.7% (1–6 bp). One *M. massiliense* sequevar was observed and identical to 1 of the *M. abscessus* sequevars. Two sequevars were found for the CV isolates and differed by 1 bp (0.3%). The CV sequevars were most closely associated with *M. immunogenum* CIP 106684^T at 98.6% (5 bp).

sodA Gene

Alignments of 426 bp of the *sodA* gene show 15 unique sequevars from 66 variable positions (Figure 4). *M. chelonae* isolates had the most variability with 9 sequevars and an intraspecies variability of 1.9% (1–8 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 3 sequevars with an intraspecies variability of 0.7% (1–3 bp). One *M. massiliense* sequevar was observed, which was identical to 1 of the *M. abscessus* sequevars. Two sequevars, which differed by 11 bp (2.6%), were found for the CV isolates. The CV sequevars were most closely associated with *M. immunogenum* CIP 106684^T at 95.8% (18 bp).

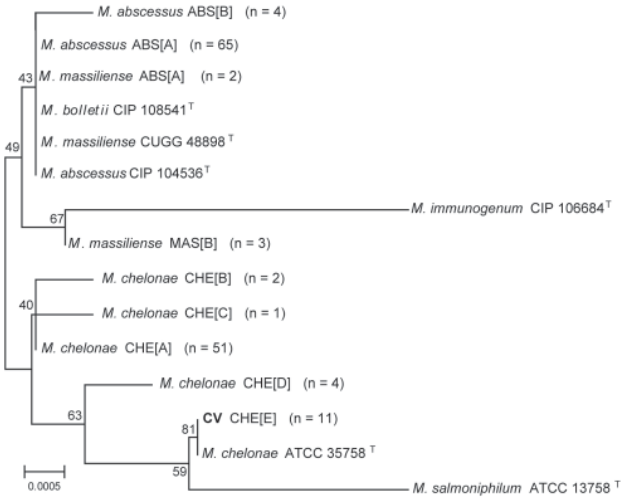

Figure 1. Neighbor-joining tree of a 1,341-bp region of unique 16S rRNA gene sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden; CV, *M. chelonae* variant; ATCC, American Type Culture Collection.

rpoB Gene

Alignments of 676 bp of region of the *rpoB* gene show 19 unique sequevars from 73 variable positions (Figure 5). *M. chelonae* isolates had the most variability with 9 sequevars and an intraspecies variability of 1.5% (1–10 bp). One *M. chelonae* clinical isolate shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 4 sequevars with an intraspecies variability of 0.7% (1–5 bp). The largest sequevar of *M. abscessus* clinical isolates had 100% identity to the type strain of *M. abscessus*. Two *M. massiliense* sequevars were observed and differed by 2 bp (0.3%). Neither sequevar shared 100% identity with the type strain for *M. massiliense*. Four sequevars were observed among the CV isolates. They had an interspecies variability of 1.8% (2–11 bp). The CV sequevars were most closely associated with *M. chelonae* CIP 106684^T at 95.1% (33 bp). Sequevar results are summarized in Table 1 in the online Technical Appendix (www.cdc.gov/EID/content/17/9/101667-Techapp.pdf).

Deep Multilocus Sequencing

Full-length genes were successfully assembled for 43 genes from 5 type strains and 1 representative clinical CV isolate. Gene names and corresponding GenBank accession

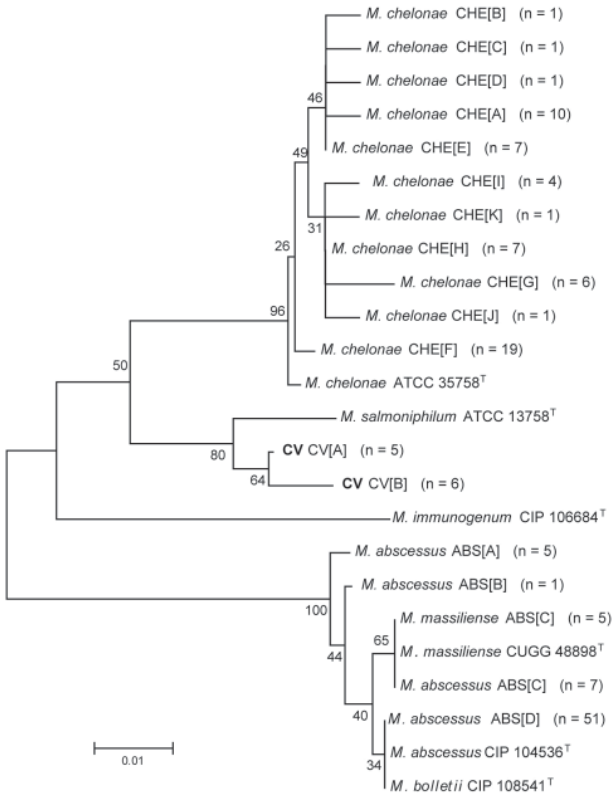

Figure 2. Neighbor-joining tree of 233 bp of unique internal transcribed spacer region sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CV, *M. chelonae* variant; CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden.

numbers are provided in the supplementary tables (online Technical Appendix Table 2). Pair-wise alignments of the DNA and amino acid sequences were performed for each isolate (online Technical Appendix Tables 3, 4). The 43 concatenated genes ranged from 41,580 to 41,619 bp and created an alignment of 41,792 nt including gaps. Comparisons of DNA percent identity ranged from 89.1% (*M. chelonae* to *M. abscessus*) to 98.3% (*M. abscessus* to *M. bolletii*). *M. massiliense*, *M. abscessus*, and *M. bolletii* showed a close association with percent identity range of 98.2%–98.3%. The novel CV isolate shared no greater than 90.5% identity with any type strain. Phylogenetic analysis shows closest relationship between *M. abscessus, M. massiliense*, and *M. bolletii* (Figure 6, panel A). The CV isolate is on a separate branch with nearly identical distances from *M. immunogenum* and *M. chelonae*.

Percent identity of amino acid comparisons ranged from 95.7% (*M. chelonae* to *M. abscessus*) to 99.6% (*M. abscessus* to *M. bolletii*). *M. massiliense*, *M. abscessus*, and *M. bolletii* showed a close association with percent identity range of 99.5%–99.6%. The novel CV isolate shared no greater than 96.1% identity with any type strain. Phylogenetic analysis shows closest relationship between *M. abscessus, M. massiliense*, and *M. bolletii* (Figure 6, panel B).

DNA-DNA Hybridization

DNA-DNA hybridization studies of clinical isolates CV004, CV005, CV006, and CV015 with labeled patient isolate CV002 showed RBRs of 66 to 96% and %D values of 0.0 to 2.0%. The reciprocal DNA-DNA hybridization of patient isolate CV002 with labeled isolate CV005 showed an RBR of 100% and a %D of 0.5%. The DNA-DNA hybridization studies of the phylogenetically (16S rRNA gene) related type strains *M. abscessus*, *M. bolletii*, *M. chelonae*, *M. immunogenum*, *M. massiliense*, and *M. salmoniphilum* performed with the patient isolate CV002 showed RBRs of 15%–69% and %D values of 5.0%–8.0%. DNA-DNA hybridization results are summarized in online Technical Appendix Table 5.

Susceptibility Testing

Susceptibilities by broth microdilution were available for most *M. chelonae, M. abscessus, M. massiliense,* and CV isolates. The MICs for the first 3 taxa were comparable

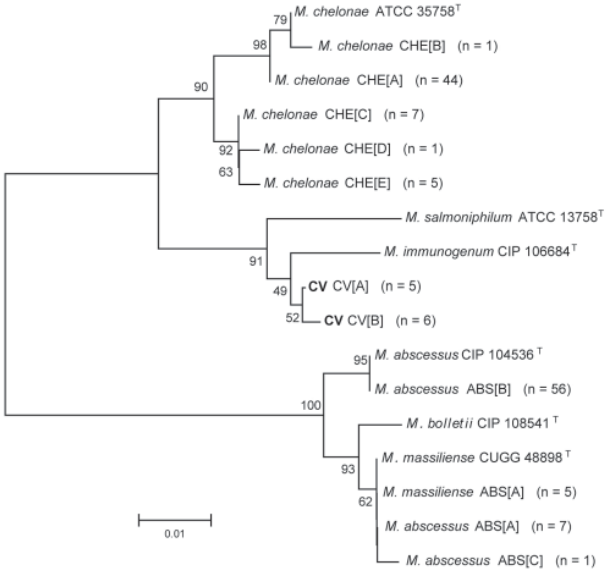

Figure 3. Neighbor-joining tree of a 361-bp region of unique heat-shock protein 65 gene sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CIP, Collection of Institute Pasteur; CV, *M. chelonae* variant; CCUG, Culture Collection, University of Göteborg, Sweden.

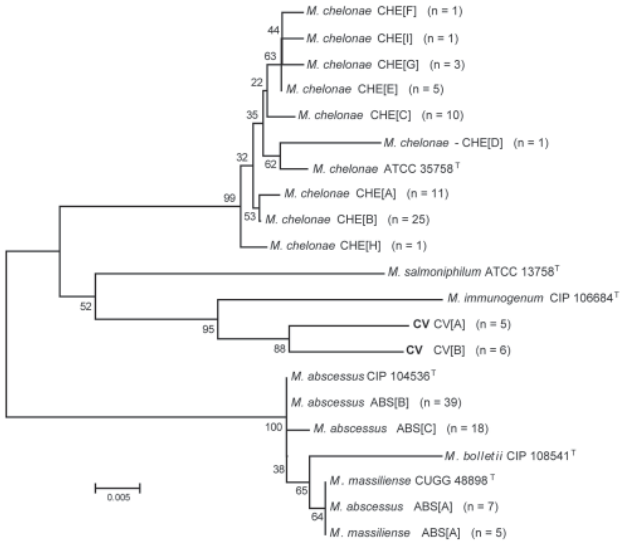

Figure 4. Neighbor-joining tree of a 426-bp region of unique *sodA* gene sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CIP, Collection of Institute Pasteur; CV, *M. chelonae* variant; CCUG, Culture Collection, University of Göteborg, Sweden.

those in to previous reports (*24*). With the exception of 1 isolate, all isolates of *M. chelonae* were cefoxitin resistant (MIC >128 μg/mL), and *M. abscessus* and *M. massiliense* were cefoxitin intermediate (MIC 32–64 μg/mL). All 3 taxa were minocycline resistant. Unlike *M. chelonae*, CV isolates were intermediate (88%) or susceptible (12%) to cefoxitin.

Clinical Spectrum and Characteristics of CV Isolates

The most common source for the CV isolates was respiratory (n = 20) (online Technical Appendix Table 6). The remaining 6 CV isolates had clinical sources that included skin (n = 2), granulomatous liver lesion (n = 1), central line infections (n = 2), and an unspecified body fluid. Most isolates (n = 15) were recovered from patients seen in 4 different hospitals or clinics in Pennsylvania. Eight patients with CV infection were seen at the Hospital of the University of Pennsylvania and 7 of 8 patients were adult females (ages 41–74 years) who acquired the mycobacterial infection as outpatients. Six charts were available for review, and partial information was available for 2 additional patients (patients CV007, CV008, CV010, CV012–CV014, CV034, and CV036).

The medical histories of the 6 patients with complete information fell into 2 groups: those with chronic sinusitis (2 patients) and those with lower respiratory symptoms (4 patients). All patients with lower respiratory symptoms and cultures positive for CV had underlying pulmonary disease (cystic fibrosis, primary ciliary dyskinesia, lung cancer, chronic obstructive pulmonary disease, recurrent pneumonia/bronchiectasis). No patients with lower respiratory symptoms received specific antibiotic therapy aimed at treating rapidly growing mycobacterial infection, although 1 patient received long term antimicrobial drug therapy for concomitant *M. avium* infection. Two patients with sinusitis were not treated with antimicrobial drugs, but both had sinus surgery with symptomatic improvement. One patient had 3 positive sputum cultures for a rapidly growing *Mycobacterium* over a 3-year period, 2 of which were shown by genetic sequencing to be CV organisms (CV014 was selected for further study). No other patient had >1 positive culture for CV, if follow-up cultures were performed. Three patients had sequential or concomitant infections with *M. abscessus, M. avium-intracellulare,* or both bacteria.

The first isolate identified as a CV was discovered in 2005 from a patient in New York. The next isolate was not identified until 2007 from Pennsylvania. Overall, 23 (88%) of 26 were isolated in patients in the northeastern United States, and the remaining 3 isolates were recovered from patients in Minnesota, Oregon, and Colorado.

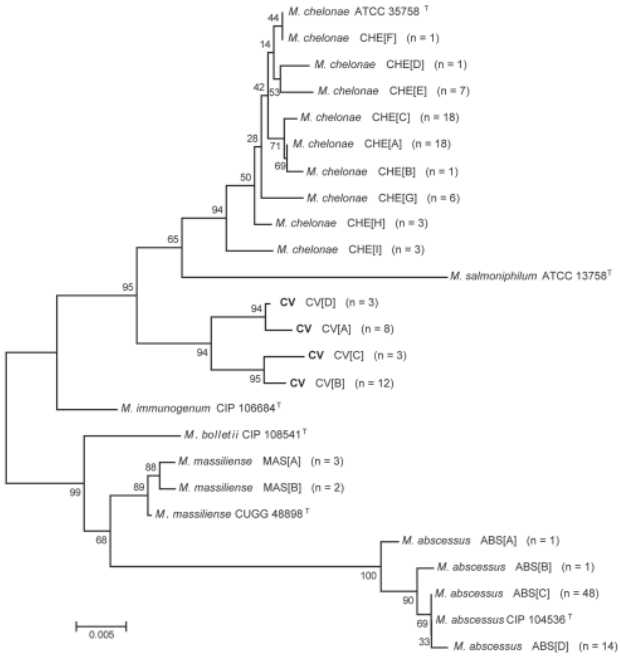

Figure 5. Neighbor-joining tree of a 676-bp region of unique *rpoB* gene sequences of 153 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CV, *M. chelonae* variant; CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden.

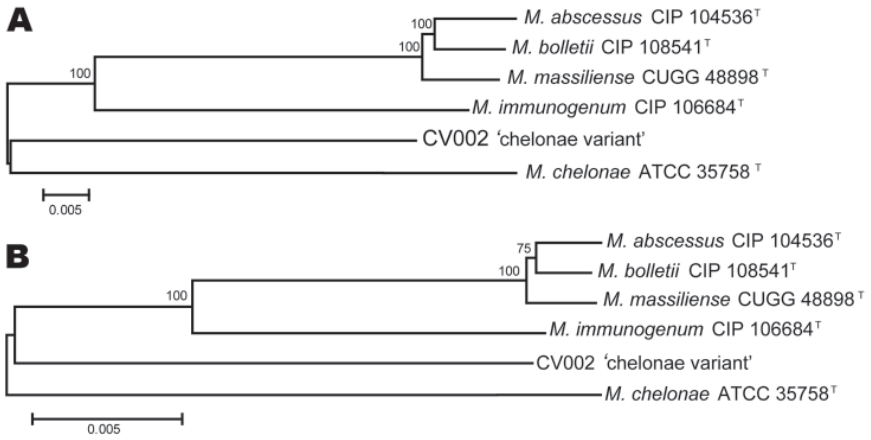

Figure 6. Neighbor-joining tree of DNA (A) and amino acid (B) concatenated gene sequences of *Mycobacterium chelonae* variant (CV) isolates and reference strains of the *M. chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Upper scale bar indicates nucleotide substitutions per site and lower scale bar indicates amino acid substitutions per site. CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden; CV, *M. chelonae* variant; ATCC, American Type Culture Collection.

Discussion

We describe the discovery of a new human pathogen with clinical features similar to *M. abscessus* that is implicated as a cause of infection for patients with chronic lung diseases, intravascular catheters, and chronic sinusitis. On the basis of our investigations, we propose CV isolates become a new member of the *M. chelonae-abscessus* complex and be named *Mycobacterium franklinii* sp. nov. *M. franklinii* (frank li′ ni I, N.L. masc. gen. n. *franklinii* of Franklin, pertaining to Benjamin Franklin, statesman, founder of the University of Pennsylvania, inventor, and scientist who helped create the nation's first public hospital in Philadelphia, Pennsylvania, USA, the origin of the isolates).

The microorganisms are acid-fast, gram-positive bacilli, and colony morphology alone is not sufficient for differentiation from other rapidly growing *Mycobacterium* spp. Colonies are nonpigmented appearing on 5% sheep blood agar, Middlebrook 7H10 agar and egg-based Lowenstein-Jensen slants in 2–5 days at temperatures between 24 and 37°C (optimally at 30°C). Even with molecular techniques, underrecognition of this new pathogen is not surprising because it shares 100% full 16S rRNA gene identity with *M. chelonae* and has sequence variation in the *hsp65* gene that results in inconsistent amplification with typical diagnostic primers for sequence or PCR restriction fragment length polymorphism analysis (*10*,*11*). However, diagnosis of *M. franklinii* infection is essential because it is more susceptible to antimicrobial drugs than other members of the *M. chelonae-abscessus* complex, and its susceptibility pattern with cefoxitin was a distinguishing characteristic leading to its discovery.

Multilocus sequencing on a population of *M. chelonae-abscessus* complex isolates using 5 DNA regions enabled us to examine a population of closely related isolates to accurately assess species variability. *M. franklinii* shared complete 16S rRNA gene sequence identity with the type strain of *M. chelonae*, but was differentiated from *M. chelonae* and other members of the *M. chelonae-abscessus* complex by partial sequencing of *rpoB, hsp65, sodA,* and ITS DNA targets. Concatenated analysis of 43 genes (≈40,000 bp) from deeper sequencing of *M. franklinii* demonstrated that this novel species shares ≤90.5% identity with any other *M. chelonae-abscessus* group member. DNA-DNA hybridization analysis also supports the novel classification with its low relative binding ratios and higher percent divergence from all other *M. chelonae-abscessus* complex type strains. Cefoxitin susceptibility or intermediate susceptibility is another distinguishing feature. Preliminary testing on 6 *M. franklinii* isolates revealed inducible resistance (data not shown) in 50% of the isolates upon prolonged clarithromycin incubation (14 days). This finding suggests that similar to *M. abscessus*, isolates of this species may have an inducible *erm* gene.

The pathophysiology of diseases associated with *M. franklinii* is largely unknown. Most *M. franklinii* isolates were from respiratory sources and from patients with underlying lung conditions. Three of these disorders (cystic fibrosis, primary ciliary dyskinesia, and recurrent pneumonia) had associated bronchiectasis, which is a known risk factor for *M. abscessus* and *M. massiliense* nodular lung disease but not for *M. chelonae* (*6*,*25*). It is unclear whether this microorganism causes respiratory tract disease, or simply colonizes damaged airways and sinuses. Similar to patients with *M. abscessus* and *M. chelonae* infections, we found 2 cases each of sinusitis and catheter-associated infection from *M. franklinii* (*26*). The association with chronic sinusitis presumably relates to sinus washes using tap water rinses in previously diseased sinuses. Although the exact reservoir of *M. franklinii* is unknown, a recent study in the Netherlands by Van Ingen et al. reported 2 isolates from tap and shower water based on *rpoB* gene sequence (*27*), and these 2 isolates shared 99.6% identity to our *M. franklinii* sequevar A *rpoB* gene

sequence. The observation in the Netherlands suggests an environmental source for this organism, and it is likely that the novel species is regionally specific and can survive in municipal water sources. This hypothesis would be supported by the large number of cases in a focused region in Pennsylvania.

Our population analyses of clinical isolates of *M. chelonae* demonstrate a lack of taxonomic clarity. Additionally, our investigations lend further evidence that species distinctions for *M. bolletii* and *M. massiliense* may be inappropriate and support the recent proposal to modify their classifications (*5*,*28*). Taxonomic uncertainty likely arises as our understanding of microbial phylogeny expands with rapid advances in technologies and often results in inconsistent standards being applied for species designations. For example, DNA-DNA hybridization is a relatively standard technique, but upon review of 14 species descriptions in 2009 only 5 were supported by using DNA-DNA hybridization (*29*–*36*).

The discovery of an emerging pathogen should be taken in the context of microbial ecology and evolution, the interaction between host and microbe, and factors of virulence. This investigation underscores the need for accurate identification of *Mycobacterium* spp. for detection of a new pathogen. The interplay between colonization and disease is not clearly defined, but we demonstrate its role in central line infections and for patients with sinopulmonary disease. *M. franklinii* may have newly emerged as a human pathogen over the past 5 years, or it has been involved in human disease previously and was unrecognized. In order to further our understanding of this pathogen and its role in disease, greater surveillance and awareness is necessary. At this time, clinical laboratories can identify *M. franklinii* by sequencing based assays that target either the ITS region (between 16S and 23S rRNA genes), *hsp65*, *rpoB*, and *sodA* genes, or by complete 16S rRNA gene sequence analysis in conjunction with cefoxitin and minocycline susceptibility patterns. The type strain, CV002 (ATCC [pending] and DSMZ 45524), was isolated from a skin lesion.

Acknowledgments

We dedicate this manuscript to medical technologists whose astute observations often serve as catalysts for our work. In particular, we thank Linda Bridge Mann, Rebecca Wilson, Ravikan Vasireddy, Steven McNulty, and Maria McGlasson and members of the Molecular Pathology and Clinical Microbiology Laboratories at the Hospital of the University of Pennsylvania and Associated Regional and University Pathologists Laboratories. Additionally, we thank Paul Bourbeau, Karen Brady, Christine Turenne, and Hans Truper for their assistance and helpful discussions.

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High Rates of Malaria among US Military Members Born in Malaria-Endemic Countries, 2002–2010

Ellen R. Wertheimer, John F. Brundage, and Mark M. Fukuda

To estimate malaria rates in association with birth country, we analyzed routine surveillance data for US military members. During 2002–2010, rates were 44× higher for those born in western Africa than for those born in the United States. Loss of natural immunity renders persons susceptible when visiting birth countries. Pretravel chemoprophylaxis should be emphasized.

Military members are at risk for malaria during assignments, deployments, and personal travel in malaria-endemic countries. They account for 5%–10% of all malaria cases reported in the United States. Immigrants from malaria-endemic countries are at risk for malaria when they visit their birth country (*1*). Visits to friends and relatives in malaria-endemic countries account for ≈50% of all malaria cases diagnosed in the United States.

Legal immigrants to the United States are eligible for military service, and many choose to serve. Malaria among US military members is tracked for health surveillance purposes by electronic records of medical encounters and notifiable medical events. These records are routinely transmitted to the Armed Forces Health Surveillance Center and integrated into the Defense Medical Surveillance System. To estimate rates of malaria in association with the birth countries of members of active components of the US military, we used these routine surveillance data for 2002–2010.

The Study

For this report, a malaria case-patient was defined as a person hospitalized with a diagnosis of malaria (code 084 in International Classification of Diseases, 9th Revision, Clinical Modification) or reported as having a case of malaria through a military notifiable medical event reporting system. Persons could be considered malaria case-patients >1 time during the surveillance period but only 1 time during any 365-day period.

To determine locations where malaria infections were acquired, we used an algorithm that considered locations of malaria-related hospitalizations, travel histories reported on notifiable medical event records, and military assignment and overseas deployment records. The methods used by the Armed Forces Health Surveillance Center to identify malaria cases and their presumed locations of acquisition have been described in detail (*2*).

Countries of birth were self-reported during personnel security investigations (Entrance National Agency Checks) conducted among applicants for US military service. Rates of malaria by birth country were expressed as the number of malaria cases among all service members born in each country of interest per 10,000 person-years of active military service of all military members who were born in the respective countries.

During 2002–2010, a total of 835 malaria cases were reported among active military members; 5 persons were affected on 2 occasions at least 365 days apart. Compared with the overall composition of the US military, the proportions of military members with malaria were overrepresented by men (95%) and those 18–34 years of age (87%). Of all cases reported during the study period, 41%, 20%, and 3% were caused by *Plasmodium vivax*, *P. falciparum*, and other *Plasmodium* spp., respectively; *Plasmodium* spp. was not determined or reported for the other cases. The proportions of infections presumably acquired in Afghanistan, South Korea, and Africa were 42%, 21%, and 20%, respectively (data not shown).

Among military members affected by malaria during the study period, 624 (74.7%) were born in the United States, 107 (12.8%) were born in malaria-endemic countries other than Mexico, and 33 (4.0%) were born in countries where malaria is not endemic or in Mexico. The birth countries of the other 71 (8.5%) were not documented in available records. The most frequent known birth countries of case-patients were the United States, Nigeria (n = 24), and Ghana (n = 21). All other birth countries were represented by <8 cases each.

The highest malaria rates were among those born in Côte d'Ivoire (54.4/10,000 person-years), Togo (39.5/10,000 person-years), Cameroon (37.6/10,000 person-years), and Ghana (36.0/10,000 person-years). Of the 15 birth countries represented by at least 4 cases each, the 7 for which rates were highest were in western Africa. The malaria rate was 44× higher among service members born in 1 of the 7 western Africa countries (30.5/10,000 person-years) than among those born in the United States (0.70/10,000 person-years) (Figure).

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DOI: http://dx.doi.org/10.3201/eid1709.110318

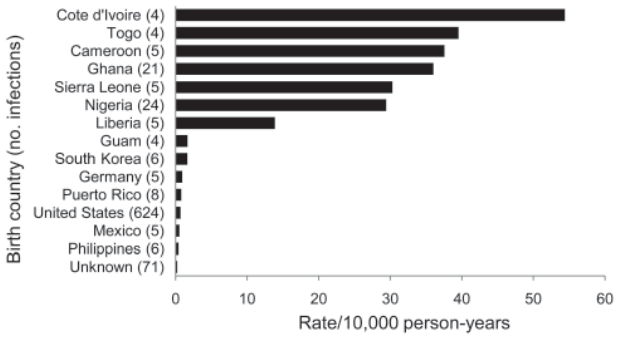

Figure. Rates of malaria infections (per 10,000 person-years of military service) by birth country, among birth countries represented by ≥4 malaria cases, active component military members, US Armed Forces, 2002–2010.

Among the 69 persons with malaria who were born in western Africa, 35 (50.7%) probably acquired the infection in their birth countries. In contrast, among the 38 malaria-infected persons born elsewhere, only 8 (21%) probably acquired the infection in their birth countries (Table).

Among the same 69 persons with malaria who were born in western Africa, the location of malaria acquisition was unknown for 27 (39%). Because these persons had no records of military assignments in or deployments to malaria-endemic areas, their infections were probably acquired during personal travel. If so, as many as 62 (90%) of these infections may have been acquired during visits to birth countries; in contrast, only ≈26% of infections of military members born in other malaria-endemic countries were potentially acquired in the respective birth countries.

Conclusions

Among US military members, malaria rates are sharply higher among those born in western Africa than in other countries. Most malaria infections of those born in western Africa were probably acquired during visits to their birth countries.

The finding that immigrants to the United States have relatively high risk for malaria when they return to their malaria-endemic birth countries is not surprising (*3*–*5*). However, the finding that malaria rates among military members were 44× greater for those born in 7 western Africa countries than for those born in the United States requires attention.

Our findings should be interpreted with consideration of limitations. For example, cases were identified from diagnoses reported on administrative records of hospitalizations in US military and civilian (i.e., purchased care) medical facilities and from reports of notifiable medical events. Records of hospitalizations in deployed medical facilities (e.g., hospitals in the field, at sea) were not available; also, malaria diagnoses reported only on outpatient records were not considered cases. In turn, the cases enumerated here may underestimate the actual malaria infections that affected US military members during the study period. In addition, for 71 (8.5%) of the 835 military members with malaria, birth places could not be ascertained from records maintained for health surveillance purposes. The missing data may bias estimates of malaria rates in association with, as well as comparisons of rates across, birth countries. Finally, rate denominators (person-years of military service) do not account for the varying times that a person is at risk for malaria infection.

Before deploying to malaria-endemic areas, US military members are informed of the risks for and countermeasures against malaria (e.g., permethrin-impregnated uniforms and bed nets, DEET [N,N-diethyl-meta-toluamide]–containing mosquito repellent, chemoprophylactic drugs). Compliance with indicated countermeasures is mandatory, and enforcement is ensured by military supervisors. In contrast, before personal travel to these areas, counseling regarding malaria prevention may not be readily available or routinely accessed, and use of countermeasures is not enforced.

For persons residing in malaria-endemic countries, partial immunity develops in response to repeated exposures to malaria parasites. These persons may not be accustomed to or feel the need for chemoprophylactic drugs. However, after leaving their countries of origin, this acquired immunity wanes, leaving them susceptible to clinically significant malaria infection during subsequent visits to their birth countries. Because immigrants from malaria-endemic countries are at risk for malaria upon return to their birth countries, pretravel counseling of immigrants should emphasize the need for personal protective measures and encourage compliance with chemoprophylactic regimens before, during, and after the visits.

Table. Numbers of malaria infections among 107 active component military members born in malaria-endemic countries, US Armed Forces, 2002–2010

	Presumed location of malaria acquisition, no. infections		
Birthplace	Birth country	Unknown	Other than birth country
Western Africa	35	27	7
Other malaria-endemic locations	8	2	28

Acknowledgment

We thank Stephen B. Taubman for his expert data analysis.

Ms Wertheimer is a surveillance epidemiologist and an editor of the Medical Surveillance Monthly Report, published by the Armed Forces Health Surveillance Center.

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Differential Risk for Lyme Disease along Hiking Trail, Germany

Dania Richter and Franz-Rainer Matuschka

To estimate relative risk for exposure to ticks infected with Lyme disease–causing spirochetes in different land-use types along a trail in Germany, we compared tick density and spirochete prevalence on ruminant pasture with that on meadow and fallow land. Risk was significantly lower on pasture than on meadow and fallow land.

In contrast to competent hosts that serve as reservoirs for Lyme disease–causing spirochetes (hereafter called Lyme disease spirochetes), ruminants exert a zooprophylactic effect on *Borrelia burgdorferi* s.l. (*1*). Fewer ticks questing on a cattle pasture harbor this pathogen than those questing elsewhere (*2*). Virtually no ticks acquire spirochetes when feeding on wild ruminants (*3*). While feeding on cattle or goats, an infected tick loses its Lyme disease spirochetes (*4*). Although wild ruminants are considered the major host of adult ticks (5,*6*), in the Netherlands fewer subadult ticks quested on a cattle pasture than on ungrazed sites (*7*).

It seems paradoxical that the presence of domestic ruminants reduces the density of host-seeking ticks, whereas wild ruminants appear to maintain tick populations. To determine whether ruminants used for extensive landscape management affect the exposure risk for Lyme disease for hikers, we compared tick density and spirochete prevalence along the waysides of a trail crossing a goat-and-cattle pasture, meadow, and abandoned fallow land.

The Study

Our study site was ≈30 km west of Rothenburg-ob-der-Tauber in southern Germany. This western-facing hillside in the Jagst Valley, with an incline of 20°–40°, was originally used as vineyard, marginal fields, and grassland. After being abandoned in the late 1960s, the site was converted into 2.16-ha pasture for Limpurger cattle in 1995. Since 2003, ≈30 Bündner and Toggenburger goats have grazed with the cattle during May, September, and October. The site is enclosed by an electric fence and is characterized by a dearth of brushy vegetation. A cultural heritage hiking trail crosses the pasture and continues through former grassland that had been left to natural succession 30 years ago. A bordering meadow is mowed each July. The waysides of the trail cutting through the meadow and fallow land served as comparison with the pasture.

Questing ticks were collected by dragging a flannel flag over the ground vegetation monthly during May–October 2006 and March–October 2007. Ticks were collected per unit time by recording the period of active flagging, identified to stage and species, and preserved in 80% ethanol. To detect and identify any spirochetes infecting these ticks, we isolated DNA and amplified and sequenced a 16S rDNA fragment (*2,8*). For differentiation of *B. bavariensis* (spec. nov. cand. [*9*]) from *B. garinii,* any DNA sample amplifying *B. garinii* was included in an outer surface protein A PCR and sequenced (*10*).

To estimate tick density, we collected questing ticks and extrapolated the number per hour of flagging. *Ixodes ricinus* was the sole tick species collected. On the pasture, questing ticks were least abundant ($p<0.0001$, Friedman test; Table 1); 12 nymphs and 2 females attached to the flag within 1 hour. On the meadow, 7× times as many questing nymphs and 5× as many females were collected in 1 hour; on the fallow land, 12× more ticks were collected. Ticks were most abundant on vegetation not modified by mowing or pasturing.

We determined the prevalence of spirochetes in questing nymphs and adults. Whereas only 7% of nymphs questing on pasture harbored spirochetes, 27% and 23% nymphs questing on the meadow and fallow land, respectively, contained these pathogens ($p<0.0005$, χ^2 for independence; Table 2). Similarly, spirochetes were less prevalent in adult ticks questing on the pasture (6%) than on the fallow land (23%) ($p<0.05$, Fisher exact test). The presence of goats and cattle seems to significantly reduce spirochete prevalence in questing ticks.

We examined the spirochete genospecies infecting the ticks. On the pasture, the non–Lyme disease spirochete *B. miyamotoi* infected more nymphs than did each of the Lyme disease genospecies (Table 2). On the meadow and fallow land, *B. afzelii* was most prevalent. *B. spielmanii, B. garinii,* and *B. valaisiana* infected ticks at each site, whereas only *B. burgdorferi* s.s. and *B. lusitaniae* infected ticks solely on the meadow and fallow land. Ticks infected with *B. bavariensis* were collected mainly from the meadow. The prevalence of particular genospecies seems to vary with the type of landscape management.

We compared the relative risk for exposure to ticks infected with pathogenic Lyme disease spirochetes. Nymphs infected with *B. afzelii, B. garinii, B. bavariensis, B. burgdorferi* s.s., and *B. spielmanii* were significantly less prevalent on the pasture than on the meadow or fallow land ($p<0.0001$, Fisher exact test; Table 1). To estimate a person's risk for exposure while walking through each of the sites for

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DOI: http://dx.doi.org/10.3201/eid1709.101523

Table 1. Density of *Ixodes ricinus* ticks, prevalence of pathogenic Lyme disease spirochetes, and theoretic risk for exposure to such infected ticks along a trail, southern Germany, May–October 2006 and March–October 2007

Study site and tick stage	Mean no. ticks collected in 1 hour*	No. ticks examined	% Ticks infected with pathogenic spirochetes	Person's risk for exposure in 1 hour†
Pasture				
Nymph	11.8	104	3.8‡	0.45
Adult	2.3	33	3.0§	0.07
Meadow				
Nymph	87.2	188	21.8	19.01
Adult	10.4	58	17.2	1.79
Fallow land				
Nymph	129.3	502	17.5	22.63
Adult	38.0	291	13.7	5.21

*Nymph or female.
†Theoretic risk for exposure, mean number of questing nymphs or female ticks infected by pathogenic genospecies of spirochetes that cause Lyme disease: *Borrelia afzelii, B. garinii, B. bavariensis, B. burgdorferi s.s., B. spielmanii.*
‡Significantly smaller (p<0.0001 by Fisher exact test).
§Comparison lacks statistical meaning because only 1 adult tick infected with pathogenic spirochetes was collected.

1 hour, we multiplied the number of ticks collected within 1 hour with the rate of infection by pathogenic spirochetes. A person would have to walk through pasture for >2 hours or >14 hours to encounter a nymph or a female, respectively, infected by pathogenic spirochetes. On the meadow land, however, a person would be exposed within 1 hour to as many as 19 such infected nymphs and ≈2 females. Walking fallow land for 1 hour, a person would be exposed to >22 such infected nymphs and 5 females. Thus, risk for exposure to a questing tick infected by pathogenic spirochetes on the pasture is 40-fold and 54-fold smaller than on the meadow and fallow land, respectively.

Conclusions

The prevalence of spirochetes in ticks questing along a hiking trail crossing a cattle-and-goat pasture, a meadow, and fallow land in southern Germany differs markedly. Where no domestic ruminants graze, ≈3.5× more ticks harbor spirochetes than on the pasture. In a previous study in the French Vosges, where cattle graze year-round on a floodplain, the zooprophylactic effect of cattle reduced prevalence of spirochetes in ticks as much as 6-fold (*2*). Not only do larvae fail to acquire spirochetes when feeding on ruminants (*3,11,12*), but previously infected ticks lose their Lyme disease spirochetes (*4*). All Lyme disease genospecies seem to be affected similarly by a tick's blood meal on a ruminant. The prevalence of *B. miyamotoi*, however, remains unchanged independent of the presence of ruminants or mowing. With a frequency of up to 2.9% in ticks questing along the hiking trail studied, *B. miyamotoi* was as prevalent along this trail as in other sites in France, northern Germany, or southern Germany (*4,8,13*). Although domestic ruminants differentially affect these 2 kinds of spirochetes, only few ticks questing on the pasture harbor Lyme disease spirochetes.

Where domestic ruminants browse extensively, they modify the habitat. They keep the vegetation low, presumably generating a somewhat drier, less suitable microclimate for host-seeking ticks. The open habitat structure of a pasture probably displaces potential reservoir hosts by increasing their risk for predation and decreasing the quality and quantity of food (*14,15*). Thus, the remaining ticks on a pasture may be more likely to feed on reservoir-incompetent ruminants than on competent reservoir hosts.

Table 2. Prevalence of *Borrelia* genospecies in questing nymphal and adult *Ixodes ricinus* ticks sampled at different sites along a trail, southern Germany, May–October 2006 and March–October 2007

Study site and tick stage	No. ticks examined	% Ticks infected	% Ticks harboring *Borrelia* spp.*									
			afz	*gar*	*val*	*bur*	*lus*	*spi*	*bav*	*bis*	*miy*	>1 genospecies
Pasture												
Nymph	104	6.7	1.9	1.0	0	0	0	1.0	0	0	2.9	0
Adult	33	6.1	3.0	0	3.0	0	0	0	0	0	0	0
Meadow												
Nymph	188	26.6	17.6	2.7	0.5	0.5	2.7	1.1	0.5	0.5	1.1	0.5†
Adult	58	17.2	10.3	3.4	0	1.7	0	0	3.4	0	0	1.7†
Fallow land												
Nymph	502	22.9	11.0	5.2	4.8	0.4	0.2	1.0	0.4	0	1.6	1.6‡
Adult	291	23.4	6.9	4.5	6.9	2.7	0	0.3	0	0	2.4	0.3†

**afz, afzelii; gar, garinii; val, valaisiana; bur, burgdorferi; lus, lusitaniae; spi, spielmanii; bav, bavariensis* (*9*); *bis, bissettii*-like, *miy,miyamotoi.*
†Co-infection of *B. afzelii* and *B. burgdorferi* s.s.
‡Co-infection of *B. garinii* and *B. valaisiana* in 4 nymphs, *B. afzelii* and *B. spielmanii* in 2 nymphs, *B. valaisiana* and *B. lusitaniae* in 1 nymph, *B. spielmanii* and *B. miyamotoi* in 1 nymph.

Fewer ticks appear to feed on rodents captured on pasture land than on those captured on woodland or grassland (*15*). This indirect effect of extensive grazing on rodent populations may amplify the zooprophylactic effect of ruminants.

Along this hiking trail, risk for exposure to ticks infected with Lyme disease spirochetes varied considerably with landscape structures. Our observations suggest that hikers are up to 54× less likely to contact a tick infected with pathogenic Lyme disease spirochetes when walking across a cattle-and-goat pasture than when walking along an abandoned area. The presence of zooprophylactic goats and cattle resulted in fewer ticks and a diminished rate of infected ticks within this local population. Extensive landscape management that uses domestic ruminants not only serves to maintain cultural and natural heritage in Germany but also seems to confer a health benefit for hikers and others seeking recreation.

Acknowledgments

We thank Michael Buß for his advice on extensive pasture sites appropriate for our study and Udo Bischoff and Mandy Pötter for expert technical assistance.

This study was funded by the Baden-Württemberg Stiftung.

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Bartonella quintana Infections in Captive Monkeys, China

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Bartonella quintana has been considered to be specifically adapted to humans. Our isolation of the organism from 2 of 36 captive rhesus macaques in China and finding antibodies against *B. quintana* in 12 of 33 indicates that the reservoir hosts of *B. quintana* may include primates other than humans.

Bartonella spp. are bacterial hemoparasites with a wide variety of mammalian hosts as reservoirs. Several members of the genus are pathogens of medical and veterinary significance. Most human infections are zoonoses; however, 2 *Bartonella* spp., *B. bacilliformis* and *B. quintana*, are considered to be specifically adapted to humans (*1*). Although, like other members of the genus, both species generally cause chronic intraerythrocytic bacteremia of little clinical consequence to their reservoir hosts, both are also associated with illness and death (*1*).

B. quintana infections most frequently cause recurrent fever and pretibial pain (trench fever), endocarditis, and bacillary angiomatosis (*2*). *B. quintana* is transmitted by the human body louse (*Pediculus humanis humanis*), which thrives in the absence of basic sanitation and hygiene; hence, infections are most frequently associated with persons who are homeless or affected by social or civil unrest (*3*), although they are increasingly encountered in rural communities in developing regions of the world (*4*,*5*).

Some reports suggest that humans are not the only reservoir hosts of *B. quintana.* In 2005, *B. quintana* was recovered from the blood of a cynomolgus monkey (*Macaca fascicularis*) imported into the United States from Southeast Asia (*6*). In addition, infections resulting in chronic bacteremia have been experimentally established in rhesus macaque monkeys (*Macaca mulatta*) inoculated with *B. quintana* isolates from infected humans (*7*,*8*).

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DOI: http://dx.doi.org/10.3201/eid1709.110133

The Study

In 2009, blood samples were collected from 36 apparently healthy rhesus macaque monkeys housed in a biologic research institute in Beijing, People's Republic of China. Breeding, delivery, and raising the young monkeys took place in an outdoor arena that housed a large group of animals; the monkeys were later moved indoors into isolation. Samples were stored at −80°C, thawed, and then plated (400 μL of each) onto 5% (vol/vol) sheep blood–enriched Columbia agar and incubated at 37°C in 5% CO_2 for as long as 45 days. After 15 days of incubation, 2 plates yielded putative *Bartonella* spp. colonies. One plate, inoculated with blood from a 3-year-old female monkey (S13), yielded 13 colonies; the other, inoculated with blood from a 2-year-old male monkey (M22), yielded 2 colonies. Microscopic examination of Gram-stained smears of these colonies revealed small, pleomorphic, gram-negative bacteria; their identity was confirmed by using molecular methods.

DNA extracts were prepared from colonies by using a QIAamp tissue kit (QIAGEN, Hilden, Germany) and incorporated into *Bartonella* genus–specific PCRs selective for fragments of the 16S rRNA–encoding gene, the 16S–23S rDNA intergenic spacer region (ISR), *gltA, ribC*, and *ftsZ* (*9*–*12*). Extraction controls (water only) were concurrently prepared with DNA extracts and incorporated into each PCR along with a reagent-only (no DNA) negative control and a reaction-positive control (*B. henselae* DNA). In all assays, DNA extracts and positive controls yielded amplification products, whereas extraction and reagent-only controls did not.

The nucleotide base sequences of all amplification products were determined. Analysis of sequence data indicated that the 2 isolates possessed indistinguishable *gltA*, 16S rDNA, and ISR sequences but that their *ribC* and *ftsZ* sequences differed from each other by 1 bp mutation. Comparison of these data with those available for validated *Bartonella* spp. indicated that the sequences of isolates S13 and M22 were most similar to those of *B. quintana* (*ribC* 97%, *gltA* 98%, ISR 98%, *ftsZ* 99%, and 16S rDNA 100% similarity). These similarity values are higher than those previously proposed as thresholds for delineation of *Bartonella* spp. (*13*), suggesting that the isolates S13 and M22 were strains of *B. quintana*.

We inferred the phylogenetic positions of the 2 isolates within the genus from alignment of concatenated sequence data (all 5 loci). In this inference, isolates S13 and M22 grouped together and formed a well-supported tight cluster with *B. quintana* and no other *Bartonella* spp. (Figure). The phylogenetic distances between *B. quintana*, isolate S13, and isolate M22 were much shorter than those between,

[1]These authors contributed equally to this article.

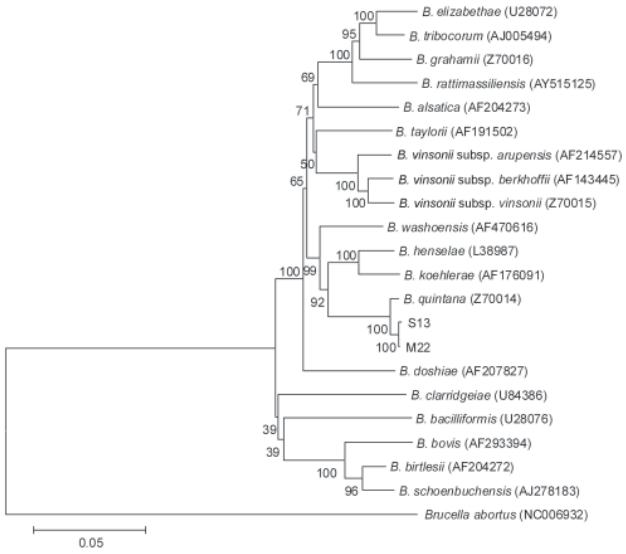

Figure. Phylogenetic dendrogram of *Bartonella* spp. inferred from alignment of concatenated sequence data (4,007 bp) by using a maximum-likelihood algorithm within the MEGA4 suite (www.megasoftware.net). The strength of proposed branching orders was tested by bootstrapping (1,000 replicates), and the percentage of samples supporting the proposed branching order are indicated at each node. Sequence data from the 5 loci studied for isolates S13 (from captive 3-year-old female rhesus macaque monkey, China) and M22 (from captive 2-year-old male rhesus macaque monkey, China) were submitted to GenBank under accession nos. HQ014621 (S13 16S rDNA), HQ014622 (S13 ISR), HQ014623 (S13 *ftsZ*), HQ014624 (S13 *gltA*), HQ014625 (S13 *ribC*), HQ014628 (M22 16S rDNA), HQ014629 (M22 ISR), HQ014630 (M22 *ftsZ*), HQ014627 (M22 *gltA*), and HQ014626 (M22 *ribC*). Scale bar indicates nucleotide substitutions per site.

for example, *Bartonella vinsonii* subsp., supporting the classification of isolates S13 and M22 as strains of *B. quintana*.

Subsequently, by using a commercial indirect immunofluorescence antibody test kit (Euroimmun, Lubeck, Germany), we found antibodies against *B. quintana* in 33 monkey serum samples. Serum from 12 monkeys yielded a high titer (≥320). Serum was available from only 1 of the culture-positive animals, M22, and this sample had a positive titer of 320. Overall, we found *B. quintana* seroprevalence to be significantly lower in sexually mature monkeys ($\chi^2 = 6.034$, $p = 0.014$), but we found no significant correlation between seroprevalence and age or gender. We obtained the *B. quintana* isolates ≈6 weeks after blood collection and immediately attempted to resample the 2 infected monkeys. We were able to obtain blood from monkey M22 only; this sample did not yield further isolates. However, the animal remained seropositive, with an anti–*B. quintana* titer of 320.

Conclusions

What is particularly intriguing about the recovery of *B. quintana* from the blood of 2 apparently healthy rhesus macaques is that the monkeys were not members of a natural wild population; rather, they had been bred in captivity in suburban Beijing and held in enclosures apart from other animals. This recovery suggests that *B. quintana* was being maintained in the colony or that monkeys acquired infection from the only other animals they had contact with: humans. High *B. quintana* seroprevalence in the colony, higher for immature than older animals, suggests that other monkeys had also been exposed to the bacterium, although the true meaning of these findings is unclear, particularly because the specificity of the immunoassay in monkeys in unknown.

Examination of the monkeys from which blood was collected failed to reveal any ectoparasites, and officials at the animal facility reported never seeing ectoparasites on the monkeys. Thus, we are no closer to identifying which species of arthropod, if any, serves as a vector for the monkey-associated *B. quintana* infection.

Although isolates S13 and M22 should best be considered strains of *B. quintana*, they are, nonetheless, apparent outliers within the currently recognized diversity of the species. Previous work surveying genetic diversity among 16 human-associated *B. quintana* isolates of diverse provenance (*14*) encountered no *gltA* sequence dissimilarity and only 0.2% *ftsZ* sequence dissimilarity, whereas isolates S13 and M22 displayed >1% dissimilarity with the *B. quintana* type strain at both loci. This level of sequence dissimilarity is similar to that reported between the *B. quintana* type strain and the isolate from a cynomolgus monkey (*6*). Unfortunately, because those data were not deposited in GenBank, we were unable to directly compare our sequence data with those previously reported for this *B. quintana* strain. Such comparisons might indicate the existence of nonhuman primate–adapted genotypes within the species.

Acknowledgments

We thank Fengxia Meng, Liang Lu, Haixia Wu, and Jimin Sun for discussing the results and Dongsheng Ren and Huailei Ma for helping with sampling.

This work was supported by Major National Science and Technology Projects of China grant 2008ZA10004-010 and National Natural Science Foundation of China grant 30371246.

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Canine Serology as Adjunct to Human Lyme Disease Surveillance

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To better define areas of human Lyme disease risk, we compared US surveillance data with published data on the seroprevalence of *Borrelia burgdorferi* antibodies among domestic dogs. Canine seroprevalence >5% was a sensitive but nonspecific marker of human risk, whereas seroprevalence ≤1% was associated with minimal risk for human infection.

Lyme disease is caused by *Borrelia burgdorferi* and transmitted in North America by *Ixodes* spp. ticks. Routine surveillance for human illness indicates that risk for infection within the United States is highly localized. Residents of 10 states accounted for >93% of the ≈248,000 cases reported to the Centers for Disease Control and Prevention (CDC) during 1992–2006 (*1*). Annual county-level incidence ranged from 0 to >1,000 cases per 100,000 population (*1*).

Accurate information about risk is necessary for targeting and motivating Lyme disease prevention efforts (*2*). In addition, health care providers require knowledge of local disease risk to properly interpret clinical and laboratory findings (*3*,*4*). Although risk often can be inferred from surveillance data, reporting practices are subject to bias. Independent measures of disease risk are therefore valuable for validating surveillance findings.

Like humans, domestic dogs are susceptible to opportunistic infection with *B. burgdorferi*. These infections are often subclinical and pose no risk for direct transmission to humans. Nevertheless, they elicit a robust antibody response. Given the greater proclivity of dogs for tick exposure, canine seroprevalence has been proposed as a sensitive and independent measure of human Lyme disease risk (*5*–*7*). We compared US national surveillance data on Lyme disease with recently published data on *B. burgdorferi* antibody seroprevalence in dogs (*8*) to determine the degree of concordance between these 2 measures of Lyme disease risk and to assess the potential for canine seroprevalence to predict areas of Lyme disease emergence among humans.

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DOI: http://dx.doi.org/10.3201/1709.110210

The Study

State and territorial health departments report Lyme disease cases to CDC as part of the National Notifiable Diseases Surveillance System (*1*). Data on canine seroprevalence of *B. burgdorferi* antibodies were obtained from a 2009 publication by Bowman et al. that reported results for 982,336 dogs tested throughout the United States by using a commercial C6-based assay during 2001–2006 (*8*). We obtained state-specific seroprevalence from Table 1 of this publication and county-specific seroprevalence as categorical values (0%, 0.1%–0.5%, 0.51%–1%, 1.1%–5%, ≥5.1%) from Figure 2 of this publication after digital enlargement. We excluded counties too small for the value to be determined reliably. We calculated average annual human Lyme disease incidence for 2001–2006 and 2007–2009 using US Census Bureau population estimates for 2004 and 2008, respectively. To evaluate county-level emergence of Lyme disease among humans, we stratified counties by the mean observed annual incidence for all counties during 2001–2006 of 4.7 cases per 100,000 population. We defined an emergent county as a county in which incidence was below this value during 2001–2006 and above this value during 2007–2009.

Detailed canine seroprevalence data were available for 46 US states. In linear regression analysis, state canine seroprevalence and human Lyme disease incidence were positively correlated (Figure 1; r^2 0.75, $p<0.001$). On the basis of this relationship, human Lyme disease incidence was effectively zero when the canine seroprevalence was ≤1.3%. States generally fell into 2 distinct categories according to canine seroprevalence (Figure 1). Median Lyme disease incidence was uniformly low (median 0.3 cases/100,000 population) and not correlated with canine seroprevalence (r^2 0.0, $p>0.4$) among 32 states with canine seroprevalence ≤5%. Among 14 states with canine seroprevalence >5%, median annual human Lyme disease incidence was ≈100-

Table. Counties meeting criteria for emergence of human Lyme disease during 2007–2009, by canine seroprevalence of *Borrelia burgdorferi* antibodies during 2001–2006, 46 US states*

Canine seroprevalence, 2001–2006, %†	No. low-incidence counties,‡ 2001–2006	No. (%) emergent counties,§ 2007–2009¶
Unknown	2,065	96 (4.5)
0	240	1 (0.4)
0.1–0.5	174	1 (0.6)
0.51–1.0	101	4 (4.0)
1.1–5.0	122	33 (27.0)
≥5.1	32	18 (56.3)

*All states except Alaska, Hawaii, Montana, and Nevada.
†Data from Figure 2 in Bowman et al. (*8*).
‡Counties with below average incidence of human Lyme disease (≤4.7 cases/100,000 population).
§Counties with below average incidence in 2001–2006 and above in 2007–2009.
¶χ^2 for trend 135.9, $p<0.0001$, for counties with known canine seroprevalence.

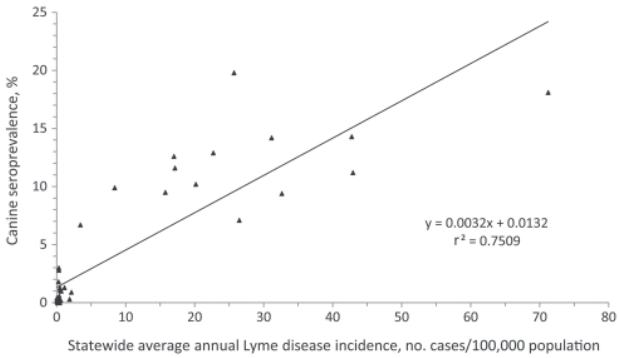

Figure 1. *Borrelia burgdorferi* antibody seroprevalence in dogs and reported Lyme disease incidence in humans, counties in 46 US states, 2001–2006.

fold higher (24.1 cases/100,000 population) and positively correlated with canine seroprevalence (r^2 0.33, p = 0.03).

Categorical canine serologic data were available for 866 (28%) of 3,141 counties in the 46 states (*8*). Median population in 2004 was 85,699 for counties for which data were available, compared with 25,505 for all counties in the 46 states. As in the state-level analysis, human incidence and canine seroprevalence were positively associated at the county level. Median annual reported Lyme disease incidence for humans was 0.2 per 100,000 population in counties with canine seroprevalence ≤1%, 1.4 in counties with canine seroprevalence 1.1%–5%, and 25.9 in counties with canine seroprevalence >5% (p<0.001; Figure 2). Five (1%) of 520 counties with canine seroprevalence ≤1% had rates of human illness above the overall county mean of 4.7 cases per 100,000 population annually, compared with 171 (85%) of 201 counties with canine seroprevalence >5%.

Overall, 153 (5%) of 2,830 counties with average annual human incidence ≤4.7 per 100,000 population during 2001–2006 met the criteria for emergence during 2007–2009. Emergence was more common in counties with higher canine seroprevalence (Table). Eighteen (56%) of 32 counties with canine seroprevalence >5% met the criteria for emergence, compared with 6 (1%) of 519 counties with seropositivity ≤1% (p<0.001). Among the 32 counties with canine seroprevalence >5%, a total of 12 (67%) of the 18 counties with emergent Lyme disease were immediately adjacent to a county with seroprevalence >5%, compared with 4 (29%) of the 14 counties with nonemergent Lyme disease.

Conclusions

Our results confirm an overall correlation between canine seroprevalence and reported human incidence of Lyme disease as measured through national surveillance. Canine seroprevalence ≤1% is associated with extremely low rates of human illness in both state- and county-level analyses. Because human cases are reported according to county of residence rather than county of exposure, infections acquired during travel will occasionally be reported from areas without local transmission. Similarly, low levels of canine seropositivity are expected on the basis of the specificity of assay (up to 2% false positivity [*9*]), data from field surveys (*7*,*10*), and relocation of dogs from areas of high endemicity (*8*). Low levels of canine seroprevalence or human incidence should not be misinterpreted as confirmation of local transmission of *B. burgdorferi*. Conversely, the overall agreement between human and canine data support the conclusion that risk for *B. burgdorferi* infection is generally low to nonexistent outside the highly Lyme disease–endemic areas of the Northeast, mid-Atlantic, and upper Midwest.

At the other end of the spectrum, canine seroprevalence >5% was invariably associated with above average Lyme disease incidence in state-level analyses. In county-level analyses, the situation was more nuanced. Although 85% of counties with canine seroprevalence >5% also had above average Lyme disease incidence, 15% did not. In more than half of these counties, incidence increased to above average rates in the following 3 years, suggesting some predictive potential for high canine seroprevalence, especially in counties geographically clustered with other high seroprevalence counties. In other counties, however, high seroprevalence appears to be an anomaly resulting from small sample sizes and local demographics. For example, Routt County, Colorado, is a small rural county in a state where locally acquired Lyme disease has never been documented. Although canine seroprevalence for the county was >5%, a survey of all county veterinarians indicated that 11 of 12 seropositive dogs had lived in or traveled to

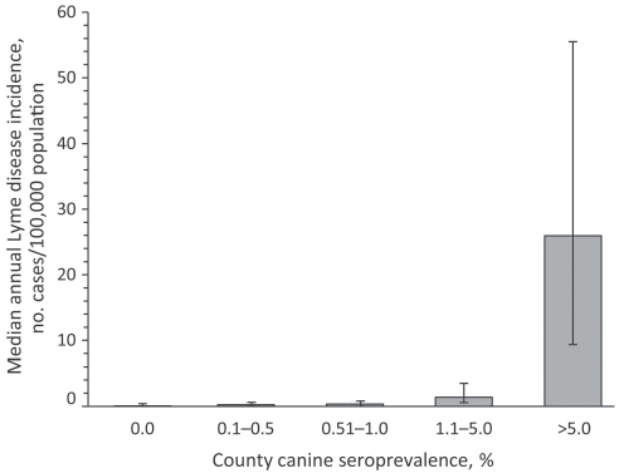

Figure 2. Median Lyme disease incidence in humans and *Borrelia burgdorferi* antibody seroprevalence in dogs in counties in 46 US states. Error bars represent 25th and 75th percentiles.

known Lyme disease–endemic areas (CDC, unpub. data). Selective testing of dogs with exposure histories may yield misleading results with respect to local endemicity.

Our findings suggest that canine seroprevalence >5% can be a sensitive but nonspecific marker of increased risk for human Lyme disease. Because dogs do not transmit infection directly to humans (or humans to dogs), this association reflects similar susceptibilities to tick-borne infection. In some circumstances, high canine seroprevalence appears to anticipate increasing rates of human infection at the county level. Conversely, canine seroprevalence ≤1% is associated with little to no local risk for human infection. Canine seroprevalence is a useful adjunct to human surveillance for Lyme disease.

Acknowledgments

We thank the anonymous reviewers for their suggestions.

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etymologia

Mycobacterium chelonae

[mi″ko-bak-tēr-eəm che′lō-nae]

From the Greek *mycēs,* fungus, *baktērion*, little rod, and *chelōnē*, turtle. German researcher Friedrich Freidmann reported isolation of this pathogen from the lung tissues of sea turtles (*Chelona corticata*) in 1903, referring to it as the turtle tubercle bacillus. In 1920, the Society of American Bacteriologists recommended that the organism be named after its discoverer, or *Mycobacterium friedmannii*. Bergey et al., however, chose in 1923 to instead recognize the host animal in the first edition of Bergey's Manual of Determinative Bacteriology and listed the bacterium as *Mycobacterium chelonei*. The spelling was changed in the 1980s to *chelonae* to make it consistent with general use.

Source: Dorland's Illustrated Medical Dictionary. 31st ed. Philadelphia: Saunders; 2007; Grange JM. *Mycobacterium chenolei*. Tubercle. 1981;62:273–6. PubMed; Topley & Wilson's Microbiology and Microbial Infections. Bacteriology, 10th ed., Vol. 2. London: Hodder Arnold; 2005.

Predominance of *Cronobacter sakazakii* Sequence Type 4 in Neonatal Infections

Susan Joseph and Stephen J. Forsythe

A 7-loci (3,036 nt) multilocus sequence typing scheme was applied to 41 clinical isolates of *Cronobacter sakazakii*. Half (20/41) of the *C. sakazakii* strains were sequence type (ST) 4, and 9/12 meningitis isolates were ST4. *C. sakazakii* ST4 appears to be a highly stable clone with a high propensity for neonatal meningitis.

Cronobacter is a genus within the family *Enterobacteriaceae* and was previously known as *Enterobacter sakazakii*. It is closely related to the genera *Enterobacter* and *Citrobacter*. *Cronobacter* spp. have been frequently isolated from the environment, plant material (wheat, rice, herbs, and spices), and various food products, including powdered infant formula (PIF). They have come to prominence because of their association with severe neonatal infections, which can be fatal (*1*–*3*). Our current knowledge of the virulence and epidemiology of this organism is limited. However, because neonates are frequently fed reconstituted PIF, this product has been the focus of attention for reducing infection risk to neonates because the number of exposure routes is limited (*1*,*2*).

Infections with *Cronobacter* spp. occur across all age groups, and most infections, albeit less severe, are in the adult population. However, neonates, particularly those of low birthweight, are the major identified group at risk, because the organism can cause meningitis, necrotizing enterocolitis (NEC), and sepsis in patients in neonatal intensive care units and has high mortality rate (*1*–*3*). Bowen and Braden (*4*) reviewed 46 cases of invasive (non-NEC) infant *Cronobacter* infections to define risk factors and provide guidance for prevention and treatment. Although these infections have been associated with intrinsically and extrinsically contaminated PIF, other environmental sources are possible and several non–infant formula–associated cases have been reported (*5*). *Cronobacter* spp. have been shown to invade human intestinal cells, replicate in macrophages, and invade the blood–brain barrier (*6*). Kucerova et al. (*7*,*8*) used comparative genomic hybridization-based analysis to describe a range of virulence traits in *Cronobacter* spp., including iron acquisition mechanisms, fimbriae, and macrophage survival.

Recently, Baldwin et al. (*9*) constructed a comprehensive multilocus sequence typing (MLST) scheme for *Cronobacter* spp. based on 7 housekeeping genes (*atpD, fusA, glnS, gltB, gyrB, infB, ppsA*; 3,036 nt concatenated length). The MLST scheme currently has 66 defined sequence types covering all *Cronobacter* spp. (www.pubMLST.org/cronobacter). However, the scheme has not been applied for any epidemiologic purposes. Therefore, we investigated whether severity of infection by *Cronobacter* spp. is associated with particular genotype(s) by compiling patient details, isolation site, and clinical signs for clinical *C. sakazakii* isolates and comparing these with the sequence type (ST) profile of the isolates.

The Study

Forty-one clinical *C. sakazakii* strains were included in the study. These strains were from 7 countries and had been isolated during 1953–2008. The strains included those of recent (*1*–*3*,*10*–*12*) and those of more historic interest (>25 years; *13*–*15*). Strains used in this study, along with patient details and clinical signs, are shown in Table 1. Details of clinical signs were collated from information in the associated publication, or supplied by the strain provider (Centers for Disease Control and Prevention, Atlanta, GA, USA). Primers and conditions for amplification and sequencing of the 7 MLST genes *atpD* (390 bp), *fusA* (438 bp), *glnS* (363 bp), *gltB* (507 bp), *gyrB* (402 bp), *infB* (441 bp) and *ppsA* (495 bp) were as described (*9*). All sequences are available for download and independent analysis through open access at www.pubMLST.org/cronobacter.

Comparative analysis with the online *Cronobacter* MLST database (covering isolates from all sources) showed that the clinical isolates were in 10 of 30 STs defined for *C. sakazakii* spp. However, the clinical strains were not evenly distributed across the STs. Of particular interest was that half (20/41) of the strains were ST4 (Table 2). The remaining strains were ST8 (7), ST1 (4), ST12 (3), ST3 (2), ST13, ST15, ST18, ST31, and ST41 (1 each). Of the 20 ST4 strains, 10 were from neonates, 7 from infants, and 1 from a child; 2 had no patient details. Similarly, most (9/12) isolates from meningitis cases were ST4 strains; 7 were isolated from cerebrospinal fluid and the others from blood and the trachea. The remaining ST4 strains were from bacteremia cases (1), NEC (2), and undefined infection (1), with 6 from unknown sources. ST4 was the main ST associated with neonates (10/18); this ST has been reported by Baldwin et al. (*9*) for the high incidence of PIF isolates.

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DOI: http://dx.doi.org/10.3201/eid1709.110260

The ST4 clinical strains were from 6 countries (the Netherlands, France, United States, New Zealand, Czech Republic, and Canada) and had been isolated during 1977–2008 (Table 1). Of the 30 strains with known patient details, only 1 isolate (ST1) was from an adult patient. To date, all other isolates from adults have been identified as *C. malonaticus* (S. Joseph, unpub. data).

Conclusions

The 7 housekeeping genes for MLST analysis are not virulence related, but a large proportion of severe neonatal infections were caused by a single sequence type. Whether this is caused by survival characteristics increasing persistence under desiccated conditions, and hence neonatal exposure or particular virulence capabilities, is uncertain.

Table 1. Strains used in study of *Cronobacter sakazakii* genotypes and disease severity and clinical details derived from original case histories*

Strain	Patient type/age (EGA)†	Clinical signs/outcome	Isolation site	Year	Country	ST	Reference
553	Neonate/1 d	UNK	UNK	1977	Netherlands	4	(*15*)
557	Neonate/5 d	UNK	UNK	1979	Netherlands	4	(*15*)
693	Neonate/13 d (41 wk)	Asymptomatic	Feces	1994	France	13	(*3*)
695	Neonate/15 d (32 wk)	Fatal NEC II	Trachea	1994	France	4	(*3*,*6*)
701	Neonate/28 d (28 wk)	Fatal NEC III	Peritoneal fluid	1994	France	4	(*3*,*6*)
709	Neonate/18 d (29 wk)	Septicemia	Trachea	1994	France	4	(*3*,*6*)
767	Neonate/19 d (31 wk)	Fatal meningitis	Trachea	1994	France	4	(*3*,*6*)
721	Neonate/2 wk	Meningitis	CSF	2003	USA	4	
978	Neonate/<1 wk	UNK	Enteral feeding tube	2007	UK	3	(*12*)
696	Neonate/17 d (32 wk)	NEC II	Feces	1994	France	12	(*3*,*6*)
984	Neonate/3–4 wk	UNK	Enteral feeding tube	2007	UK	3	(*12*)
690	Neonate/27 d (31 wk)	Asymptomatic	Feces	1994	France	12	(*3*)
1218	Neonate/<1 mo (30 wk)	Fatal meningitis	CSF	2001	USA	1	
1219	Neonate/<1 mo (36 wk)	Fatal meningitis	CSF	2002	USA	4	
1221	Neonate/<1 mo	Meningitis, adverse neurologic outcome	CSF	2003	USA	4	
1225	Neonate/<1 mo (35 wk)	Fatal meningitis	Blood	2007	USA	4	
1231	Neonate (33 wk)	Fatal neurologic damage	Feces	2004	New Zealand	4	(*2*)
HPB 3290	Neonate (33 wk)	Meningitis	CSF	2001	USA	1	(*1*)
1249	Neonate	Fatal infection	UNK	2009	UK	31	
1220	Infant/6 wk (37 wk)	Brain abscess, nonfatal	CSF	2003	USA	4	
1223	Infant/6 wk (31 wk)	UNK, in ICU	Blood	2004	USA	4	
1240	Infant/7 wk	Fatal meningitis	CSF	2008	USA	4	(*11*)
1242	Infant/7 wk	Fatal meningitis	Brain	2008	USA	4	(*11*)
1241	Infant/7 mo	Sudden infant death syndrome	Blood	2008	USA	1	(*11*)
1222	Infant/8 mo	Fever, recovered	Blood	2003	USA	4	
1224	Infant/10 mo	Fever, severe combined immunodeficiency	Blood	2004	USA	4	
HPB 2856	Child/6 y	UNK	UNK	2002	Canada	15	(*10*)
ATCC 29544	Child	UNK	Throat	1980	USA	8	(*13*)
20	Child/6 y	UNK	Feces	2004	Czech Rep	4	
12	Adult/74 y	UNK	Feces	2004	Czech Rep	1	
CDC 0743–75	UNK	Foot wound	Wound	1975	USA	41	(*13*)
CDC 407–77	UNK	UNK	Sputum	1977	USA	8	(*13*)
CDC 996–77	UNK	UNK	Spinal fluid	1977	USA	8	(*13*)
NCTC 9238	UNK	UNK	Abdomen pus	1953	UK	18	(*15*)
HPB 2852	UNK	UNK	UNK	1990	Canada	8	(*10*)
HPB 2853	UNK	UNK	UNK	1990	Canada	4	(*10*)
511	UNK	UNK	UNK	1983	Czech Rep	8	(*14*)
513	UNK	UNK	UNK	1983	Czech Rep	8	(*14*)
520	UNK	UNK	UNK	1983	Czech Rep	12	(*14*)
526	UNK	UNK	UNK	1983	Czech Rep	8	(*14*)
558	UNK	UNK	UNK	1983	Netherlands	4	(*15*)

*EGA, estimated gestational age; ST, sequence type; UNK, unknown; NEC, necrotizing enterocolitis; CSF, cerebrospinal fluid; ICU, intensive care unit; Czech Rep, Czech Republic; CDC, Centers for Disease Control and Prevention.
†Numbers in parenthesis are estimated gestational age. Values <37 weeks are considered premature.

Table 2. Summary of *Cronobacter sakazakii* sequence types and source details from study of *C. sakazakii* genotypes and disease severity*

		Patient details					Clinical signs					
ST	No. infections	Neonate†	Infant‡	Child	Adult	UNK	Meningitis	Bacteremia	NEC	Infection	Asymptomatic	UNK
1	4	2	1		1		2	1				1
3	2	2										2
4	20	10	7	1		2	9	1	2	2		6
8	7			1		6	1					6
12	3	2				1			1		1	1
13	1	1									1	
15	1			1								1
18	1					1				1		
31	1	1								1		
41	1					1				1		
Total	41	18	8	3	1	11	12	2	3	5	2	17

*ST, sequence type; UNK, unknown; NEC, necrotizing enterocolitis.
†Age <28 d.
‡Age 28–364 d.

It is plausible that different age groups are exposed to different genotypes of *C. sakazakii* according to their diet and lifestyle. *C. sakazakii* ST4 appears to be a stable clone because strains have been isolated from 7 countries for >50 years. The earliest (1951) nonclinical isolate was from a can of dried milk (*13*). Whether this clonal nature occurs in other *Cronobacter* spp. awaits future investigation.

Acknowledgments

We thank Nadia Chuzhanova for providing statistical advice and those who provided cultures, particularly Maria-Francoise Preré, Harry Muytjens, Ivo Safarík, Jeff Farber, and Matthew Arduino.

This study was supported by Nottingham Trent University.

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Dr Forsythe is professor of microbiology at Nottingham Trent University. His research interests are foodborne pathogens, especially emergent bacterial pathogens and the origin of their virulence.

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Wild Rodents and Novel Human Pathogen *Candidatus* Neoehrlichia mikurensis, Southern Sweden

Martin Andersson and Lars Råberg

We examined small mammals as hosts for *Anaplasmataceae* in southern Sweden. Of 771 rodents, 68 (8.8%) were infected by *Candidatus* Neoehrlichia mikurensis, but no other *Anaplasmataceae* were found. *Candidatus* N. mikurensis has recently been found in human patients in Germany, Switzerland, and Sweden, which suggests that this could be an emerging pathogen in Europe.

The rickettsial family *Anaplasmataceae* contains several tick-transmitted bacteria of considerable medical and veterinary importance, including known human pathogens such as *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*. Recently, a case of human disease in a Swedish patient with recurrent fever episodes caused by another member of *Anaplasmataceae* was reported. The infectious agent was shown by PCR amplification of the 16S rRNA gene to be identical to *Candidatus* Neoehrlichia mikurensis (*1*).

This published report was followed by the descriptions of a patient from Switzerland (*2*) and 2 German patients with severe febrile illness caused by *Candidatus* N. mikurensis (*3*). This member of the family *Anaplasmataceae* was first discovered in ticks in the Netherlands in 1999; it was originally designated as an *Ehrlichia* spp.–like species (*4*). Similar organisms were later detected in Russia (*5*) as well as in other parts of Europe (*6*). In 2004, a closely related organism was detected in Japan in wild rodents and was named *Candidatus* N. mikurensis (*7*). In the United States, a similar organism (*Candidatus* N. lotori) has been detected in wild raccoons (*8*). The distribution and reservoir hosts of *Candidatus* N. mikurensis in Europe are largely unknown. It has, however, been detected in a blood sample from a single bank vole in Italy (*9*), indicating that rodents are possible reservoir hosts.

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DOI: http://dx.doi.org/10.3201/eid1709.101058

In this study, we collected blood samples from 7 small mammal species (rodents and shrews) at 5 localities in southern Sweden to investigate their role as hosts for *Anaplasmataceae* and to determine whether these small mammals serve as natural hosts for *Candidatus* N. mikurensis.

The Study

From May through October 2008, we trapped small mammals at 5 sites in southern Sweden (Figure 1). Animals were captured with live traps and released after sampling. Our primary objective was to obtain samples from bank voles, *Myodes glareolus,* but other species were also sampled when captured. A total of 829 animals of 7 species were caught (Table). Approximately 20 μL of blood was taken from each animal. A nested PCR was performed with primers for *Anaplasmataceae* specific for the 16S rRNA gene (*10*).

All amplified fragments were sequenced, and a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search showed that 68 animals were infected by *Candidatus* N. mikurensis. The primers were chosen to be specific for bacteria belonging to the families *Rickettsiaceae* and *Anaplasmataceae* (*10*), but

Figure 1. Collection locations for rodents and shrews tested for *Candidatus* Neoehrlichia mikurensis and *Bartonella* spp. infections, southern Sweden, 2008. Prevalence of infection: Häglinge, n = 45 infections, 0% *Candidatus* N. mikurensis, 44.4% *Bartonella* spp.; Revinge, n = 623 infections, 9.3% *Candidatus* N. mikurensis, 33.7% *Bartonella* spp.; Istaby, n = 53 infections, 3.8% *Candidatus* N. mikurensis, 34% *Bartonella* spp.; Hemmeströ, n = 64 infections, 4.7% *Candidatus* N. mikurensis, 39.1% *Bartonella* spp.; Herseby, n = 49 infections, 12.5% *Candidatus* N. mikurensis, 45.0% *Bartonella* spp.

Table. Species tested and number of animals infected with *Candidatus* Neoehrlichia mikurensis or *Bartonella* spp., Sweden, 2008

Species	No. sampled	*Candidatus* N. mikurensis	*Bartonella* sp.
Bank vole (*Myodes glareolus*)	705	64	232
Field vole *(Microtus agrestis*)	24	2	11
Wood mouse (*Apodemus sylvaticus*)	10	1	4
Yellow-necked mouse (*A. flavicollis*)	25	1	14
Apodemus sp.	7	0	5
Common shrew (*Sorex araneus*)	43	0	28
Pygmy shrew (*S. minutus*)	12	0	0
Water shrew (*Neomys fodiens*)	3	0	0
Total	829	68	294

they also amplified *Bartonella* spp. under the given PCR conditions. In total, 35.5% of the animals were infected by *Bartonella* spp. Double infections with both *Candidatus* N. mikurensis and *Bartonella* spp., as indicated by double peaks on the sequencing chromatogram, occurred in 12 cases.

To further characterize the obtained *Candidatus* N. mikurensis, we sequenced 1,426 bp of the 16S rRNA and 1,233 bp of the *groEL* gene (*1*). The obtained 16S rRNA *Candidatus* N. mikurensis sequences in this study were identical (1,426/1,426 bp) to sequences obtained from human patients in Germany and Switzerland (*2*,*3*). The *groEL* sequence was identical to the isolate from Germany (1,233/1,233 bp) but differed slightly from the isolate from Switzerland (1,072/1,084 bp, 98.9% pairwise identity). A phylogenetic network containing unique 16s rRNA sequences from *Candidatus* N. mikurensis available at the National Center for Biotechnology Information was made with the program Network 4.5.1.6 (www.fluxus-engineering.com) by using the median-joining algorithm (*11*) (Figure 2).

The prevalence of *Candidatus* N. mikurensis at the 5 sites ranged from 0% to 12.5%, and *Bartonella* spp. occurred in 33.7% to 45.0% of the animals (Figure 1). The prevalence of *Candidatus* N. mikurensis and *Bartonella* spp. in each host species is given in the Table. *Candidatus* N. mikurensis occurred in all 4 rodent species, but not in the shrews; the difference in prevalence between rodents and shrews was statistically significant (p = 0.011, by Fisher exact test). *Bartonella* spp. were found in all rodent species and the common shrew.

Conclusions

In the present field survey of *Anaplasmataceae* in Swedish rodents, we have amplified 16s rRNA and *groEL* sequences identified as *Candidatus* N. mikurensis identical to sequences obtained from humans. This organism has been amplified from humans with febrile illness on ≥4 occasions (*1*–*3*), demonstrating that *Candidatus* N. mikurensis can cause human infections, at least occasionally.

Apart from the first human case, to our knowledge, *Candidatus* N. mikurensis has not been detected in Sweden. However, we identified *Candidatus* N. mikurensis at 4 of 5 sites, indicating that this organism is widespread in southern Sweden. Identical or similar sequences have been detected in *Ixodes ricinus* ticks from several European countries (*3*–*6*,*13*), showing that it is distributed over a large area of Europe. In contrast, we found no evidence of infection by any other *Anaplasmataceae*. *I. ricinus* is the dominating tick species in Sweden, and animals at all our study sites were heavily infested with larvae of this species and occasionally with nymphs (M. Andersson and L. Råberg, unpub. data). Reservoir hosts are essential for several tick-borne pathogens that lack the capacity for transovarial transmission from female ticks to larvae, such

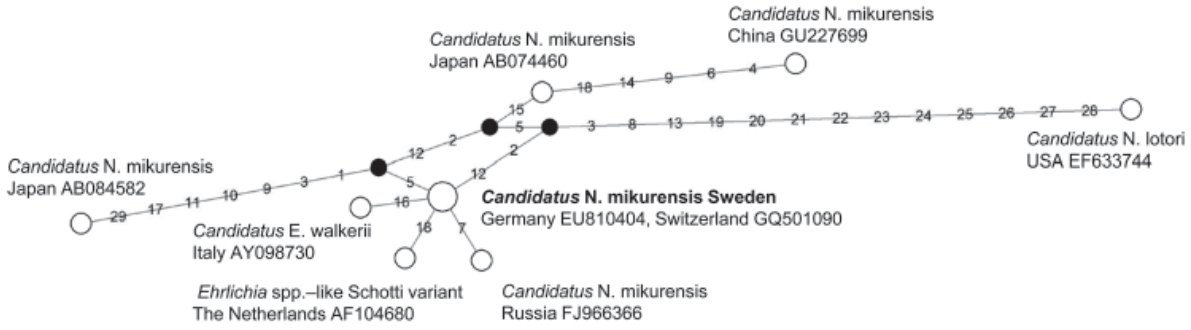

Figure 2. Phylogenetic network of 16s rRNA sequences (1,231 bp) from *Candidatus* Neoehrlichia mikurensis, southern Sweden, 2008. Black nodes indicate intermediate inferred sequences on the most parsimonious route between observed sequences. Numbers on branches represents mutations, numbered according to nucleotide position in the alignment. The sequence obtained in this study is shown in **boldface** and is identical with sequences from human patients in Germany (*3*) and Switzerland (*2*). The Japanese reference strain TK4456 (*7*) showed 99.2% similarity with our sequence, and the North American *Candidatus* N. lotori strain (*12*) showed 98.3% similarity.

as *A. phagocytophilum* (*14*). Approximately 70% of the tick larvae in Sweden engorge on small mammals, such as voles, mice, and shrews (*15*).

The prevalence of *Candidatus* N. mikurensis was similar in all investigated rodent species in our study with a mean value of 8.8%. These results are consistent with several studies in Japan, which also found *Candidatus* N. mikurensis in several different rodent species (*7*,*10*). This finding indicates that rodents play a role in the natural cycle of *Candidatus* N. mikurensis in Europe and that rodents are likely to be competent reservoir hosts. In contrast, the closely related *Candidatus* N. lotori, found in the United States, seems to use raccoons rather than rodents as hosts (*12*). Whether the European variant of *Candidatus* Neoehrlichia is capable of infecting animals other than rodents and humans remains to be investigated. We found the organism was completely absent in shrews, which suggests that they might not be competent hosts for these bacteria.

We conclude that *Candidatus* N. mikurensis is geographically widespread in southern Sweden and that several rodent species, the main source of blood meals for larvae of *I. ricinus* in Sweden (*15*), can be infected. The relatively high prevalence in common rodent species suggests that the risk for humans and domestic animals to encounter infected *I. ricinus* nymphs and adults is substantial. Thus, *Candidatus* N. mikurensis should be considered when diagnosing bacterial infections associated with tick bites.

Acknowledgments

We thank Kristin Scherman and Staffan Bensch for comments on the manuscript and Olof Hellgren, Barbara Tschirren, Mimi Lannefors, Pär Söderberg, and Sandra Chiriac for field assistance.

Financial support was provided by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning and the Swedish Research Council to L.R.

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Q Fever among Culling Workers, the Netherlands, 2009–2010

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In 2009, dairy goat farms in the Netherlands were implicated in >2,300 cases of Q fever; in response, 51,820 small ruminants were culled. Among 517 culling workers, despite use of personal protective equipment, 17.5% seroconverted for antibodies to *Coxiella burnetii*. Vaccination of culling workers could be considered.

Q fever is caused by the bacterium *Coxiella burnetii*. Since 2007 in the Netherlands, annual outbreaks originating from dairy goat and sheep farms have occurred. In 2009, a total of 2,354 cases in humans were reported, 20% of patients were hospitalized, and at least 6 died (*1*). Among acute cases, ≈2% become chronic, and fatality rates for untreated chronic patients are high (*2*). To stop spread, culling was conducted from December 19, 2009, through June 22, 2010, on 87 infected commercial dairy goat farms and 2 dairy sheep farms (Figure 1). A total of 50,355 pregnant goats and sheep and 1,465 bucks were culled (*3*). Animal pregnancies were confirmed by abdominal ultrasound; pregnant animals were sedated and euthanized, and their corpses were transported to a destruction facility. Culling workers were provided with personal protective equipment (PPE) and advised to read occupational health and hygiene regulations (*4*). To determine seropositivity of workers before culling, incidence of symptomatic and asymptomatic *C. burnetii* infection during culling, and risk factors associated with occupational exposure, we conducted a prospective cohort study.

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DOI: http://dx.doi.org/10.3201/eid1709.110051

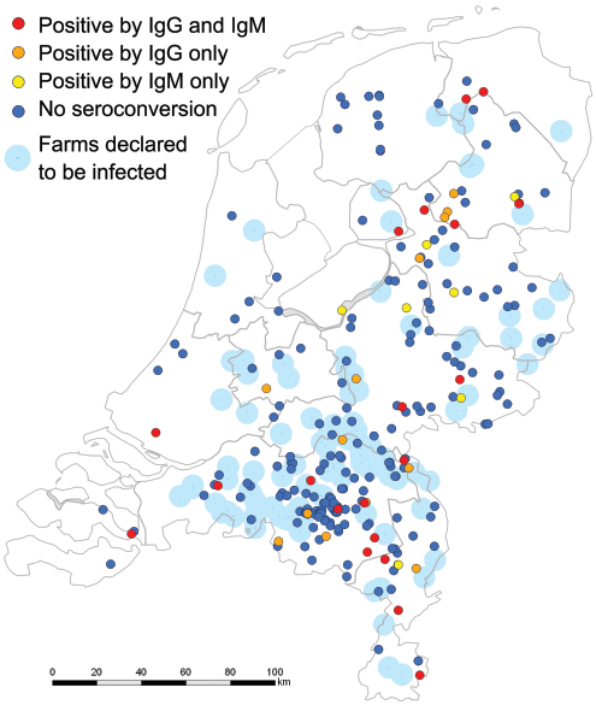

Figure 1. Residential location of 246 culling workers who were seronegative in December 2009 and their serostatus in June 2010 with location of 89 farms declared to be infected (by PCR-positive bulk-milk monitoring) in 2009 and 2010, the Netherlands. Ig, immunoglobulin. Seroconversion detected by ELISA was confirmed by immunofluorescence assay for 40 persons (38 [95%] at titers >128 and 2 [5%] at titers of 32).

The Study

Participants were 517 workers who culled goats and sheep during December 2009–June 2010. Serum samples were required from workers before employment in December 2009 (pre-cull) (*4*), and voluntary post-cull samples were requested in June 2010. In June, workers were asked to complete a questionnaire about symptoms, occupational exposure, adoption of hygiene measures and PPE use (filtering facepiece masks, gloves, overalls, hairnets), demographics, medical history, and other animal contact. Written informed consent was obtained. Information about farms (animal numbers and abortions) and workers (hours worked per person, job description) was available from occupational records.

Serum was tested for immunoglobulin (Ig) G and IgM against *C. burnetii* phase II by using ELISA (Virion/Serion, Würzburg, Germany). According to manufacturer instructions, IgG phase II seropositivity was defined as negative for titers ≤30 IU/mL and positive for titers >30 IU/mL. IgM phase II was qualitatively positive or negative. A worker was considered seronegative if a phase II sample was IgM and IgG negative and seropositive if IgM and/

or IgG positive. Positive results were confirmed by immunofluorescence assay (Focus Diagnostics, Cypress, CA, USA) titers ≥32. Symptomatic infection was defined as fever or rigors and ≥1 of the following after December 1, 2009: malaise, headache, cough, nausea, diarrhea, shortness of breath, pleuritic chest pain, or myalgia. Intensity of occupational exposure was summarized as follows: hours worked, weighted mean farm size (animal number), whether animal abortions were reported, and whether work was performed on average inside or outside the stable (proxy for direct/indirect animal contact). Months worked were dichotomized as cold (December 2009–March 2010) (*5*) and warm (April–June 2010) (*6*). Use of PPE was classified as compliant or noncompliant.

To calculate distance of workers' residence to the nearest infected farm, we used ArcGIS software (www.esri.com/software/arcgis/index.html). We used Stata version 11 (StataCorp LP, College Station, TX, USA) to examine univariable associations (Pearson χ^2 or Fisher exact test). Variables with probability $p<0.2$ and known risk factors for Q fever were selected for binomial regression analyses. Interactions between significant variables in the multivariable model were investigated. Missing values were excluded.

Of 517 participants, 453 gave pre-cull blood samples, 246 of these gave post-cull samples, and 351 completed the questionnaire. Age, gender, and residential distance from the nearest infected farm were available from occupational records. Participant median age was 47 years (range 19–67 years); 97% were male. Before culling, 14 (3.1%) were IgM II and IgG II positive, 8 (1.8%) were IgM II positive only, 36 (8%) were IgG II positive only, and 395 (87%) were IgG II and IgM II negative; i.e., any seropositivity was found for 13.0%. Pre-cull blood samples indicated more seropositivity among workers who lived within 5 km of an infected farm and had regular work contact with sheep and goats (excluding culling). Prior culling experience was more common among seronegative than seropositive workers (Table 1). Among those who were IgG seropositive before culling, none became IgM seropositive after culling.

Among the 395 workers who were seronegative before culling, 246 (62%) provided a follow-up blood

Table 1. Baseline characteristics of workers before culling small ruminants, the Netherlands, December 2009*

Characteristic	Total no. workers	No. (%) workers		p value†
		Seronegative, n = 395	Seropositive, n = 58	
Sex‡				
M	342	303 (89)	39 (11)	
F§	11	10 (91)	1 (9)	0.812
Age group, y¶				
<40	114	95 (83)	19 (17)	
40–49	157	137 (87)	20 (13)	
50–59	154	139 (90)	15 (10)	
≥60	26	22 (85)	4 (15)	0.398
Distance of residence from nearest infected farm, km¶				
≤5	116	95 (82)	21 (18)	
>5	317	282 (89)	35 (11)	0.052
Level of education¶				
Low	48	43 (90)	5 (10)	
Medium	132	117 (89)	15 (11)	
High	53	45 (85)	8 (15)	0.725
Medical history¶#				
No	159	140 (88)	19 (12)	
Yes	57	47 (83)	10 (18)	0.288
Current smoker¶				
No	189	162 (86)	27 (14)	
Yes	53	48 (91)	5 (9)	0.357
Previous culling experience¶				
No	116	94 (81)	22 (19)	
Yes	135	124 (92)	11 (8)	0.011
Regular occupational contact with sheep or goats¶				
No	202	182 (90)	20 (10)	
Yes	34	24 (71)	10 (29)	0.002

*Missing values excluded from analysis.
†Pearson χ^2.
‡Maximum 453 respondents. Data available from occupational records.
§No female respondents were pregnant.
¶Maximum 251 respondents. Data available from questionnaire responses.
#History of cardiorespiratory disease, liver disorders, diabetes, cancer, immunosuppression, allergies, skin conditions.

sample in June 2010, and 199 (80.8%) of these completed the questionnaire. Those who participated in June were more likely to be male (p = 0.015) and 40–60 years of age (p<0.001). Seroconversion among 246 seronegative respondents occurred as follows: 23 (9.4%) became IgG and IgM seropositive, 7 (2.9%) became IgM positive only, 13 (5.3%) became IgG positive only, and 203 (82.5%) remained seronegative; i.e., any seroconversion was found

Table 2. Variables associated with Q fever seroconversion among 246 workers who were seronegative before culling small ruminants, the Netherlands, 2009*

	No. (%) workers		Univariable analysis		Multivariable analysis†	
Variable	Total	Seroconversion	RR (95% CI)	p value‡	RR (95% CI)	p value
Total	246 (100)	43 (17)				
Sex						
F	6 (2)	2 (33)	Reference			
M	240 (98)	41 (17)	0.51 (0.16–1.64)	0.301		
Age, y						
≤45	96 (39)	14 (15)	Reference			
>45	150 (61)	29 (19)	1.33 (0.74–2.38)	0.339	2.0 (0.93–4.16)	0.07
Level of education						
Low	39 (21)	5 (13)	Reference			
Medium	103 (55)	18 (17)	1.36 (0.54–3.42)			
High	44 (24)	8 (18)	1.42 (0.51–3.98)	0.765		
Minimum distance of residence from nearest infected farm, km						
>5	174 (73)	32 (18)	Reference			
≤5	63 (27)	9 (14)	0.78 (0.39–1.53)	0.460		
Medical history§						
No	128 (75)	23 (18)	Reference			
Yes	42 (25)	5 (12)	0.66 (0.27–1.63)	0.358		
Current or past smoker						
No	84 (44)	16 (19)	Reference			
Yes	108 (56)	16 (15)	0.78 (0.41–1.46)	0.435		
Total hours worked inside farm perimeter¶						
0–20	81 (33)	5 (6)	Reference		Reference	
21–100	82 (34)	18 (22)	3.56 (1.39–9.12)		5.53 (0.71–42.77)	0.102
>100	80 (33)	20 (25)	4.05 (1.60–10.26)	0.003	7.75 (1.02–58.99)	0.048
Mean farm size ≥1,500 animals#						
No	167 (68)	22 (13)	Reference			
Yes	79 (32)	21 (27)	2.02 (1.18–3.44)	0.010	1.75 (0.93–3.30)	0.081
Worked mostly inside stable						
No	110 (45)	11 (10)	Reference			
Yes	133 (55)	31 (23)	2.33 (1.23–4.42)	0.006	2.58 (1.04–6.37)	0.040
Animal abortions on farm						
No	208 (85)	33 (16)	Reference			
Yes	38 (15)	10 (26)	1.66 (0.89–3.07)	0.119	0.93 (0.45–1.91)	0.844
Any previous culling experience						
No	85 (43)	15 (18)	Reference			
Yes	114 (57)	17 (15)	0.84 (0.45–1.59)	0.603		
Adherence to hygiene and preventive measures**						
Fully compliant	91 (50)	13 (14)	Reference			
Not compliant	91 (50)	17 (19)	1.31 (0.68–2.53)	0.424	0.94 (0.51–1.72)	0.829
Months spent culling						
2009 Dec–2010 Mar only (mean temperature 3.2°C)	105 (54)	18 (17)	Reference			
2010 Apr–Jun only (mean temperature 13.9°C)	2 (1)	1 (50)	2.92 (0.69–12.41)			
2009 Dec–2010 Jun	87 (45)	21 (24)	1.41 (0.80–2.47)	0.288		

*Total for each category may be <246 because of missing data. RR, risk ratio; CI, confidence interval.
†All data available for n = 180 in multivariable analysis.
‡Pearson χ^2.
§History of cardiorespiratory disease, liver disorders, diabetes, cancer, immunosuppression, allergies, skin conditions.
¶Data only available for n = 194, those who worked inside the farm perimeter.
#Weighted mean number of animals on farms worked by participants.
**Includes wearing mask, gloves, overalls, hairnet, showering after exposure.

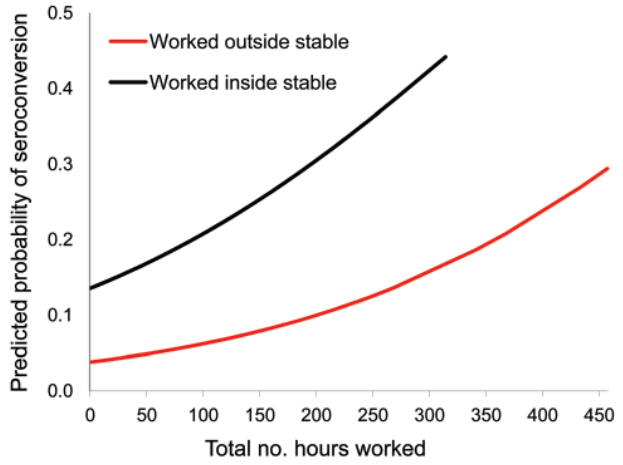

Figure 2. Predicted probabilities of seroconversion among small ruminant culling workers by total hours worked, weighted mean farm size, and location on farm while working during December 2009–June 2010, the Netherlands. Seroconversion probabilities calculated by multivariable model adjusted for age group, occurrence of animal abortions on the farms worked, and compliance with wearing personal protective equipment.

for 17.5%. Questionnaire respondents who seroconverted had more symptoms after December 1, 2009, (9 [31%] of 29) than nonseroconverters (17 [11%] of 150; relative risk 2.7, 95% confidence interval 1.4–5.5, p = 0.005). Symptomatic seroconverters reported fever and/or rigors and malaise (n = 7), headache (n = 6), cough (n = 6), or myalgia (n = 4). Mean duration of illness was 7.6 (range 1–14) days.

Univariable model indicated significance for total hours worked, farm size, and working inside the stable ($p<0.05$; Table 2). Multivariable model indicated significance for working >100 hours on the farm and working inside the stable (Table 2; Figure 2). Interaction effects were not significant.

Conclusions

Seroconversion for *C. burnetii* among 17.5% of culling workers who were seronegative before culling provides evidence of high-risk work. Before culling, seroprevalence was 13%, similar to that among blood donors in a high-incidence area in the Netherlands in 2009 (H.L. Zaaijer, pers. comm.) and in similar high-risk occupational groups (*7*). Laboratory testing by using ELISA is an accepted method in an acute setting (*8*), and positive results (including positive IgM only) were confirmed by immunofluorescence assay. Nonparticipants were in the youngest and oldest age groups; their effect on the proportion of seroconversion is uncertain. Eighteen workers (excluded for not providing a follow-up blood sample) completed the questionnaire in June. Symptom incidence for these 18 workers was the same as that for included participants.

Symptomatic infection (31% of seroconverters) was probably underestimated. A diagnosis of Q fever was self-reported (unconfirmed) to the occupational health service by 8 workers who did not participate in the study. During December–July 2010, the national infectious disease surveillance system reported 11 culling-related cases of acute Q fever; 2 of these patients were hospitalized.

A strong association was shown between risk for seroconversion and total hours worked on the farms and working inside the stable. In other settings internationally, a risk gradient has also been shown for close direct and indirect animal contact over time (*9,10*). In our study, half the participants had experience with previous animal epidemics (avian influenza, foot-and-mouth disease, classical swine fever) and using PPE. Their compliance with PPE was reportedly high; however, a key problem was not wearing PPE while taking work breaks but remaining on the farm.

Given the high risk for infection despite extensive personal protective measures during culling, additional preventive measures are needed. The Health Council of the Netherlands issued guidelines for persons in risk groups who would benefit from vaccination against Q fever (*11*). Culling workers were not included in these guidelines. The efficacy of human Q fever vaccine has been shown to be high for young and healthy persons in similar occupational groups (*12–14*). Vaccination of culling workers could be considered if further animal culling is advised.

Acknowledgments

We thank Ben Bom for providing the map and distance calculations; Daan Notermans, Marianne van der Sande, Ioannis Karagiannis, and Biagio Pedalino for their scientific input; the culling workers and their affiliated organizations for their participation; and the Food and Consumer Product Safety Authority for facilitating this study.

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Syndromic Surveillance during Pandemic (H1N1) 2009 Outbreak, New York, New York, USA

Marlena Gehret Plagianos, Winfred Y. Wu, Colleen McCullough, Marc Paladini, Joseph Lurio, Michael D. Buck, Neil Calman, and Nicholas Soulakis

We compared emergency department and ambulatory care syndromic surveillance systems during the pandemic (H1N1) 2009 outbreak in New York City. Emergency departments likely experienced increases in influenza-like-illness significantly earlier than ambulatory care facilities because more patients sought care at emergency departments, differences in case definitions existed, or a combination thereof.

Health departments perform syndromic surveillance to provide early warning of emerging outbreaks and provide situational awareness for ongoing outbreaks to help characterize magnitude and geographic scope of outbreaks over time. The New York City (NYC) Department of Health and Mental Hygiene, New York, New York, USA, conducts syndromic surveillance by using emergency department (ED) visits (*1*,*2*) and electronic health record data from ambulatory clinics in its Primary Care Information Project (*3*) and the Institute for Family Health (IFH), a network of community health centers (*4*,*5*).

The pandemic (H1N1) 2009 outbreak was first detected in NYC through traditional surveillance, a report of increasing influenza-like illness (ILI) at a high school in Queens on April 24 (*6*). After this report, Department of Health and Mental Hygiene syndromic surveillance detected citywide increases in patients with ILI seeking care at EDs. To assess the performance characteristics of ambulatory-based syndromic surveillance, we performed retrospective analyses to determine if the ambulatory-based system was also able to detect an increase in ILI during the spring 2009 outbreak, and, if so, determine whether it provided earlier notification or greater magnitude of detection compared with ED data.

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DOI: http://dx.doi.org/10.3201/eid1709.101357

The Study

Ambulatory surveillance data originated from 9 IFH facilities located in Manhattan and the Bronx and 49 primary care practices enrolled in the Primary Care Information Project and located throughout NYC. ED surveillance data originated from 50 EDs across the city. ED and ambulatory care facilities were similarly distributed (Figure 1). EDs had a high volume of patients (mean 247/day, mean age 34 years). Ambulatory care facilities saw fewer patients (mean 34.5/day, mean age 33 years).

ILI case definitions were based on previous correlations to seasonal influenza and differed slightly between systems. Within ambulatory clinics, ILI was defined as presence of fever (either measured temperature >99.9°F, or fever as a reason for visit) plus reason for visit of cough, "flu" or influenza, or ILI-related International Classification of Diseases, 9th Revision, encounter diagnosis (codes 079.99, 466.0, 487.1, 382.00, 465.9). The ED ILI case definition was based on a chief report of fever plus sore throat or cough, or chief complaint mentioning influenza.

For both systems, we calculated the percentage of ILI visits (number of ILI-related visits/total number of encounters) at each facility on weekdays (weekends were excluded because many ambulatory clinics were closed) and determined the first day each facility experienced a increase in the percentage of ILI visits, on the basis of

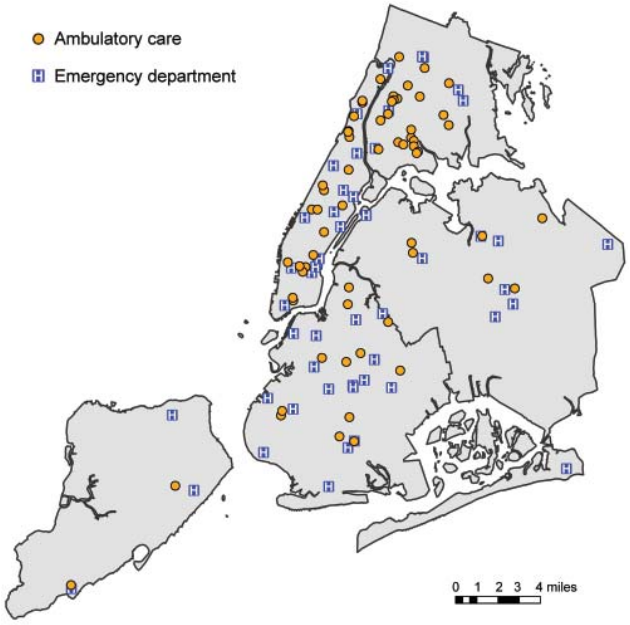

Figure 1. Locations of ambulatory care facilities and emergency departments used in analysis of syndromic surveillance of pandemic (H1N1) 2009, New York, New York, USA, May 2009.

28-day moving averages and z-score. A significant increase was defined as a *z*-score >2 (*7*).

ED surveillance showed elevated ILI activity in 2 distinct phases during the spring 2009 pandemic (H1N1) 2009 outbreak (*8*). We counted the number of days during April 24–May 8 (representing the first phase of elevated ILI activity) when each facility experienced its first increase in ILI visits and the number of days during May 14–June 4 (representing the second phase) when each facility experienced its next increase in ILI visits. Because not all facilities experienced increases in ILI, we used the survival analysis method of the log-rank test to compare time to significant increase in ILI visits between EDs and ambulatory clinics. We repeated the analysis for each borough to assess potential differences within boroughs in the timing between systems to initial ILI increase. To compare the magnitudes of the signals, we used the Wilcoxon matched-pairs test to compare facility *z*-scores between systems.

Before April 24, syndromic surveillance data from both systems had shown decreasing levels of ILI (*8*). The survival curves (Figure 2) show that in the first phase of the pandemic (H1N1) 2009 outbreak, most EDs rapidly experienced noticeable increases in ILI, whereas the increase in ILI at ambulatory clinics occurred in fewer sites and was more gradual. During the second phase, most EDs immediately experienced substantial increases in ILI. Although more ambulatory facilities experienced a substantial increase in ILI compared with the first phase, the response was again more gradual. The survival curves differed significantly by the log rank test ($p<0.001$). When the analysis was repeated for each borough, EDs experienced an increase before ambulatory clinics across all boroughs except Staten Island during the first phase and in all boroughs during the second phase (Table).

The magnitude of the signals' *z*-scores were significantly greater at EDs during the first phase ($p = 0.004$). However, they were not different during the second phase ($p = 0.121$).

Conclusions

The results of this analysis confirm that ambulatory syndromic surveillance detected increases in ILI activity during both phases of the pandemic (H1N1) 2009 spring outbreak in NYC. However, the timeliness of detection appeared significantly earlier in EDs during both phases, and the magnitude of ILI signaling was significantly greater at the EDs during the first phase of the outbreak. During previous influenza seasons, the EDs and IFH ambulatory care facilities tracked well together (*4*,*5*).

There are several limitations worth noting. First, coverage of NYC EDs for syndromic surveillance is comprehensive (50 of 55 EDs), whereas the proportion of all NYC ambulatory clinics in this analysis is small. Better representation of NYC ambulatory clinics would possibly affect these results as there might be factors associated with electronic health record-based practices in the ambulatory surveillance system that resulted in the differences seen. Geographic distribution of the ambulatory clinics and EDs in this analysis is similar and differential sampling by location alone is unlikely to explain the differences.

Second, there are several EDs not participating in the syndromic surveillance network in eastern Queens where the pandemic (H1N1) 2009 outbreak first emerged. Given the proximity of these nonreporting hospitals to where the outbreak began, their inclusion might have altered the findings reported toward an earlier or stronger signal among EDs.

Third, ambulatory care facilities are able to triage telephone calls from patients and may have instructed patients with mild ILI symptoms to stay home. In addition, some ambulatory care patients may have had to wait several days between requesting a visit and receiving care. Either telephone triage or appointment delays could have reduced

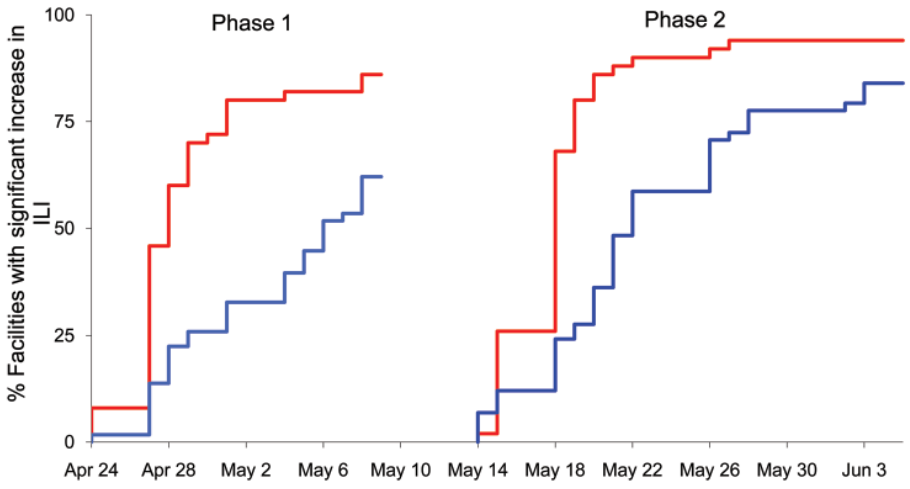

Figure 2. Percentage of emergency departments (red lines) and ambulatory clinics (blue lines) with substantial increases in patients with influenza-like illness (ILI) during phases 1 and 2 of pandemic (H1N1) 2009, New York, New York, USA, spring 2009.

Table. Borough-specific results for syndromic surveillance during pandemic (H1N1) 2009 outbreak, New York, New York, USA, 2009*

Borough and pandemic phase	Median days to increase in visits for ILI		p value†
	ED	AC	
Phase 1: Apr 24–May 8			
All	4	12	<0.001
Bronx	5	12	0.045
Brooklyn	3	14	0.025
Manhattan	4	13	0.008
Queens	3	7	0.007
Staten Island	14	10	0.902
Phase 2: May 14–Jun 4			
All	4	8	<0.001
Bronx	1	6	0.004
Brooklyn	4	12	0.039
Manhattan	4	7	0.016
Queens	4	8	0.091
Staten Island	5	8	0.012

*ILI, influenza-like illness; ED, emergency department; AC, ambulatory care.
†1-sided log rank test.

the number of ILI visits in these settings, which would not have been possible at EDs.

Fourth, the case definitions for ILI differ slightly between systems. The ED case definition is less specific because it includes patients reporting a chief complaint of "flu" alone, whereas the ambulatory care definition requires both febrile and respiratory symptoms and diagnoses. Thus, EDs may have detected greater increases in ILI because of higher sensitivity. Such an increase, especially in worried well patients, may have occurred during the outbreak, contributing to more ILI cases captured by the ED syndromic surveillance system.

Although earlier detection at EDs might be the result of persons choosing to go to EDs instead of ambulatory care clinics, it may have occurred because the less specific ED case definition was able to capture more events, or it may be a combination of these 2 factors. The findings reported here do not definitively demonstrate that ED syndromic surveillance is inherently timelier than ambulatory syndromic surveillance in detecting emerging influenza outbreaks. The heightened awareness of pandemic (H1N1) 2009 influenza during the spring 2009 outbreak may have affected the findings we reported. Further investigation during future outbreaks will help to better assess the innate abilities of the systems to provide early warning and situational awareness of emerging infectious disease outbreaks.

Acknowledgments

We thank Don Weiss for reading and commenting on the article. We also thank the New York City Department of Health syndromic surveillance team for their contribution in coding and managing the emergency department data.

This work was funded by the New York City Department of Health and Mental Hygiene.

At the time of this study, Ms Plagianos was a data analyst at the Primary Care Information Project at the New York City Department of Health and Mental Hygiene. She now does statistical consulting. Her research interests are data modeling and infectious disease epidemiology.

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Tubulinosema sp. Microsporidian Myositis in Immunosuppressed Patient

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The Phylum Microsporidia comprises >1,200 species, only 15 of which are known to infect humans, including the genera *Trachipleistophora*, *Pleistophora*, and *Brachiola*. We report an infection by *Tubulinosema* sp. in an immunosuppressed patient.

Initially designated as primitive eukaryotic protozoa, the microsporidia are now classified as fungi (*1*,*2*) with >1,200 known species. The ribosomes of microsporidia resemble prokaryotic ribosomes in terms of size but lack a 5.8S subunit (*3*). The microsporidia infect many different animals and insects, but human infections were rarely reported before the HIV/AIDS epidemic when *Enterocytozoon bieneusi* was shown to be a major cause of diarrhea in patients with low CD4+ lymphocyte counts (*4*). Since then, 14 other species of microsporidia have been reported to infect the human gastrointestinal tract, eye, or muscle and to cause disseminated infection, most commonly in immunocompromised hosts (*5*–*12*). We report an infection by *Tubulinosema* sp. in an immunosuppressed patient.

The Study

Our patient was a 67-year-old woman with known high-grade non-Hodgkin lymphoma since 1993 and chronic lymphocytic leukemia since 2003. She had received multiple courses of chemotherapy, including fludarabine, cyclophosphamide, rituximab, pentostatin, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone, and alemtuzumab. During 2004 through 2008, she had multiple hospitalizations with neutropenic complications. Her chemotherapy regimen was switched to bendamustine and cyclophosphamide in January 2009 because of persistent neutropenia.

In February 2009, the patient was hospitalized with fever and abdominal pain. No clear infectious etiology could be ascertained despite an extensive workup; she was treated with cefepime, vancomycin, and metronidazole without any improvement. At the end of the antimicrobial drug course, she noticed 2 painful white lesions on her tongue and also had fever and chills. The lesions consisted of two 1.0- × 1.5-cm nodules on the anterior aspect of the tongue. These were initially treated as oral thrush with fluconazole without resolution. At the same time, the patient experienced a relapse of herpes zoster that was treated with valacyclovir.

In April 2009, biopsy samples of the lesions were obtained. Results of the initial histopathology report were consistent with an inflamed granulation type tissue with collections of epithelioid histiocytes resembling naked-type granulomatous changes. Numerous intracellular microorganisms were seen in the myocytes. Culture for bacteria and fungi was negative and culture for microsporidia was not attempted. Serologic test results for *Toxoplasma gondii* and *Trypanosoma cruzi* were also negative.

Paraffin-fixed tissue and slides were sent to the laboratories of the Parasitic Diseases Branch and the Infectious Disease Pathology Branch, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA. Detailed results of the evaluations performed at CDC are given below. On the basis of a preliminary diagnosis of microsporidia, the patient was treated with albendazole 400 mg daily without any improvement.

In June 2009, the patient sought treatment for decreased urine output and acute kidney injury attributed to acute interstitial nephritis of unknown etiology based on eosinophils in her urine. A renal biopsy showed lymphocytic infiltrates negative for CD5 and CD20 and positive for paired box gene-5 and p53 expression, consistent with Richter's transformation of the kidney. She was also found to have anterior mediastinal lymphadenopathy, interval increase in splenomegaly, ascites, pleural effusions, and bilateral interstitial infiltrates. Specimens from a bronchoscopy did not show any evidence of malignancy. Cultures were negative for bacteria and fungi. The patient died the next day. The family chose not to have an autopsy done. The cause of death was transformed chronic lymphocytic leukemia with acute renal failure as a contributory cause.

Muscle tissue after fixation was stained with hematoxylin and eosin, periodic acid–Schiff, mucicarmine, Grocott methenamine silver, Giemsa, Warthin-Starry silver,

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DOI: http://dx.doi.org/10.3201/eid1709.101926

acid-fast, and Lillie-Twort Gram stains. Granulomatous inflammation with focal infiltrates by neutrophils and eosinophils was seen. Within the myofibrils, there were abundant clusters of small, ovoid, basophilic organisms (Figure 1, panel A) measuring 2 μm stained positive by Lillie-Twort Gram and Warthin-Starry stains (Figure 1, panel B). The organisms stained faintly with Giemsa and were negative by Grocott methenamine silver, periodic acid–Schiff, and mucicarmine stains.

Results of immunohistochemical analysis (immune alkaline phosphatase technique) were negative, and autoimmune histochemical analysis was not performed. The primary antibodies used in the tests were an antibody against *T. cruzi* and an antibody against *T. gondii*. Appropriate positive and negative controls were run in parallel.

Electron microscopy at CDC revealed numerous spores but few developing stages (Figure 2, panel A). All stages were in direct contact with the host cell cytoplasm. The spores ranged from 1.4 to 2.4 μm in length and were characterized by an outer electron-dense exospore and a thick electron-lucent endospore. Within the endospore, a thin plasma membrane surrounded the polar filament coils and a polaroplast. These features are diagnostic characteristics of microsporidia. The endospore was considerably thinner near the anchoring disk. The polar filament had 11 coils arranged mostly in single rows, although in a few spores double rows were also seen. Three of the coils were slightly smaller than the others, which indicated the polar filaments are anisofilar (Figure 2, panel B). The polar filament coils measured 83.3–102 nm. At higher magnification, the polar filament coil exhibited a lucent ring around a dense core (Figure 2, panel C). A salient feature of the spore was the presence of a diplokaryotic nucleus with 2 nuclei closely opposed in a coffee bean–like appearance. The cytoplasm surrounding the nucleus was densely packed with ribosomes. Additional morphologic features included a posterior vacuole (Figure 2, panel B) and lamellar polaroplast consisting of tightly coiled membranes encircling the polar filament (Figure 2 panel, D).

Molecular analysis was performed on a specimen of human muscle only. Taxonomically, the isolated spores' small subunit of rRNA sequence on BLAST analysis (www.ncbi.nlm.nih.gov/BLAST) was found to be 100% identical (within a 500-nt sequenced fragment) to *Tubulinosema acridophagus* (GenBank accession no. AF024658), which usually infects North American grasshoppers (*Schistocerca americana* and *Melanoplus* spp.). It was also 96% identical to that of *Tu. ratisbonensis* (GenBank accession no. AY695845) obtained from a *Drosophila melanogaster* fruit fly and to *Tu. kingi* (GenBank accession no. DQ019419 and L28966) obtained from a *D. willistoni* fruit fly (Table).

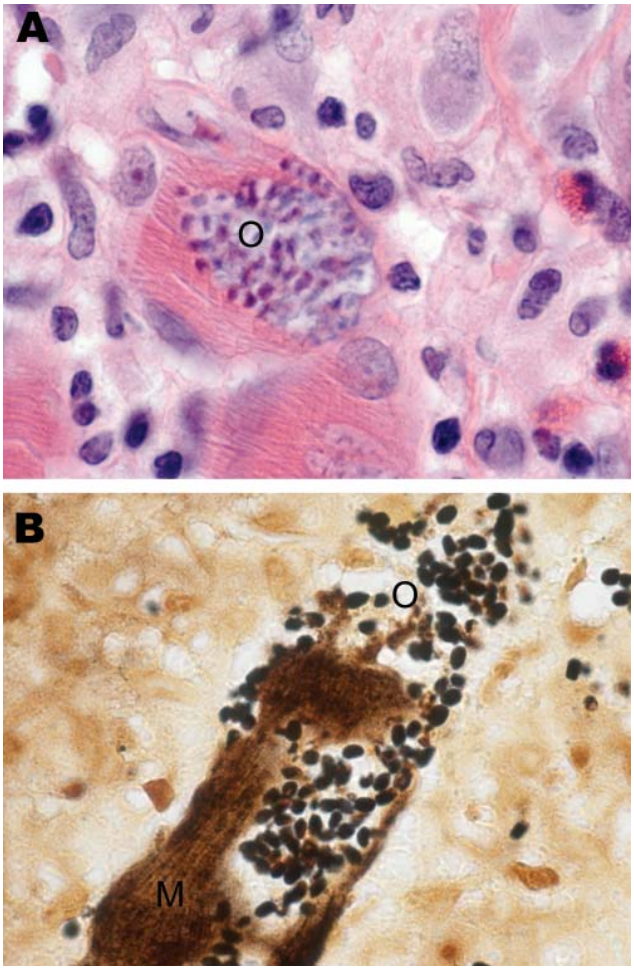

Figure 1. Skeletal muscle tissue samples from a 67-year-old woman with *Tubulinosema* sp. infection, 2009. A) Hematoxylin and eosin stain shows inflamed fibers with mononuclear infiltrate (O). B) Warthin-Starry silver stain shows abundant clusters of ovoid, basophilic organisms (O) within the muscle fibers (M). Original magnifications ×1,000.

Conclusions

We report a case of microsporidian myositis caused by *Tubulinosema* sp. in a patient with chronic lymphocytic leukemia and subsequent Richter's transformation. Franzen et al. in 2005 proposed a new genus and species (*13*), *Tu. ratisbonensis*, for a microsporidium that parasitizes the fruit fly *D. melanogaster*. Subsequently, phylogenetic analyses of ribosomal RNA sequences determined that *Tu. ratisbonensis* was similar to several species included in the genus *Nosema* (*13*). Two other species of *Nosema* (*N. kingi* and *N. acridophagus*), both parasites of insects fitting the generic description of *Tubulinosema*, were transferred to the new genus (*13*). On the basis of limited ultrastructural studies, the microsporidia described here resemble *Tu. acridophagus*, *Tu. kingi*, and *Tu. ratisbonensis* (Table).

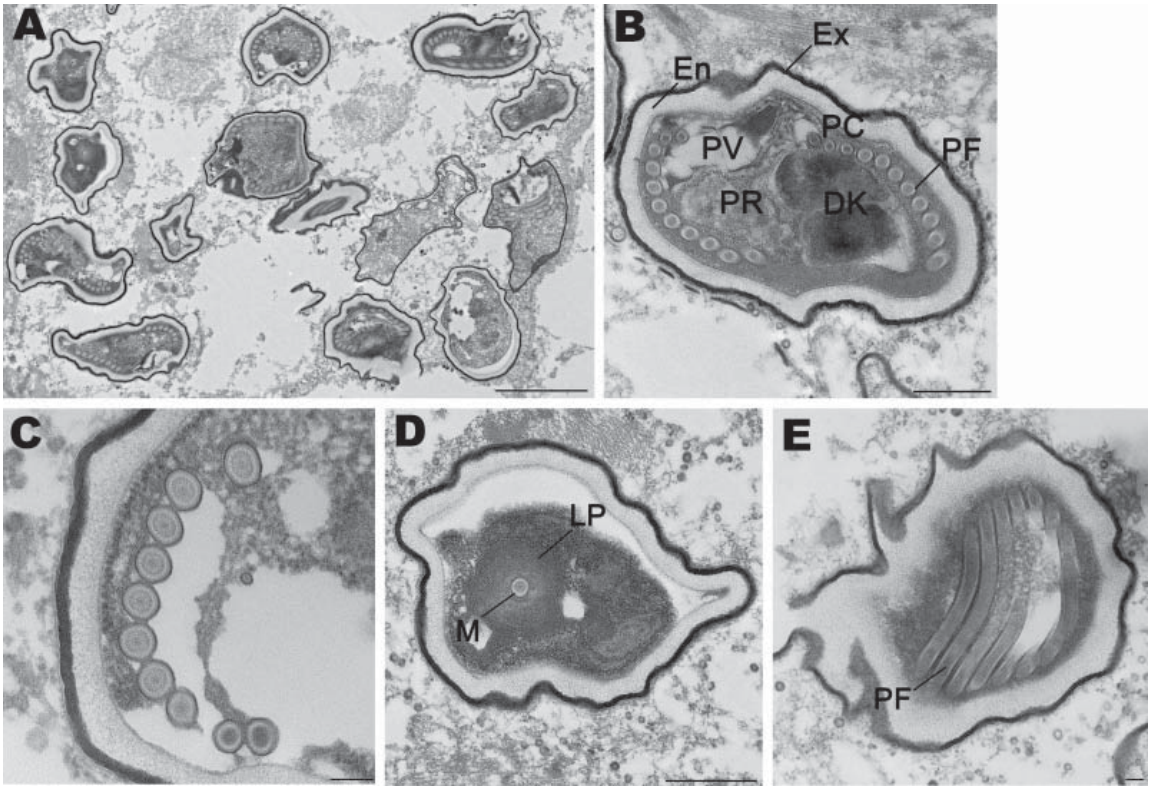

Figure 2. Spores of *Tubulinosema* sp. from a 67-year-old woman with *Tubulinosema* sp. infection, 2009. A) Electron micrograph of numerous spores in various stages in muscle tissue. Scale bar = 2 μm. B) An immature spore showing an electron-dense exospore (Ex) and a thick electron-lucent endospore (En), which together compose the spore wall. Diplokaryon (DK), posterior vacuole (PV), ribosomes in crystalline clusters as polyribosomes (PR) along with the polaroplast and polar filaments (PF) constitute the sporoplasm. A small difference in polar filament diameter in the anterior and posterior coils (PC), with the last 3 or 4 coils being smaller (anisofilar), is also apparent. Scale bar = 500 nm. C) Polar filaments exhibiting a lucent ring around a dense core. Scale bar = 100 nm. D) Cross-section of the manubroid (M), which is the straight portion of the polar filament surrounded by the lamellar polaroplast (LP). Scale bar = 500 nm. E) Longitudinal sections of polar filaments (PF) stained with uranyl acetate-lead citrate. Scale bar = 100 nm.

Moreover, PCR of formalin-fixed, paraffin-embedded tissue showed that it was closely related to *Tu. acridophagus*, a parasite of the fruit fly *D. melanogaster*, suggesting an insect source of this infection.

To the best of our knowledge, before this case microsporidia belonging to the genus *Tubulinosema* had not been associated with human infection. However, members of another genus *Anncaliia* (*Brachiola*), recently classified

Table. Comparison of various *Tubulinosema* spp. with organism isolated from a 67-year old woman in 2009*

Characteristics	*Tu. ratisbonensis*	*Tu. kingi*	*Tu. acridophagus*	2009 isolate
First described	2005	1962	1967	NA
Previously named	NA	*Nosema kingi*	*Visvesvaria acridophagus*, *Nosema acridophagus*	NA
Host in which identified	*Drosophila melanogaster* fruit fly (Diptera)	*D. willistoni* fruit fly	*Schistocerca Americana* grasshopper (Orthoptera)	Human (skeletal muscle)
Known human infections	None	None	None	None
Microtubules on plasma lemma	Present	Present	Present	Not seen
Spore shape	Pear shaped	Oval	Oval	Round-to-pear shaped
Meronts nuclei	1, 2, or 4	1, 2, or 4	1, 2, or 4	Meronts not seen
Sporonts nuclei	2–4	2–4	2–4	Sporonts not seen
Polar tube	9–14	13	10–12	11
Coils/rows	Single	Single/ double (anterior)	Single	Single
Polar filament arrangement	Anisofilar	Isofilar	Isofilar	Anisofilar

*NA, not applicable.

under the same family *Tubulinosematidae* as *Tubulinosema*, have been known to cause myositis and keratitis in humans (*14*). *A. algerae* is a well known parasite of mosquitoes and has also been described as causing infections in humans (*15*). Currently, microsporidia belonging to 8 genera are known to cause human infections (*4*). Therefore, clinicians managing immunodeficient patients who have fatigue, weakness, and other nonspecific symptoms, including unexplainable lesions, should consider microsporidiosis as a possible differential diagnosis.

Dr Choudhary is a second-year resident in internal medicine at the Cleveland Clinic Foundation in Cleveland, Ohio. Her research interests include transplant infections.

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Listeriosis, Taiwan, 1996–2008

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During 1996–2008, a total of 48 patients with listeriosis were identified at a Taiwan hospital. Average annual incidence increased from 0.029 to 0.118 cases per 1,000 admissions before and after January 2005. Serotype 1/2b predominated; serotype 4b emerged since 2004. Food monitoring and disease surveillance systems could help control listeriosis in Taiwan.

Listeria monocytogenes is a gram-positive bacillus that exists in contaminated food and animal products (*1*). Certain serotypes (1/2a, 1/2b, 1/2c, and 4b) are associated with most human diseases and have caused several outbreaks (*1*). Clinical features of human listeriosis include self-limiting gastroenteritis in outbreak cases, spontaneous abortion in pregnant women, and severe infections (sepsis or meningitis) in immunocompromised persons and in elderly persons. In the latter, the case-fatality rate is 20%–30% (*1*). The incidence of nonpregnancy-associated listeriosis has increased recently in Europe despite strict food regulations (*2*,*3*).

In Taiwan, unlike in other Asian countries, human listeriosis was rarely reported, although *L. monocytogenes* has been isolated from local farm products (*4–6*). Recent surveillance of neonatal listeriosis in Taiwan identified an increase in cases after 2000 (*5*). However, there are a paucity of data on serotyping and molecular epidemiology of human listeriosis in Taiwan because the disease is not nationally notifiable (*7*). We investigated nonpregnancy-associated listeriosis in adults, as well as serotyping and genetic relatedness for all isolates identified in our hospital.

The Study

We reviewed the medical records of patients who had *L. monocytogenes* isolated from blood and body fluids from sterile sites during 1996–2008 at the National Taiwan University Hospital (NTUH), a 2,500-bed hospital in Taiwan. Demographic and clinical data of nonpregnant adults with listeriosis were retrieved for further analysis. We evaluated disease severity using modified Acute Physiology And Chronic Health Evaluation II scores (*8*). Incidence of nontyphiodal *Salmonella* bacteremia (NTSB) during 2000–2008 in NTUH was calculated for trend comparison of the 2 foodborne illnesses. Only 1 episode was calculated during the same admission for NTSB to avoid the influence of repetitive bacteremia.

Isolates from patients with fetomaternal listeriosis (i.e., paired isolates from mother and neonate who had listeriosis) were considered to be the same and only 1 of them was analyzed. All isolates were analyzed for their serotype by PCR as described, and genetic relatedness was evaluated by pulsed-field gel electrophoresis (PFGE) by using PulseNet standardized protocols and 2 restriction enzymes (*Asc*I and *Apa*I) (*2*,*9*). Strains were considered to be of the same cluster if their bands had indistinguishable restriction patterns by both enzymes. Strains with PFGE patterns with >80% similarity by *Asc*I and *Apa*I profiles were considered to be closely related. A forward stepwise model with a p value of 0.1 was used, and $p<0.05$ was considered statistically significant in the multivariate Cox proportional hazards model.

During the study period, listeriosis was diagnosed in 48 patients, and 46 nonduplicated isolates were obtained for further microbiological analysis. Average annual incidence increased from 0.0287 cases per 1,000 admissions during 1996–2004 to 0.118 cases per 1,000 admissions during 2005–2008 (Figure 1). The increase in annual incidence of listeriosis was significantly correlated with years ($p = 0.0045$). The average annual incidences of NTSB were 1.189 and 1.118 per 1,000 admissions during 2000–2004 and 2005–2008, respectively; it was not significantly correlated with years ($p = 0.50$). Age-specific incidence of listeriosis increased at both extremes of age, but especially among patients ≥80 years (Figure 2). All of the patients with listeriosis lived in northern Taiwan, and no obvious geographic correlation was observed between listeriosis patients in each year.

We reviewed 43 cases of listeriosis in nonpregnant adults (Table). All 43 patients had underlying predisposing conditions, and 18 (42%) were >65 years of age. Of the 30 patients with malignancies, 23 (77%) developed listeriosis within 4 weeks after receiving chemotherapy.

Among the 46 isolates, serotype 1/2b was identified most frequently (46%), followed by 1/2a (28%) and 4b (26%). No serotype 1/2c was detected by PCR. Serotype 4b was noted beginning in 2004. PFGE results showed no shared pulsotypes. All 46 isolates were susceptible to ampicillin, ertapenem, meropenem, and vancomycin; 3 isolates were intermediately susceptible to trimethoprim/sulfamethoxazole; and 4 isolates were nonsusceptible to linezolid (*10*).

The all-cause death rate at day 14 of hospitalization was 28%. Sixteen (37%) patients received cephalosporin

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DOI: http://dx.doi.org/10.3201/eid1709.110093

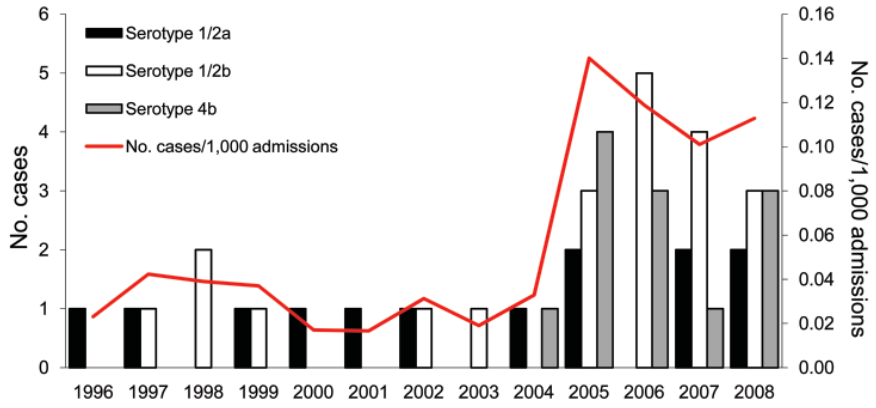

Figure 1. Incidence (cases per 1,000 admissions) of human listeriosis and serotype distribution of all isolates, National Taiwan University Hospital, Taipei, Taiwan, 1996–2008. Forty-six isolates were available for analysis, including 2 serotype 4b isolates from fetomaternal transmission in 2005 and 2006 and 1 serotype 4b from a pediatric patient (2005). Isolates from fetomaternal transmission were considered to be the same.

alone as initial treatment regimens and empirical treatment was effective for only 14 (33%). The presence of solid-organ malignancies was a significant negative prognostic factor for 14-day mortality in the univariate analysis (95% confidence interval [CI] 1.165–16.694; p = 0.029) but not in the multivariate analysis. The results of the multivariate analysis for 14-day mortality showed that hepatic decompensation at disease onset was a significant negative prognostic factor (hazard ratio 12.02, 95% CI 1.842–78.470; p = 0.009) and that the use of effective antimicrobial drugs after culture results were reported was a significant positive prognostic factor (hazard ratio 0.014, 95% CI 0.002–0.131; p<0.001). Log-rank tests performed to compare the difference in survival between patient groups for the 2 variables also had the same results (p<0.001).

Conclusions

We observed an upsurge of listeriosis beginning in 2005 in NTUH. The increase might not be attributable to common-source outbreaks because no clustering was detected. The annual incidence of listeriosis has been on the rise in Europe since 2000 (*2*,*3*,*11*). The reason is not clear because the increase could not be attributed to outbreak clusters and no increase in pregnancy-related listeriosis was observed (*2*,*3*).

L. monocytogenes isolates are not uncommon in domestic food products in Taiwan. Wong et al. found that *L. monocytogenes* was isolated in >50% of pork samples and chicken carcasses (*6*). Semiready foods (dumplings and meatballs) and frozen dim sum examined also carried the pathogen (34.0% and 4.4%, respectively), and >60% of the isolates were serotype 1 or 4 (*6*). If served undercooked, these foods could be potential transmission sources. Taiwan currently has no strict regulatory policy regarding listeriosis in the food industry and no disease surveillance system.

In France, serotype 4b was the predominant serotype (42%–56%), whereas serotype 1/2b was more common (46%) in our study (*2*). Detection of resistance is not routinely performed in most laboratories. In our study, none of the isolates were resistant to the tested agents, except for 4 isolates, which were resistant to linezolid. The clinical efficacy of these new agents should be carefully evaluated.

The contributions of disease severity and antimicrobial drug treatment are difficult to evaluate in population-based studies (*12*). Brouwer et al. reported that up to 30% of adults with *L. monocytogenes* meningitis did not receive initial adequate antimicrobial drug therapy, and they found no association between that variable and outcome (*13*). A high proportion (37%) of patients in our study also received inadequate antimicrobial drug therapy initially. Initial disease severity and initial adequate antimicrobial drug therapy was not associated with overall mortality. We found that mortality was related to hepatic decompensation and effective antimicrobial drug therapy after culture results were reported. However, further studies comprising larger patient populations are necessary to confirm our findings.

Our data were based on patients in a single hospital. Therefore, the incidence of and risk factors for human listeriosis in Taiwan could not be determined precisely,

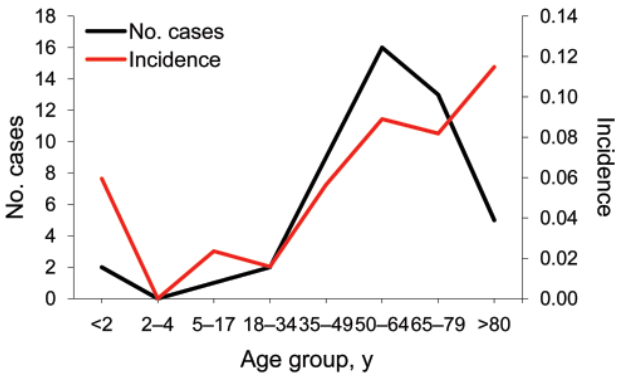

Figure 2. Patient distribution and incidence of human listeriosis, by age group, National Taiwan University Hospital, Taipei, Taiwan, 1996–2008.

Table. Demographic data for 43 nonpregnant adults with listeriosis, National Taiwan University Hospital, Taipei, Taiwan, 1996–2008*

Characteristic	No. (%) patients, n = 43
Sex	
M	29 (67)
F	14 (33)
Underlying condition	
Steroid use >0.5 mg/kg/day	19 (44)
Diabetes mellitus	11 (26)
Surgery in previous 2 mo	10 (23)
Peptic ulcer disease with or without bleeding	9 (21)
Liver cirrhosis†	9 (21)
Renal insufficiency‡	8 (19)
Alcoholism	4 (9)
Ulcerative colitis	2 (5)
Antiphospholipid syndrome	1 (2)
Hematologic malignancy	15 (35)
Multiple myeloma	5 (12)
Non-Hodgkin lymphoma§	5 (12)
Leukemia	4 (9)
Myelodysplastic syndrome	1 (2)
Solid cancer	19 (44)
Lung cancer	4 (9)
Malignancy of unknown primary hepatocellular carcinoma	3 (7)
Gastric cancer§	2 (5)
Colorectal cancer§	2 (5)
Bladder cancer§	2 (5)
Ovarian cancer	2 (5)
Chemotherapy within 4 weeks	23 (53)
Diagnosis¶	
Bacteremia	29 (67)
Meningitis/meningoencephalitis	8 (19)
Spontaneous bacterial peritonitis	4 (9)
Brain abscess	2 (5)
Crude mortality rate	
7 d	11 (26)
14 d	12 (28)

*Mean age, y, ± SD: 62.12 ± 15.10.
†Including 4 patients with hepatic decompensation.
‡Including 3 patients with end-stage renal insufficiency who were receiving hemodialysis.
§One female patient had coexisting non-Hodgkin lymphoma and colon cancer; 1 male patient had coexisting non-Hodgkin lymphoma, gastric cancer, and bladder cancer.
¶Initial modified Acute Physiology And Chronic Health Evaluation II score, mean ± SD: 18.8 ± 7.3.

and potential outbreaks might have been overlooked. The retrospective design of our study limited the possibility of identifying the potential vehicles and disease-acquiring behaviors.

An increase of listeriosis was noted since 2005 in our hospital, and all of the affected patients had predisposing factors that hampered their immunity. Dietary education and food management information should be provided to high-risk groups in Taiwan. Food monitoring and human disease surveillance systems need to be established in Taiwan to control this potentially fatal foodborne disease.

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Tattoo-associated *Mycobacterium haemophilum* Skin Infection in Immunocompetent Adult, 2009

Meagan K. Kay, Tara R. Perti, and Jeffrey S. Duchin

After a laboratory-confirmed case of *Mycobacterium haemophilum* skin infection in a recently tattooed immunocompetent adult was reported, we investigated to identify the infection source and additional cases. We found 1 laboratory-confirmed and 1 suspected case among immunocompetent adults who had been tattooed at the same parlor.

Mycobacterium haemophilum, a nontuberculous mycobacterial species, typically affects immunocompromised persons. It produces subcutaneous nodules, papules, and pustules; less commonly it produces septic arthritis, osteomyelitis, pneumonitis, and disseminated infection (*1,2*). This organism causes lymphadenitis in healthy children (*3*) but rarely affects immunocompetent adults (*4*). Although other species of nontuberculous mycobacteria, predominantly rapidly growing species, have been associated with wound infections, cosmetic surgery, body piercing, and tattooing (*5–7*), *M. haemophilum* infection rarely has been reported as a complication of tattooing (*8,9*).

In November 2009, Public Health–Seattle and King County was notified of a chronic skin infection in an immunocompetent adult who had been recently tattooed; *M. haemophilum* had been isolated from the patient's skin lesions. We investigated to characterize the clinical features of the case, determine the source of the infection, and identify additional cases.

The Study

In August 2009, a healthy 44-year-old man (patient 1) received a tattoo on his left forearm at a commercial tattoo parlor. Three days later, a painless rash developed at the tattoo site. He applied antibacterial ointment, but the rash did not resolve; 12 days after rash onset, he sought care from his health care provider. The patient denied fever and other focal or constitutional symptoms. Erythematous nodules of 3–5 mm diameter in the region of the tattoo were noted, and the patient was given ceftriaxone and trimethoprim/sulfamethoxazole for presumed pyogenic infection. Two weeks later, the lesions were unimproved. Aerobic culture of the lesions was conducted and clindamycin was prescribed; no organisms grew from the culture. In mid-September, the patient again visited his health care provider because the nodules remained unimproved. Ceftriaxone was administered, and oral cephalexin was prescribed; an aerobic bacterial culture was repeated. Two weeks later, the numerous nodular pustules confined to the tattoo region remained (Figure).

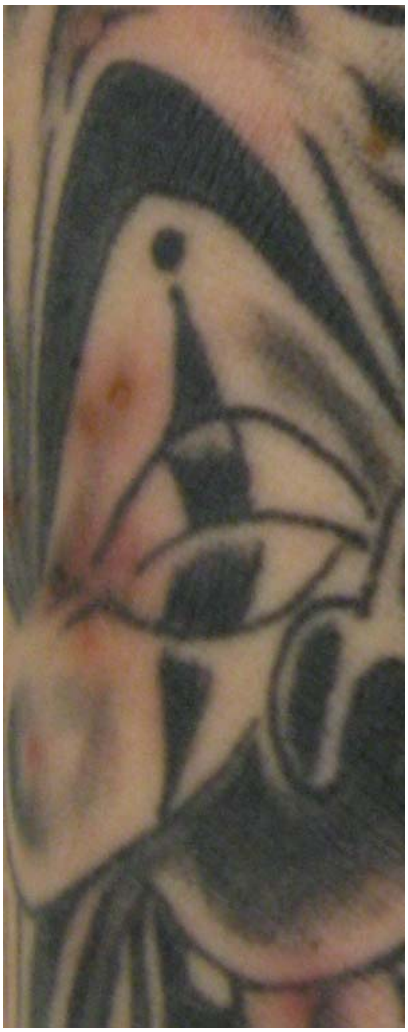

Figure. Pustular rash caused by *Mycobacterium haemophilum* confined to the tattooed region of the forearm. Photograph taken in October 2009, two months after tattooing. Expanded photograph available at www.cdc.gov/EID/content/17/9/102011-F.htm.

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DOI: http://dx.doi.org/10.3201/eid1709.102011

Test results for hepatitis B and C viruses and HIV were negative. A swab of purulent material from 2 pustules was submitted for aerobic bacterial and fungal culture, an acid-fast bacilli (AFB) culture and smear, and a varicella-zoster virus direct fluorescent antibody assay and culture; clindamycin was prescribed. Samples were spread onto Middlebrook and chocolate agar plates and incubated at 30°C and onto Middlebrook agar plates and incubated at 37°C. After 3 weeks, AFB were recovered from only the plates incubated at 30°C. Using 16S rRNA gene sequencing, we identified the isolates as *M. haemophilum*. The organism was sensitive to clarithromycin (≤15 μg/mL), rifampin (≤1 μg/mL), trimethoprim/sulfamethoxazole (≤0.5/9.5 μg/mL), amikacin (≤12 μg/mL), linezolid (≤6 μg/mL), ciprofloxacin (≤2 μg/mL), and moxifloxacin (≤5 μg/mL) (*10*).

In December 2009, treatment with rifampin, ciprofloxacin, and clarithromycin was initiated. In February 2010, the rash had improved, although healing papules and erythema were still present. In March 2010, the patient discontinued treatment because of nausea. By May 2010, the lesions had healed.

In mid-October 2009, the same health care provider evaluated a healthy 35-year-old man (patient 2) with a pustulo-nodular skin infection confined to shaded areas in a tattoo received in August 2009 at the same tattoo parlor. During November–December 2009, standard aerobic bacterial or mycobacterial cultures from this patient's lesions were performed, but no organisms were recovered. We considered this to be a suspected case.

During December 2009, both patients were interviewed; no other potential epidemiologic links were identified. Each patient denied exposure to recreational water, aquarium water, water with rusty sediment, or any other potential skin irritants.

To identify additional *M. haemophilum* cases, Public Health–Seattle and King County asked physicians to report atypical skin infections that developed after receipt of tattoos performed during June 1–December 1, 2009, and asked clinical laboratories to report atypical mycobacterial species recovered during the same period. No additional cases were identified.

During an investigation of the tattoo parlor on December 10, 2009, the operator reported having used similar procedures to tattoo each patient. No deviations from Washington State safety and sanitation standards were recognized (*11*). Municipal water was used in a rinse solution applied during and after tattooing and to dilute ink for shading. Eleven environmental samples collected during the site visit included ink (1.5 L); tap water (1.5 L); liquid soap (1 L); petroleum jelly; and swabs of equipment, the soap dispenser port, and the tip of a reusable black-ink container. All samples were submitted to the Centers for Disease Control and Prevention (Atlanta, GA, USA) for mycobacterial culture; no mycobacteria were recovered. The tattoo parlor operator was instructed to use only sterile water for rinse solutions and dilution of tattoo dye.

Conclusions

Although the infectious agent was confirmed by culture for patient 1 only, the infection for patient 2 was consistent with *M. haemophilum* infection and patient 2 was epidemiologically linked to patient 1. The nonspecific rash that developed 3 days after tattooing for patient 1 might be unrelated to *M. haemophilum*; however, the development of pustular nodules after 2 weeks is consistent with the incubation period for this infection. Although punch biopsies are typically required for diagnosis of nodular lesions, *M. haemophilum* was cultured from a swab of the lesions. The pustules were similar to those previously reported for tattoo-associated *M. haemophilum* infection (*8*) and might be associated with the presumed mode of inoculation.

Although the environmental reservoir for *M. haemophilum* is unknown, the organism is thought to be widespread in the environment (*2*). Water has been a suspected reservoir because of the epidemiology of other environmental mycobacteria and because *M. haemophilum* has been detected by PCR in biofilms from research aquariums (*12*). However, in most investigations, culture of *M. haemophilum* from environmental samples has been futile (*2,5*). The interval of >4 months between the time patient 1 was tattooed and the environmental sample was collected might have further reduced the likelihood of recovering *M. haemophilum*. Molecular methods such as PCR might be more successful than culture alone for detecting *M. haemophilum* infections.

No tattoo industry standards exist for the practice of diluting tattoo ink. Washington State does not specifically require tattoo artists to use steam-distilled or sterile water when rinsing needles or diluting ink; tap water is often used (*11*). However, legislation enacted in July 2010 prohibits mixing ink and pigments with improper ingredients (*11*). Although infections attributable to water appear uncommon, we advise against using tap water for tattoo procedures.

Treatment for *M. haemophilum* infection among immunocompetent adults should be based on that used for immunocompromised patients for whom multidrug regimens, including clarithromycin, rifampin, rifabutin, and ciprofloxacin, are recommended (*1,2,13*). Agents that seem to be active in vitro are amikacin, clarithromycin, ciprofloxacin, rifampin, and rifabutin (*1,14*). Isolates have variable susceptibility to doxycycline and sulfonamides and are typically resistant to ethambutol, isoniazid, and pyrazinamide (*1,13*). However, because no standardized

methods for assessing antimicrobial drug susceptibility of *M. haemophilum* exist, in vitro susceptibility data must be used with caution.

Clinicians should consider *M. haemophilum* in the differential diagnosis of skin infections after tattooing, particularly chronic skin infections that are unresponsive to treatment with antimicrobial drugs, regardless of the patient's immune status. *M. haemophilum* infections can be difficult to diagnose because the organism is slow growing and fastidious and requires iron supplementation and a lower incubation temperature for growth (30°–32°C) than other mycobacteria (*15*). Laboratory practices vary, and hemin might not be routinely added to all AFB cultures. Therefore, for suspected cases, clinicians should alert the laboratory to use appropriate procedures to culture for *M. haemophilum* and other AFB.

Acknowledgments

We thank Heather O'Connell and Brandi Limbago for their help with testing of environmental samples, the staff at the University of Washington Medical Center Clinical Microbiology Laboratory for their assistance with identifying the organism from clinical specimens, and Troy Amundson for valuable assistance.

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Pandemic (H1N1) 2009 Transmission during Presymptomatic Phase, Japan

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During an epidemiologic investigation of pandemic influenza (H1N1) 2009 virus infection in May 2009 in Osaka, Japan, we found 3 clusters in which virus transmission occurred during the presymptomatic phase. This finding has public health implications because it indicates that viral transmission in communities cannot be prevented solely by isolating symptomatic case-patients.

The first indigenous cases of pandemic (H1N1) 2009 in Japan were detected in Kobe City (*1*) and Osaka Prefecture on May 16, 2009. In response to the outbreak, the National Institute of Infectious Diseases, Infectious Diseases Surveillance Center, and its Field Epidemiology Training Program began epidemiologic investigations in both areas. Clinical manifestations of these infections were described in 2 previous articles (*2*,*3*).

In general, pandemic influenza (H1N1) 2009 virus is considered to be infectious during patients' presymptomatic phase (*4*,*5*). However, to our knowledge, no epidemiologic studies about infectiousness during the presymptomatic phase have been reported. The aim of this study was to provide scientific evidence to ascertain the infectious period of pandemic influenza (H1N1) 2009 through epidemiologic investigation in Osaka, Japan.

The Study

We began an epidemiologic investigation in Osaka on May 17, 2009, and conducted face-to-face interviews. Thirty-six confirmed cases had occurred by May 22. The definition of a confirmed case-patient was a person with influenza-like illness (ILI) and laboratory confirmation of pandemic (H1N1) 2009 virus infection by real-time reverse transcription PCR. ILI was defined as the presence of a fever (≥38.0°C) and acute respiratory symptoms (cough or sore throat). Local public health staff also conducted face-to-face or telephone interviews, or both, with other patients and persons in close contact with the case-patients until the end of May, when the local epidemic appeared to wane (*1*,*6*). Interviews were conducted by using a standard questionnaire to collect data on patient demographics, clinical course of illness, patient behavior, and history of patient contacts. By analyzing these data and careful investigation of contact history and epidemiologic links, we made a transmission tree. During the epidemiologic investigation, we found 3 clusters in which disease transmission could have occurred before symptom onset in the index case-patients (Figure).

The first outbreak in Osaka occurred in 1 high school, and no community transmission had been verified in mid-May. Case-patient 1, a student at the high school, had symptoms on May 13. Case-patient 2, the sister of case-patient 1, had no symptoms while she was at school, according to her answer on the self-report survey as well as face-to-face interview carried out with a classmate who sat right behind her (case-patient 3). Symptoms developed in the evening of May 15 after she returned home, and the school was closed proactively the following day. She later received a diagnosis of infection with the virus. No other students or staff had ILI symptoms at that time. For case-patient 3, who sat immediately behind case-patient 2 in class and chatted with her, influenza-related symptoms developed on May 17. Our investigation suggested that transmission of infection from case-patient 2 to case-patient 3 occurred during the presymptomatic phase of infection in case-patient 2.

Case-patients 4, 5, and 6 were previously healthy boys and schoolmates of case-patients 2 and 3. They did not have contact with anyone with ILI symptoms at school or

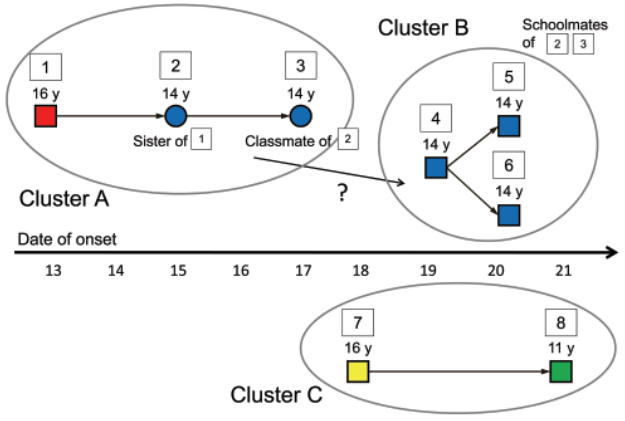

Figure. Three clusters of pandemic (H1N1) 2009 presymptomatic transmission in May 2009 in Osaka, Japan. All cases were confirmed as pandemic (H1N1) 2009 virus infection by real-time reverse transcription PCR. Squares indicate male case-patients, and circles indicate female case-patients. Colors of the squares and circles denote the similar or different schools the students attended.

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DOI: http://dx.doi.org/10.3201/eid1709.101411

elsewhere. They played video games together in a small room on May 18 for several hours. They took turns using the same game consoles and chatted together. Case-patient 4 became febrile on the morning of May 19. He may have been infected by case-patient 2 at school, but we cannot confirm this. Case-patients 5 and 6 became febrile on May 20. They had no opportunity to be in contact with symptomatic persons other than case-patient 4. Thus, we concluded that case-patients 5 and 6 were likely infected by case-patient 4 before symptom onset. Even if transmission from case-patient 2 were the reason that case-patients 5 and 6 became ill, transmission from a presymptomatic person likely occurred because case-patient 2 would have been asymptomatic when she met case-patients 5 or 6, or both, at school.

Case-patient 7 attended a different high school than case-patients 1–6. Case-patient 8 was an elementary school pupil who lived in a different region from case-patients 1–7. No cases of pandemic (H1N1) 2009 had been reported in the area where he lived, including at his school, in mid-May.

Investigation results indicate that case-patient 7 was infected by a classmate other than case-patients 1–8 around May 16; this classmate was infected by a student at the same high school that case-patient 1 attended. On May 17, ≈20 persons, including case-patients 7 and 8, went on a 1-day trip to Okayama Prefecture, which is ≈180 km west of Osaka. The trip took ≈3 hours by train, which was not crowded. No positive results were reported by active surveillance for ILI in Okayama in mid-May. Case-patients 7 and 8 stayed close together during the trip. In the evening of May 18, case-patient 7 had a sore throat and cough, and he became febrile on May 19. Case-patient 8 had symptoms on May 21, 3 days after case-patient 7 exhibited symptoms. No one around them had respiratory symptoms during the trip. The trip was the only opportunity for case-patient 8 to have been exposed to the virus. This indicates that transmission from case-patient 7 to case-patient 8 occurred on the day before symptom onset.

Conclusions

Our epidemiologic investigation results indicate that pandemic influenza virus (H1N1) 2009 is infectious during the presymptomatic phase. Our investigation was conducted in the early phase of the outbreak in Japan. During this period, almost all persons with reported cases in Osaka were students and their close contacts (*1*). Surveillance data from the Osaka Prefectural Institute of Public Health supported this finding. The institute had been conducting active surveillance for patients with ILI and suspected pandemic (H1N1) 2009 virus infection since April 2009 (*7*). Since mid-June, many community-transmitted cases had been reported in Japan, thereby limiting the ability to find single exposure cases. Furthermore, most cases were no longer laboratory confirmed. As such, our results are valuable in terms of collecting information before the community spread of the virus.

The mode of transmission of pandemic (H1N1) 2009 is considered to be similar to that of seasonal influenza. The infectious period of seasonal influenza is measured from 1 day before symptom onset to 5–7 days after onset or until symptoms resolve; this period is mainly based on experimental data on viral shedding (*4*,*5*). Regarding seasonal influenza, we found only 1 report documenting that a transmission took place during the presymptomatic phase, but the index case-patient of the report could have been in a "prodromal," not "presymptomatic," state because this case-patient did not feel completely well when the transmission took place (*8*).

Our epidemiologic results provide useful clues for understanding the transmission of pandemic (H1N1) 2009 virus. This finding of presymptomatic transmission has critical implications for public health because it indicates that viral transmission in communities cannot be completely prevented solely by isolating symptomatic case-patients.

Two possible limitations of this report are the presence of unidentified case-patients and asymptomatic infected persons because our epidemiologic investigation was based on interviews with symptomatic patients and their close contacts. However, we could not find any cases that suggested infection from these kinds of cases during the early phase of the outbreak in Japan. Additionally, social attention was very high during the period.

In conclusion, our epidemiologic investigation results suggest that pandemic (H1N1) 2009 is probably infectious during the presymptomatic period, at least 1 day before symptom onset. Our results are consistent with the description of the infectious period of the virus.

Acknowledgments

We are grateful for the cooperation and support of the residents of both cities and staff at Osaka Prefectural Institute of Public Health, Osaka Prefecture Public Health Centers, Osaka City Public Health Center, Sakai City Public Health Center, and Takatsuki City Public Health Center. In addition, we appreciate the valuable comments and input provided by Francisco Averhoff and Daphne Copeland.

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Increased Extent of and Risk Factors for Pandemic (H1N1) 2009 and Seasonal Influenza among Children, Israel

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During the pandemic (H1N1) 2009 outbreak in Israel, incidence rates among children were 2× higher than that of the previous 4 influenza seasons; hospitalization rates were 5× higher. Children hospitalized for pandemic (H1N1) 2009 were older and had more underlying chronic diseases than those hospitalized for seasonal influenza.

We compared the extent and pattern of pandemic (H1N1) 2009 with the previous 4 influenza seasons (2005–2009) among Israel's child population, both for community-based surveillance and pediatric hospitalizations. We also sought a possible association between the pandemic waves and schools closure. The study was approved by the Institutional Review Board Committee of Hadassah Medical Center.

The Study

Israel's Center for Disease Control seasonal influenza surveillance system operated throughout our 5-year study. The system is based primarily on 1) anonymous patient visits for influenza-like illnesses (ILI) to Maccabi Community Clinics, Israel's second largest health maintenance organization, insuring ≈1 of every 4 Israelis; and 2) nasopharyngeal swabs from sample ILI patients at designated sentinel clinics countrywide. ILI was defined as fever (≥37.8°C) with ≥1 of the following: cough, coryza, sore throat, or myalgia. Swab samples were tested for influenza viruses at the Health Ministry's Central Virology Laboratory (*1*) by using multiplex real-time reverse transcription PCR (RT-PCR) (TaqMan chemistry quantitative RT-PCR) (*2*).

ILI rates constituted 3 escalating waves of infection, all at times atypical for seasonal influenza (Figure 1). The first peaked early August (week 32). Israel's schools close July/August, but children stay together in summer frameworks during July. Wave 2 peaked mid-September (week 38), 2 weeks into the school year, declining when schools closed for holidays until the end of week 41. During week 42, the third, largest wave began, peaking in mid-November (week 46).

The cumulative incidence (cases/10,000 population) of ILI in children 0–18 years of age during the pandemic (week 25, 2009 to week 7, 2010) was 369.3 (95% confidence interval [CI] 365.7–373.1), far higher than average rates documented in earlier influenza seasons (143.4, 95% CI 140.7–146.2). Incidence was 295.8 (95% CI 285.7–306.1) for children <2 years of age, 347.1 (95% CI 338.4–355.9) for children 2–4 years of age, and 389.4 (95% CI 382.5–391.4) for children 5–18 years of age, compared with 107.8 (95% CI 100.9–115.1), 179.5 (95% CI 172.5–186.7), and 140.2 (95% CI 140.7–146.2), respectively, for each age group in earlier seasons.

Israel identified its first pediatric pandemic (H1N1) 2009 cases in June 2009 (week 24) and recorded local transmission the following week (Figure 2). During weeks 28–43, the weekly percentage of positive influenza samples among children was 40%–60%, peaking at

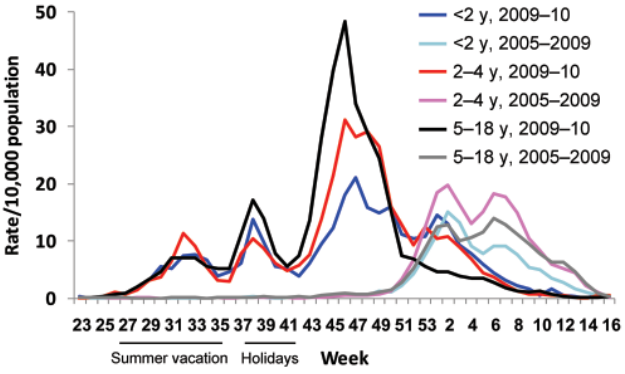

Figure 1. Rates of weekly visits to community health clinics for influenza-like illness, by age group, June 2009–April 2010, compared with the 2005–2009 average (Maccabi Health Services), with school holidays indicated, Israel.

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DOI: http://dx.doi.org/10.3201/eid1709.102022

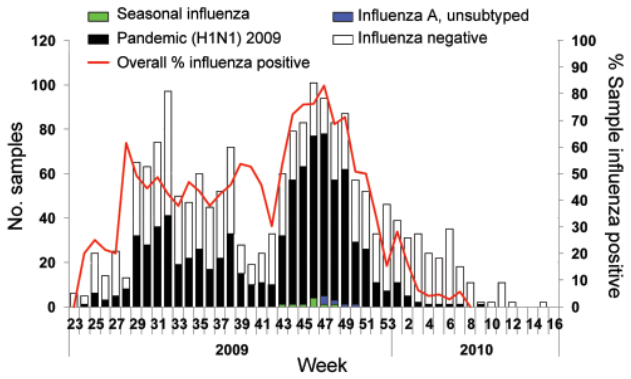

Figure 2. Positive influenza samples among total samples collected by the sentinel network, children 0–18 years of age, Israel, June 2009–April 2010.

70%–80% during weeks 44–49 (late October to early December). This finding correlates with the return to school for a continuous period.

Pandemic (H1N1) 2009 in Israel was present for 36 weeks; the overall percentage of positive influenza samples among children was 50%. Earlier influenza seasons were shorter (13–22 weeks) but with similar overall positive percentages (52%–57%) of influenza A/B. Most (98%) of the influenza cases during 2009 were the pandemic strain; in previous years a mixture of various influenza A/H1N1, H3N2, and B strains occurred.

We compared hospitalization of children with laboratory-confirmed influenza infection during the pandemic with the previous 4 influenza seasons in the pediatric departments of Hadassah's 2 hospitals in Jerusalem. These departments provide primary medical care for ≈250,000 children (1 of every 10 children in Israel), as well as tertiary care for chronic diseases. We performed our study at these hospitals because respiratory specimens were routinely taken year-round for laboratory confirmation from all children with suspected influenza or respiratory virus infection during the 5-year study. Direct immunofluorescence assay was used at Hadassah in previous years for detection of influenza and other respiratory viruses and multiplex real-time PCR (TaqMan chemistry quantitative RT-PCR) for detection of influenza viruses during the pandemic.

Findings from pandemic (H1N1) 2009 were retrospectively compared with those from previous influenza seasons. Two-sample *t*-tests and the Mann-Whitney nonparametric tests compared continuous variables. Categorical variables were compared with χ^2 and the Fisher exact tests. All tests applied were 2-tailed; a p value <0.05 was considered statistically significant.

During June 29, 2009–January 25, 2010, a total of 127 children were admitted to Hadassah hospitals with documented pandemic (H1N1) 2009 infection; most hospitalizations (77 of the total 890 children hospitalized during that period) occurred during the third peak, October–November 2009. Table 1 summarizes the major clinical manifestations for the 127 children; 33/124 (26.6%) had leukocyte counts <5,000 cells/mm^3. All patients received oseltamivir treatment; 1 child in whom resistance to oseltamivir developed was given zanamivir. Children ≥2 years of age had a significantly higher rate of underlying illness compared with children <2 years of age (49/79 [62.0%] vs. 17/48 [35.4%]; p = 0.006). All survived, including 2 children who were mechanically ventilated (11 and 25 days, respectively) and another 6 who required intensive care. Nationwide, 9 children (median age 12.5 years) died from pandemic (H1N1) 2009, a mortality rate of 3.69/1,000,000. No deaths were reported during previous influenza seasons, including among infants, for which reporting is mandatory.

In previous shorter influenza A/B seasons, fewer children were hospitalized; none were treated with antiviral agents, and statistically significant differences included age, underlying chronic diseases, underlying chronic lung disease, and neonatal fever as the initial symptom (Table 2). No significant differences were found regarding history of prematurity (<33 weeks), weight percentile, pediatric intensive care unit admission, evidence of pneumonia, oxygen saturation ≤90%, and leukopenia. In previous seasons, 6 nosocomial influenza infections and 2 co-infections with respiratory syncytial virus were reported; none were seen for pandemic (H1N1) 2009.

Conclusions

Children, mainly those 5–10 years of age, were affected by pandemic (H1N1) 2009 markedly more so than by seasonal influenza, similar to results reported from the United States, Spain, and Switzerland (*3–6*). During the 1918 Spanish influenza pandemic, the highest incidence rates were among older children (*7*). In our study, hospitalized children infected with pandemic (H1N1) 2009 were older, and findings were compatible with reports from several other countries (*8,9*), but

Table 1. Major clinical manifestations in 127 children hospitalized with pandemic (H1N1) 2009 infection, Hadassah University Hospitals, Israel, June 29, 2009–January 25, 2010

Symptom/finding	No. (%) patients
Fever (>38°C)	119 (93.7)
Cough	86 (67.7)
Decreased appetite	49 (38.6)
Weakness	47 (37.0)
Rhinorrhea	43 (33.9)
Vomiting/nausea	43 (33.9)
Oxygen saturation ≤90%	36 (28.3)
Dyspnea	34 (26.8)
Diarrhea	28 (22.0)
Abdominal pain	27 (21.3)

Table 2. Comparison of children hospitalized at Hadassah University Hospitals, Israel, during pandemic (H1N1) 2009 and during influenza seasons for 2005–2009*

Parameter	Pandemic (H1N1) 2009	Seasonal influenza	p value
No. patients during 1 season	127	Median 20 (range 18–39)†	NA
Children with influenza A/B	127/0	68/28	NA
Duration of hospitalization, wks	34	Median 16 (range 13–22)	NA
No. (%) children who received antiviral treatment	98/127 (77.2)	0/96	<0.001
Patient age, mo, median (range)	51 (0.23–197.00)	6 (0.3–206.0)	<0.001
No. (%) children with underlying chronic diseases	66 (52.0)	24 (25.0)	<0.001
No. (%) children with underlying chronic lung disease	39/127 (30.7)	14 (14.6)	0.007
No. (%) children with neonatal fever as initial symptom	6/127(4.7)	26 (27.1)	<0.001

*NA, not applicable.
†Total for all 4 years = 96.

findings were unlike those from Argentina, where 60% were infants (*9*). The age of children who died in Israel also underlines the impact on older children, as reported elsewhere (*10,11*). Although pandemic (H1N1) 2009 virus may cause severe, life-threatening disease in previously healthy children of all ages (*12*), the children we studied had significantly more underlying chronic diseases than did children hospitalized for seasonal influenza (*13*).

We, like others (*3*), found no increase in pneumonia or pediatric intensive care unit admissions caused by pandemic (H1N1) 2009. However, this finding could be because antiviral therapy was administered during the pandemic but not in previous years; 98/127 (77.2%) of children hospitalized for pandemic (H1N1) 2009 received oseltamivir (Table 2).

The nationwide pandemic (H1N1) 2009 influenza mortality rate in Israel is similar to that reported for the United Kingdom (*14*) but cannot be compared with previous years because laboratory data are lacking and there was no requirement to report the death of children >12 months of age. Our study is limited in that it was retrospective. During the pandemic, parents were advised not to attend the clinic for mild disease, although anxiety may have increased visits. There may have been differences between diagnoses of ILI among different Maccabi physicians. The 2 hospitals studied, which represented 10% of hospitalized children, were selected not as nationally representative but because of the feasibility of viral diagnosis since 2005. Influenza detection during the pandemic in patients hospitalized at Hadassah was based on PCR; immunofluorescent antibody assay was used for previous seasons.

Awareness that pandemic influenza may have unique clinical characteristics, risk factors, and increased incidence, mainly among children 5–18 years of age, is advocated. Because school opening in late summer 2009 triggered the wave of pandemic (H1N1) 2009 influenza (*15*), closing or delaying opening schools until vaccine is available should be considered among mitigation strategies in future influenza pandemics, especially for more virulent viruses**.**

Acknowledgments

We thank Maccabi Health-Care Services for providing anonymous data regarding outpatient visits for ILI and thank all physicians and nurses who collected samples from ILI outpatients for laboratory confirmation of influenza viruses throughout the study as part of the influenza surveillance network of Israel's Center for Disease Control. We also thank Zalman Kaufman who helped analyze these data.

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Hospitalized Patients with Pandemic (H1N1) 2009, Kenya

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To describe the epidemiology and clinical course of patients hospitalized with pandemic (H1N1) 2009 in Kenya, we reviewed medical records of 49 such patients hospitalized during July–November 2009. The median age (7 years) was lower than that in industrialized countries. More patients had HIV than the general Kenyan population.

Since pandemic (H1N1) 2009 influenza virus emerged in April 2009, virus-associated hospitalizations and deaths have been reported in many countries (*1*). However, little is known about severe cases of pandemic (H1N1) 2009 in sub-Saharan Africa. We describe the epidemiology, clinical characteristics, and clinical course of the disease in patients hospitalized with laboratory-confirmed pandemic (H1N1) 2009 infection in Kenya during July–November, 2009.

The Study

Following detection of pandemic (H1N1) 2009 virus in 2 patients in California, USA, in April 2009, the Kenyan Ministry of Health intensified surveillance at 26 existing influenza sentinel surveillance sites where surveillance was being conducted for influenza-like illness and severe acute respiratory illness. An additional 29 hospitals and clinics in the country were trained to conduct surveillance for these conditions. A hospitalized case-patient with pandemic (H1N1) 2009 was defined as a patient hospitalized for ≥24 hours with acute respiratory illness who had an oropharyngeal or nasopharyngeal swab specimen positive for pandemic (H1N1) 2009 virus by real-time reverse transcription PCR at either the Kenya Medical Research Institute (KEMRI)/National Influenza Center laboratory, or the KEMRI/Centers for Disease Control and Prevention, Kenya laboratory. Testing was performed according to World Health Organization methods (*2*).

We reviewed all available medical records for hospitalized patients with laboratory-confirmed pandemic (H1N1) 2009 using a standardized case report form that included demographic, clinical, laboratory, and epidemiologic information. For hospitalization duration calculation, the day of admission was hospital day 0. For patients for whom height and weight measurements were available, the body-mass index (BMI, weight in kilograms divided by the square of height in meters) was calculated. Obesity was defined as a BMI ≥30 in adults ≥18 years old (*3*).

From June 29, 2009, when pandemic (H1N1) 2009 infection was confirmed in Kenya, through November 29, 2009, 690 patients with laboratory-confirmed pandemic (H1N1) 2009 were identified. Of these patients, 88 (13%) were hospitalized in 12 surveillance hospitals. Most hospitalizations (61 [69%]) occurred during October and November (Figure). The median patient age was 5.1 years (range 1 month–61 years). Thirty-four (39%) patients were <2 years, and 19 (22%) were 18–49 years (Table).

We could not obtain clinical records for 39 (44%) case-patients. We describe clinical data for 49 (66%) case-patients. No significant differences were found in median age (7 years vs. 3 years; p = 0.39) or sex (51% females vs. 54% females; p = 0.40) between patients with clinical data and patients without clinical data.

The 49 case-patients were from 4 public and 3 private hospitals in 4 of Kenya's 8 provinces. Half (51%) of case-patients were admitted to Siaya District Hospital in Nyanza Province. Thirteen (26%) patients had an underlying medical condition (Table). Of 20 patients with known HIV infection status, 4 (20%) were HIV positive. Of 20 patients with BMI information, 4 (20%) were obese. None of the adult female patients was pregnant.

The median duration between onset of symptoms and hospitalization was 3 days (range: 0–14 days). The most common symptoms at admission were self-reported fever (43 patients [88%]), cough (45 patients [92%]), and vomiting (20 patients [41%]). Eighteen (37%) patients reported diarrhea. Of 22 patients with malaria blood smear results, 5 (23%) had positive results (median patient age 5 months). Of 42 patients with data on antiviral treatment, 10 (24%) had received oseltamivir either before or during hospitalization.

The median duration of hospitalization was 4 days (range 1–51 days). All 4 HIV-infected patients survived without known complications. Two (4%) patients died.

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DOI: http://dx.doi.org/10.3201/eid1709.100992

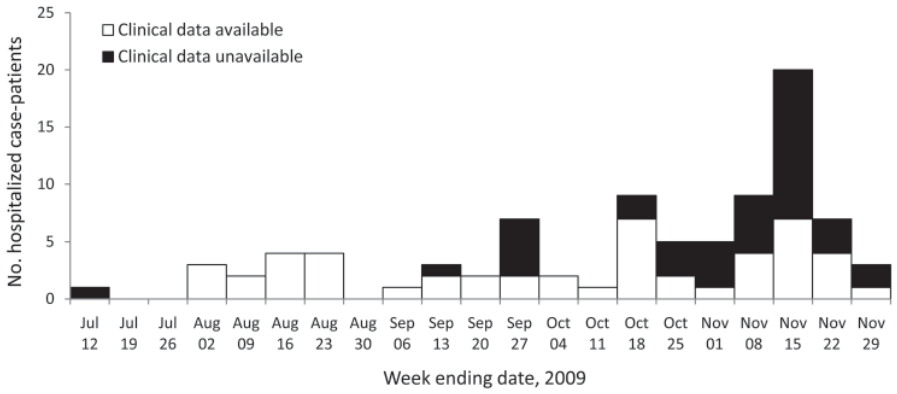

Figure. Pandemic 2009 (H1N1) case-patients hospitalized by week, Kenya, July–November 2009 (n = 88).

The first was a 55-year-old HIV-negative man without underlying illness who had been admitted with acute respiratory illness. He was transferred to the intensive care unit after secondary bacterial infection (*Pseudomonas aeruginosa*) developed. The patient died after 45 days while receiving mechanical ventilation in the intensive care unit. The next person who died was a 4-year-old boy without underlying illness (HIV status unknown) who had been admitted with a cough, vomiting, and fever. He had malaria confirmed by blood smear and died 4 days after admission. Neither patient received oseltamivir.

Conclusions

This study describes the epidemiology, clinical features, and clinical course of pandemic (H1N1) 2009 infections in hospitalized patients in a sub-Saharan African country outside South Africa. The large proportion of pandemic (H1N1) 2009 hospitalizations among young adults in Kenya is consistent with findings from New Zealand, the United States, and Australia (*4*–*6*). However, the median age of 5.1 years is lower than median ages found in Australia (15 years) and the United States (20 years) (*4*,*5*). The lower median age in Kenya could be explained by varying patterns of health care use by age in Kenya. Studies in western Kenya and Nairobi have shown that adults are less likely to seek medical care at hospitals for acute respiratory illness than are young children (*7*,*8*).

One fourth of those hospitalized with pandemic (H1N1) 2009 influenza in Kenya had an underlying medical condition, a lower proportion than in the United States (73%), Ireland (50%), and Chile (37%) (*5*,*9*,*10*). This difference could be due to undetected chronic illnesses; in many health care facilities in Kenya, patients are not routinely screened for chronic diseases. Additionally, data about chronic illness may not have been recorded on hospital charts.

Twenty percent of hospitalized pandemic (H1N1) 2009 patients with available HIV data were positive for the virus. Although this percentage is higher than the national HIV prevalence (7%) (*11*), the HIV-positive patients were all from Nyanza Province, which has an HIV prevalence of 15% (*11*). Nevertheless, our findings suggest that HIV patients could be at risk for severe pandemic (H1N1) 2009 influenza, supporting results of a study in South Africa which reported that 53% of pandemic (H1N1) 2009 patients who died were HIV positive (*12*). However, our report included a relatively small number of patients who had been tested for HIV, and CD4 counts and information on use of antiretroviral drugs were unavailable.

The proportion of hospitalized pandemic (H1N1) 2009 patients who were obese (20%) is higher than the estimates of the prevalence (5%–10%) of obesity in adults in Kenya (*13*). These findings are consistent with recent studies in the United States that have shown obesity to be a risk factor for severe outcomes from pandemic (H1N1) 2009 (*14*).

Although 50,000 treatment courses of oseltamivir were available in Kenya in May 2009, oseltamivir was underused during the study period, likely because

Table. Characteristics of hospitalized patients with pandemic (H1N1) 2009, Kenya, July–November 2009*

Characteristic	No. patients with characteristic/ no. with data available (%)
Female sex	46/88 (52)
Age group, y	
0–<2	34/88 (39)
2–4	9/88 (10)
5–9	10/88 (11)
10–18	13/88 (15)
19–49	19/88 (22)
>49	3/88 (3)
Underlying medical condition†	
Asthma	5/49 (10)
HIV	4/20 (20)
Obesity	4/20 (20)
Tuberculosis‡	1/25 (4)
Valvular heart disease	1/49 (2)
Oseltamivir treatment	10/42 (24)
ICU care	1/49 (2)

*ICU, intensive care unit.
†Two patients had asthma and were obese.
‡In continuation phase of treatment.

laboratory confirmation of pandemic (H1N1) 2009 was not available quickly enough to inform patient management. In addition, clinicians may not have been aware of the availability of and indications for oseltamivir. The Kenya Ministry of Health has recommended and made available oseltamivir for empirical treatment of hospitalized patients with suspected pandemic (H1N1) 2009 (*15*).

Nevertheless, our study has some limitations. Some medical charts had incomplete data and others could not be accessed. The reported cases therefore may not be representative of all hospitalized persons with pandemic (H1N1) 2009 in Kenya. In addition, reported cases were limited to hospitals where influenza surveillance was conducted and hospitals where clinicians were aware that pandemic (H1N1) 2009 testing was available. Most Kenyan hospitals have not been conducting systematic surveillance for pandemic (H1N1) 2009; thus, hospitalized patients with pandemic (H1N1) 2009 were likely missed.

Existing and enhanced sentinel hospital surveillance for influenza in Kenya made it possible to describe severe outcomes of pandemic (H1N1) 2009 in this country. Yet, the number of severe outcomes was likely much higher than reported here. Continued surveillance is essential in clarifying the full clinical spectrum and evolution of the pandemic in Kenya, including the role of coexisting conditions such as HIV infection and malaria. In addition, increasing clinician awareness of pandemic (H1N1) 2009 treatment guidelines could improve patient management**.**

Acknowledgments

We thank the staff at the Division of Disease Surveillance and Response of the Kenya Ministry of Public Health and Sanitation and the influenza laboratory and surveillance staff of the National Influenza Center/Kenya Medical Research Institute, the Kenya Medical Research Institute/Centers for Disease Control and Prevention–Kenya, and the Walter Reed Project–Kenya for their contributions to the influenza surveillance and testing described in this article.

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Ciprofloxacin-Resistant *Shigella sonnei* among Men Who Have Sex with Men, Canada, 2010

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In 2010, we observed isolates with matching pulsed-field gel electrophoresis patterns from 13 cases of ciprofloxacin-resistant *Shigella sonnei* in Montréal. We report on the emergence of this resistance type and a study of resistance mechanisms. The investigation suggested local transmission among men who have sex with men associated with sex venues.

Shigella spp. are enteropathogen bacteria that are transmitted person-to-person and require a low infectious inoculum (*1*). Fluoroquinolones are among the first-choice antimicrobial drugs for treatment of *Shigella* spp. infections in adults (*1*), but resistance to these agents has been documented, primarily in Asia (*2*). Among men who have sex with men (MSM), *Shigella* spp. infection is, in most cases, sexually transmitted, and clusters are regularly reported (*3–5*). We investigated an outbreak of ciprofloxacin-resistant *Shigella sonnei* among MSM and studied its resistance mechanisms.

The Study

Laboratories report shigellosis to the Montreal public health department (Quebéc, Canada). When a cluster is suspected, isolates are sent to the provincial laboratory to conduct pulsed-field gel electrophoresis (PFGE) to identify links between patients.

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DOI: http://dx.doi.org/10.3201/eid1709.102034

In July 2010, microbiology services at the Hôpital Saint-Luc alerted the public health department to *S. sonnei* resistant to ciprofloxacin and trimethoprim/sulfamethoxazole and susceptible to ampicillin. The *S. sonnei* had been isolated 2 days apart from stool cultures of 2 HIV-positive MSM.

Public health officials sent a notice to physicians, clinics, and laboratories in Montréal to report the presence of ciprofloxacin-resistant *S. sonnei* among MSM and to describe the antimicrobial treatment with ampicillin or azithromycin, procedures for case reporting, and preventive measures (*6*). Confirmed cases were defined as infection by *S. sonnei* with resistance to ciprofloxacin and trimethoprim/sulfamethoxazole and susceptibility to ampicillin (later specified as pulsovar 72). Probable cases were defined as infection by *S. sonnei* with a resistance profile identical to that of confirmed cases but where PFGE was not conducted.

Retrospective searching of the notifiable disease database found ciprofloxacin-resistant *S. sonnei* with a different PFGE pattern that had been isolated in February 2010 from a female patient who had traveled to a country where shigellosis is highly prevalent. Hence, this case was not from this outbreak. The provincial laboratory searched their records to identify cases elsewhere in Québec. During June–October 2010, nine confirmed cases and 4 probable cases were identified in Montréal and the surrounding regions (Table 1). Most patients had an onset date from the end of June to mid-July 2010 (Figure 1). All 13 patients were interviewed. Most patients were men (11/13; 85%) with a mean age of 40 years (range 20–65 years). All male patients were MSM, and 4 (36%) of 11 reported being HIV positive. Travel to a European country during August 2010 was mentioned by 1 MSM patient. Eight (73%) of 11 MSM patients mentioned participation in anal sex or contact during the exposure period. The use of sex venues was indicated by 4/11 MSM patients, and 3 mentioned a common sex venue. In addition, 1 other MSM patient reported that his sex partners frequented the common sex venue. This suggests that unprotected anal sex, associated with local sex venues, was the primary mode of transmission.

Two female patients (45 and 50 years of age) were reported. *S. sonnei* was detected in a food sample from a restaurant where 1 female patient ate during the exposure period, but the isolate did not match the outbreak PFGE pattern and was not related to any known human patients. No epidemiologic links between the female and male patients could be identified.

The public health interventions included a weekly analysis of incident shigellosis infections, resistance profiles, and risk factors. Given the preponderance of infections among MSM visiting sex venues, kits of condoms, soap, and information on prevention were distributed

Table 1. Characteristics of 13 cases of ciprofloxacin-resistant *Shigella sonnei* and results of susceptibility testing, Montreal, Québec, Canada, June–October 2010*

Patient ID	Patient age, y/sex	Stool sample date	Patient's signs and symptoms	Antimicrobial agent			PFGE group
				Amp	TMP/SMX	Cip	
1	52/M	Jun 24	Diarrhea	S	R	R	72
2	48/M	Jun 26	Nausea, vomiting, diarrhea, blood in stools, abdominal pain	S	R	R	NT
3	22/M	Jun 28	Nausea, diarrhea, abdominal pain, fever	S	R	R	NT
4	38/M	Jul 5	Nausea, vomiting, diarrhea, abdominal pain, fever, fatigue	S	R	R	72
5	52/M	Jul 4	Diarrhea, abdominal pain, fever	S	R	R	72
6	32/M	Jul 14	Diarrhea	S	R	R	NT
7	50/F	Jul 11	Nausea, diarrhea, blood in stools, abdominal pain, fever	S	R	R	72
8	45/F	Jul 14	Diarrhea, blood in stools, abdominal pain	S	R	R	72
9	25/M	Aug 30	Diarrhea, blood in stools, abdominal pain	S	R	R	NT
10	50/M	Sep 5	Vomiting, diarrhea, abdominal pain, fever	S	R	R	72
11	65/M	Sep 8	Nausea, vomiting, diarrhea, abdominal pain	S	R	R	72
12	38/M	Sep 23	Diarrhea	S	R	R	72
13	20/M	Oct 22	Diarrhea, blood in stools, abdominal pain, fever	S	R	R	72

*ID, identification; Amp, ampicillin; TMX/SMX, trimethoprim/sulfamethoxazole; Cip, ciprofloxacin; PFGE, pulsed-field gel electrophoresis; S, sensitive; R, resistant; NT, not typed.

at sex venues in August 2010 (*3*,*6*). Community-based organizations that work with MSM living with HIV/AIDS were contacted to disseminate information on preventive measures. As a potential effect, few cases were declared in September, although sporadic cases continued to appear until October 2010.

The resistance profile investigation identified 14 *S. sonnei* isolates from 13 patients by using commercial biochemical kit tests. Identification of *S. sonnei* from 9 patients was confirmed at the provincial laboratory. Antimicrobial susceptibility testing was done by agar dilution or disk diffusion method (*7*), Vitek 2 (bioMérieux, Marcy l'Étoile, France), or Etest (AB Biodisk, Solna, Sweden) (ampicillin, trimethoprim/sulfamethoxazole, and ciprofloxacin) for 14 isolates and by Etest (AB Biodisk) (azithromycin, cefotaxime, and tetracycline) and with nalidixic acid (30-μg disk) for 7 or 8 isolates. The susceptibility of *S. sonnei* isolates to antimicrobial agents is reported in Tables 1 and 2.

PFGE was done by the provincial laboratory according to international standards set by the US Centers for Disease Control and Prevention (*8*). The *Xba*I and *Bln*I patterns were interpreted using the standards of Tenover et al. (*9*). The *Salmonella enterica* serotype Braenderup strain (H9812) was used as the size marker in each gel (*10*). Band position tolerances and optimization values of 1% were used for all analyses. Similarity coefficient was obtained within BioNumerics (www.applied-maths.com/bionumerics/bionumerics.htm) by calculating Dice coefficients. Cluster analysis was done by using with the unweighted pair group method with arithmetic averages. The *S. sonnei* isolates from 9 patients for whom typing was done were indistinguishable for the 2 enzymes (Figure 2). PulseNet Canada accession numbers for the isolate from our study are SSOXAI.0067 and SSOBNI.0040 for the *Xba*I and *Bln*I patterns, respectively.

For the study of the mechanisms of drug resistance, bacterial DNA was extracted using MasterPure Complete DNA Purification Kit (Epicenter Biotechnologies, Madison, WI, USA). The *gyrA* and *parC* genes were analyzed by direct DNA sequencing procedures as described (*11*) on an ABI Prism Genetic Analyzer 3130xl (Applied

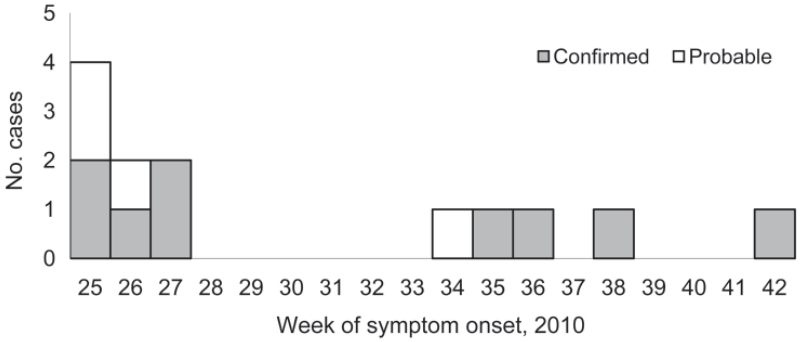

Figure 1. Confirmed and probable cases of ciprofloxacin-resistant *Shigella sonnei* infection, by week of onset, Montréal, Québec, Canada, June–October 2010.

Table 2. Antimicrobial drug susceptibility of *Shigella sonnei* isolates to 7 agents, Montreal, Québec, Canada, 2010*

Antimicrobial agent	No. isolates tested	MIC	Interpretation
Ampicillin	11	2–8 mg/L	S
TMP/SMX	11	>32 mg/L	R
Ciprofloxacin	11	≥4–16 mg/L	R
Azithromycin	8	8–16 mg/L	NA
Cefotaxime	8	0.06–0.125 mg/L	S
Tetracycline	7	256–>256 mg/L	R
Nalidixic acid	8	6 mm†	R

*The susceptibility and resistance breakpoints were those for *Enterobacteriaceae,* except for azithromycin, for which these breakpoints are not available (*7*). TMP/SMX, trimethoprim/sulfamethoxazole; S, susceptible; R, resistant; NA, not available.
†By disk diffusion method (*7*).

Biosystems, Foster City, CA, USA). The DNA sequences were converted into amino acid sequences by using the EMBOSS Transeq tool (European Molecular Biology–European Bioinformatics Institute), aligned by using ClustalW (DNAStar, Madison, WI, USA), and compared with that of the reference quinolone-susceptible strain (GenBank accession no. NC_008258). The 8 *S. sonnei* strains from 7 patients harbored the same nonsynonymous substitutions in comparison with the quinolone-susceptible reference strain: S83L and D87G for *gyrA* and S80I for *parC*. These amino acid substitutions have been previously associated with ciprofloxacin resistance in *Escherichia coli* (*11*) and in *S. dysenteriae*, *S. flexneri*, and *S. boydii* (*2*) but not in *S. sonnei* isolates.

Blood in stools or fever was reported by 9 (69%) of 13 patients (Table 1). Of the known treatment outcomes, 2 of the 4 patients treated with oral ampicillin had a negative stool culture 48 hours and 72 hours after completion. One of the 2 patients treated with oral amoxicillin experienced a clinical and microbiologic treatment failure 48 hours after completion, but a clinical and microbiologic cure was achieved after treatment with oral azithromycin. Two other patients were treated with azithromycin and 1 other with ciprofloxacin.

Conclusions

Sporadic ciprofloxacin-resistant *Shigella* has been infrequently documented (*2*,*12*–*14*). In India, ciprofloxacin-resistant *S. dysenteriae*, *S. flexneri,* and *S. boydii* have been isolated since 2002, and their fluoroquinolone-resistant mechanisms have been determined (*2*). Ciprofloxacin-resistant *Shigella* remains rare and was found among 0.2% of isolates in the United States (2000–2009) (*12*) and 0.5% of isolates in Canada (1997–2000) (*13*). In the United States, 10 ciprofloxacin-resistant *Shigella* spp. isolates (6 *S. flexneri*, 3 *S. sonnei*, and 1 *Shigella* spp.) were documented by the National Antimicrobial Resistance Monitoring System (2000–2009) (*12*). In New York, NY, in 2006, ciprofloxacin resistance was detected among 4 *S. sonnei* and 1 *S. flexneri* acquired locally (*14*). In 2010 in South Carolina, ciprofloxacin-resistant *Shigella flexneri* 2a was isolated from 3 patients (*15*).

We report the suspected transmission of ciprofloxacin-resistant *S. sonnei,* among MSM in Montreal, Québec. Some authors suggest the antimicrobial drug treatment of all patients infected with *Shigella* spp. (*1*), but others disagree with this recommendation (*14*). It is essential that physicians request bacterial stool cultures when *Shigella* spp. enteric infection is suspected in MSM even without blood in stools or fever.

Acknowledgments

We thank Michèle Tremblay, Elisabeth Lacombe, Patricia Hudson, Sylvie Habash, Suzanne Rajotte, Michel Poisson, Claude Lemieux, M.-D. Baptiste-Desruisseaux, Johanne Ismaïl, the medical microbiologists from Clinique l'Actuel and the hospitals Verdun and Maisonneuve-Rosemont, Pierre-Boucher, and Jean-Talon for their assistance.

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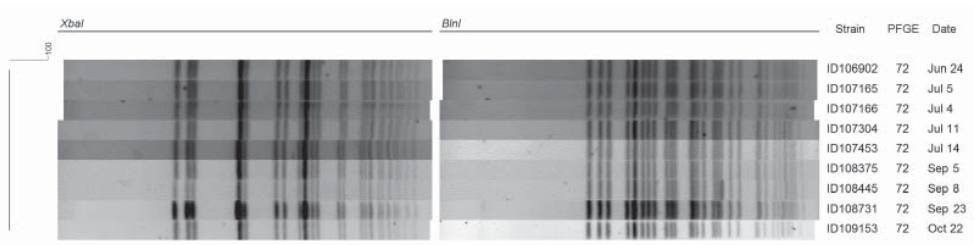

Figure 2. Digestion pattern of ciprofloxacin-resistant *Shigella sonnei* isolated from 9 patients for *Xba*I and *Bln*I, Montréal, Québec, Canada, June–October 2010. PFGE, pulsed-field gel electrophoresis.

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Multidrug-Resistant *Acinetobacter baumannii* in Veterinary Clinics, Germany

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An increase in prevalence of multidrug-resistant *Acinetobacter* spp. in hospitalized animals was observed at the Justus-Liebig-University (Germany). Genotypic analysis of 56 isolates during 2000–2008 showed 3 clusters that corresponded to European clones I–III. Results indicate spread of genotypically related strains within and among veterinary clinics in Germany.

Within the genus *Acinetobacter, A. baumannii* is clinically the most relevant species, frequently involved in hospital outbreaks and affecting critically ill humans (*1*,*2*). The strains involved are usually multidrug resistant, which limits therapeutic options (*3*). Many outbreaks in Europe and beyond have been associated with the European clones I–III (*4*–*6*).

Nosocomial infection in veterinary medicine is an emerging concern. The role of acinetobacters in diseases of hospitalized animals is largely unknown. Recent reports have documented occurrence of or infection with *Acinetobacter* spp., including *A. baumannii*, in hospitalized animals (*7*,*8*). The internal laboratory records of the microbiology department of the Giessen Veterinary Faculty (Institute for Hygiene and Infectious Diseases of Animals, Giessen, Germany) noted an increase in antimicrobial drug–resistant *Acinetobacter* isolates. To assess the species and type diversity of these organisms, we investigated a set of isolates from Giessen and other veterinary clinics obtained during a 9-year period by a combination of genotypic methods and compared the isolates for their susceptibility to antimicrobial drugs.

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DOI: http://dx.doi.org/10.3201/eid1709.101931

The Study

The Institute for Hygiene and Infectious Diseases of Animals in Giessen receives samples for investigation from other veterinary departments of the university (mainly referral clinics) and from external veterinary clinics throughout Germany. During 2000–2008, *Acinetobacter* spp. were obtained from 137 hospitalized animals. From these animals, 56 isolates were selected for further characterization. The selection was made to reflect the diversity in epidemiologic origin of the collection regarding date of isolation, animal species, specimen, and veterinary clinic (82% from Giessen) (online Appendix Table, www.cdc.gov/EID/17/9/101931-appT.htm). Only isolates with possible clinical significance were included as inferred from the fact that they were the only or the dominating agent within the sample. Furthermore, according to data from the diagnostic laboratory, the selected isolates were highly resistant.

Confirmatory susceptibility testing of isolates was conducted by using the Clinical Laboratory Standards Institute broth dilution method (*9*) (Table). For precise species identification, amplified ribosomal DNA restriction analysis was performed. By this method, the 16S rDNA sequence was amplified by using PCR, followed by restriction of the amplified fragment by 5 restriction enzymes: *Cfo*I, *Alu*I, *Mbo*I, *Rsa*I, and *Msp*I. The combination of electrophoretic patterns of the respective enzymes was compared with a library of profiles (*10*).

Fifty-two isolates were identified as belonging to *A. baumannii* and 3 to *A. pittii* (*Acinetobacter* gen. sp. 3) (*11*); 1 with a yet undescribed profile remained unclassified. Amplified fragment length polymorphism (AFLP) DNA fingerprint analysis was performed as described for confirmative species identification, for strain typing, and for clone identification (*4*,*12*,*13*). Briefly, *Eco*RI and *Mse*I were used to generate restriction fragments that were selectively amplified by using a Cy-5–labeled Eco-A and an Mse-C primer. Amplification products were separated by electrophoresis and subjected to cluster analysis with the BioNumerics software package 5.1 (Applied Maths, St-Martens-Latem, Belgium). For species identification, isolates were compared with reference strains of all described *Acinetobacter* species included in the Leiden University Medical Center AFLP database (Leiden, the Netherlands). Isolates with profiles ≥50% similar were considered to belong to the same species (*1*).

To assess the type diversity of the organisms, isolates were typed by pulsed-field gel electrophoresis (PFGE) (*14*) and by AFLP analysis. For PFGE, DNA was digested with the restriction endonuclease *Apa*I. Digitized profiles were analyzed with the BioNumerics software. For AFLP typing, a subset of 27 isolates was analyzed (online Appendix Table). The profiles obtained were compared with each

Table. Resistance profiles of 56 animal *Acinetobacter* spp. isolates for 19 antimicrobial agents, obtained by CLSI broth microdilution test *

Profile; no. isolates	Tested antimicrobial agents																		
	Oxa	Pen	Ctn	Ery	Cli	Chl	Cst	Cvf	Amp	Amc	Tet	Enr	Orb	Dif	Kan	Sxt	Gen	Ipm	Amk
1; 1	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	S
2; 1	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	S	R
3; 28	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
4; 2	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	S	S
5; 2	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	S	S
6; 1	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	S	S
7; 1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S
8; 1	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	S	S	S
9; 3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S
10; 1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	S	S	S	S
11; 1	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	S	S	S	S
12; 1	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	S	S	S
13; 1	R	R	R	R	R	R	R	R	R	I	R	S	S	S	R	R	R	S	S
14; 3	R	R	R	R	R	R	R	R	I	I	R	R	R	R	S	S	S	S	S
15; 1	R	R	R	R	R	R	R	R	I	I	R	R	R	R	S	S	S	S	S
16; 2	R	R	R	R	R	R	R	R	I	I	S	S	S	S	S	S	S	S	S
17; 1	R	R	R	R	R	R	R	R	I	S	S	S	S	S	S	S	S	S	S
18; 1	R	R	R	R	R	R	R	I	I	S	S	S	S	S	S	S	S	S	S
19; 1	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
20; 1	R	R	R	R	R	R	R	I	S	S	S	S	S	S	S	S	S	S	S
21; 1	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S
22; 1	R	R	R	R	R	R	S	I	S	S	S	S	S	S	S	S	S	S	S

*CLSI guidelines M31-A2 (*9*). CLSI, Clinical Laboratory Standards Institute; Oxa, oxacillin; Pen, penicillin; Ctn, cephalotin; Ery, erythromycin; Cli, clindamycin; Chl, chloramphenicol; Cst, colistin; Cvf, cefovecin; Amp, ampicillin; Amc, amoxicillin/clavulanic acid; Tet, tetracycline; Enr, enrofloxacin; Orb, orbifloxacin; Dif, difloxacin; Kan, kanamycin; Sxt, trimethoprim/sulfamethoxazole; Gen, gentamicin; Ipm, imipenem; Amk, amikacin; R, resistant; I, intermediate; S, susceptible.

other and with those of the Leiden database, including those of the European clones I–III. A similarity cutoff level ≥80% was used to delineate members of the same clone and ≥90% to delineate organisms related at the strain level (*4*,*12*,*13*).

For PFGE, at a similarity level of 86%, 3 major clusters (A, B, and C) and 6 unique isolates were distinguished (Figure 1). Within major cluster C, 2 main subclusters (C1 and C6) and 4 single profiles (C2–C5) were observed at 97% similarity (online Appendix Table; Figure 1). Despite some band differences, the patterns in major cluster C were strikingly similar. The maximum number of band differences in subcluster C1 was 3, which indicates that the organisms were genetically closely related. In subcluster C6, only minor differences in size of the fragments were observed (Figure 1).

For AFLP, we investigated a subset of 27 isolates, including at least 1 isolate of each of the 16 different PFGE profiles and the 3 isolates nontypeable by PFGE. Seventeen AFLP types were distinguished at the 90% similarity cutoff level for strain delineation. Identification by AFLP showed full agreement with amplified ribosomal DNA restriction analysis species identification (online Appendix Table). Comparison of isolates to those of the Leiden AFLP database grouped isolates with AFLP profile 8 (corresponding PFGE profiles A1, A2) with isolates of European clone I, those with profiles 10–16 (corresponding PFGE profile C1–C6) with clone II, and with profile 7 (corresponding PFGE profiles B1, B2) with clone III (online Appendix Table). Examples are shown in Figure 2.

Conclusions

The occurrence of PFGE type C in different animals admitted to 3 different clinical wards of the Justus-Liebig-University Giessen over 9 years might indicate endemic occurrence of these organisms on these wards. Survival in the hospital environment (*15*), patient-to-patient transfer, and transfer from 1 animal clinic to another may have contributed to their persistence and spread. Because veterinarians, stockmen, and students rotate between the various clinics and departments, transmission by hands or equipment should be considered. Frequent transport of colonized animals to and from shared examination rooms, e.g., for computer-assisted tomography, might also have contributed to the chain of spread. Because type C isolates also were found in samples from animal clinics throughout Germany (online Appendix Table), limited genetic variation in animal strains of *A. baumannii* also is possible.

AFLP data were, further to comparative typing of the animal isolates, also used to assess the relatedness of the

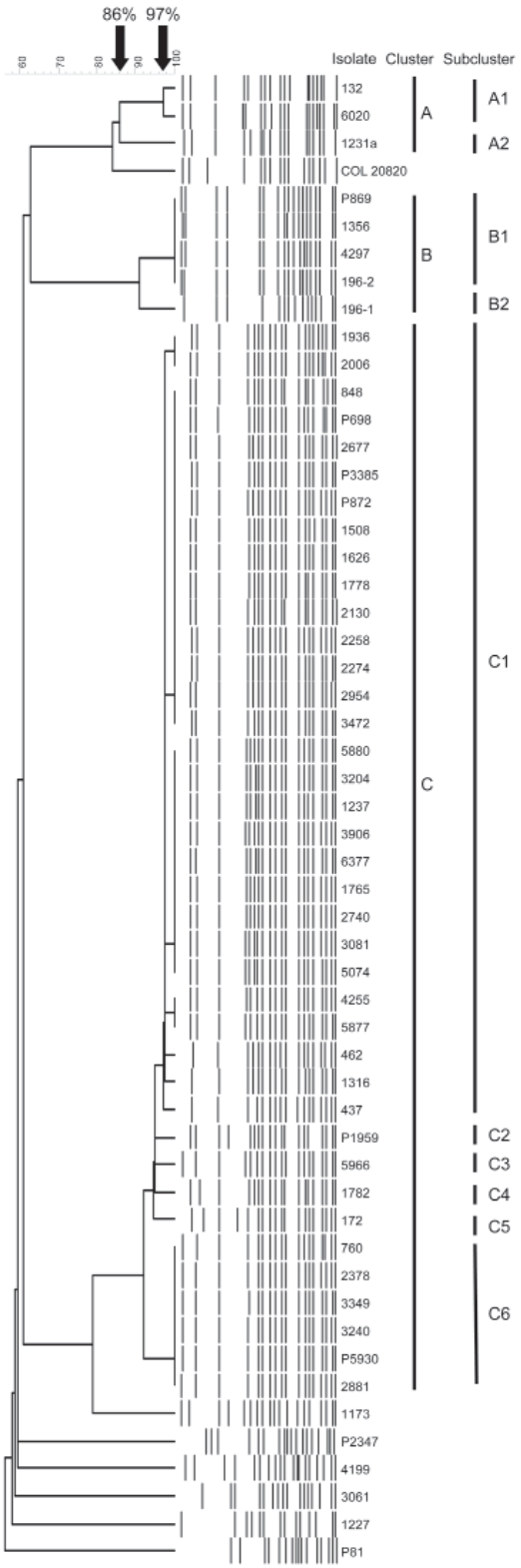

Figure 1. Computer-assisted cluster analysis of pulsed-field gel electrophoresis fingerprints of 53 *Acinetobacter baumannii* and 2 *Acinetobacter* spp. pittii isolates. COL 20820 was used as the reference standard for normalization of the digitized gels (*14*).

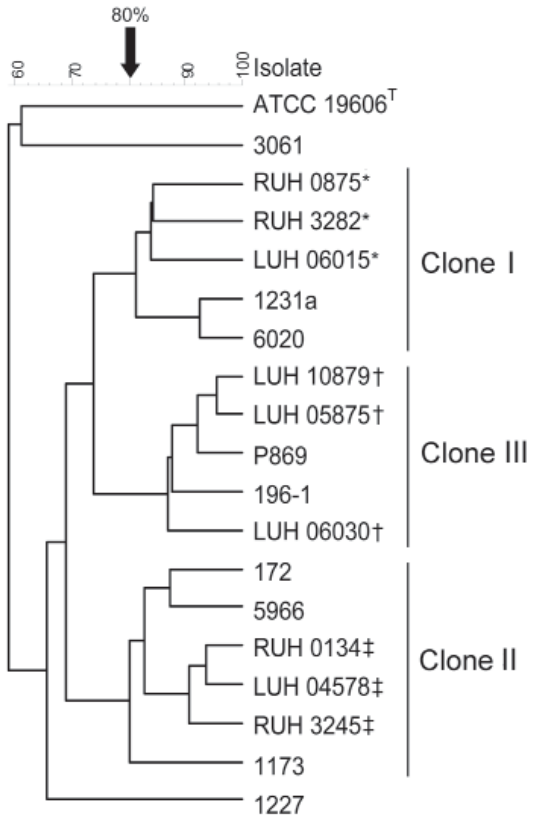

Figure 2. Amplified fragment length polymorphism analysis of 9 animal *Acinetobacter baumannii* isolates belonging the major pulsed-field gel electrophoresis types and 9 reference strains of the European clones I–III from the Leiden University Medical Center collection. *Reference strains of European clone I; †reference strains of European clone III; ‡reference strains of European clone II.

isolates in our study to those of the widespread European clones I–III that represent genetically related but not identical strains that are frequently multidrug resistant and associated with epidemic spread in human clinics (*1*,*4*–*6*). Although not all strains were characterized by AFLP, we conclude by inductive generalization of results that the findings apply to all isolates of the PFGE types from which the organisms were selected. Thus, a large proportion of the animal *A. baumannii* isolates were genetically congruent with the European clone I, II, or III. Occurrence of such isolates in ill, hospitalized animals of various species might indicate that, as in human medicine, *A. baumannii* is an emerging opportunistic pathogen in veterinary medicine. The occurrence of clones I–III in animals and humans also raises concern about whether the organisms can spread from animals to humans or whether the animals have acquired the organisms from humans.

The occurrence of genotypically related, antimicrobial drug–resistant *A. baumannii* strains in hospitalized animals suggests that these organisms are most likely nosocomial

pathogens for animals. If so, veterinary clinics face a great challenge regarding prevention, control, and treatment of infections with these organisms, similar to situations in human hospitals. Finally, the possibility of spread from humans to animals or vice versa requires special attention.

Acknowledgments

We thank Gabriele Köpf and Beppie van Strijen for excellent technical assistance.

The work was supported by a grant from the Akademie für Tiergesundheit e. V.

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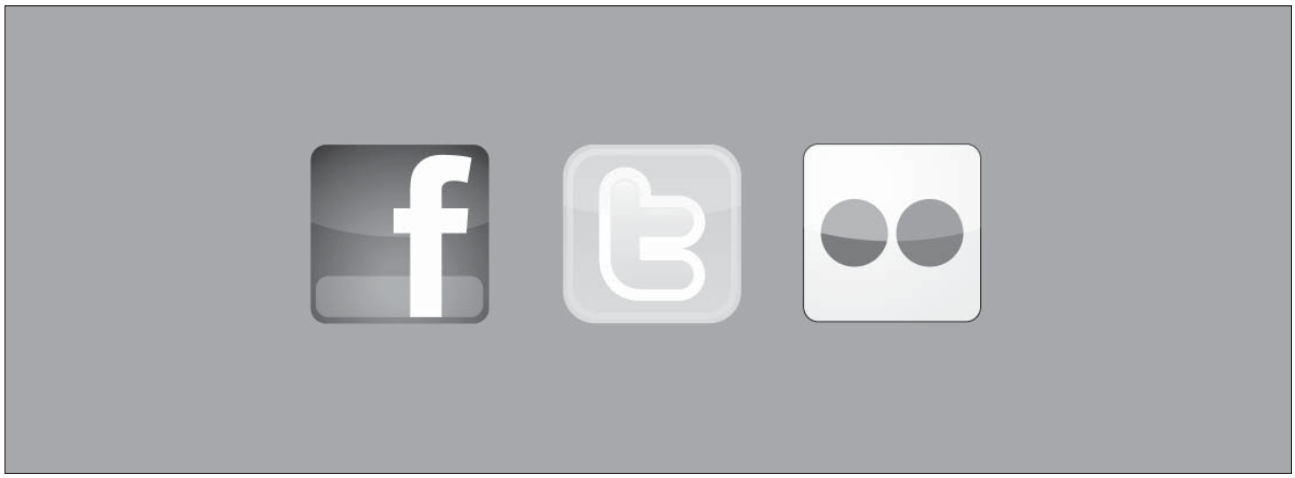

Escherichia coli O104:H4 from 2011 European Outbreak and Strain from South Korea

To the Editor: Beginning in early May 2011, an outbreak caused by Shiga toxin–producing *Escherichia coli* O104:H4 was reported in Germany and other countries in Europe. In this outbreak, the number of hemolytic uremic syndrome (HUS) cases has been unusually high (*1*). As of June 9, 2011, a total of 722 cases of HUS, 19 deaths, and 2,745 cases of enterohemorrhagic *E. coli* (EHEC) infection were reported (*2*).

A case of HUS caused by *E. coli* O104:H4 was first reported in South Korea in 2004 (*3*). Because infections caused by *E. coli* O104:H4 have been reported rarely, interest has arisen in the *E. coli* O104:H4 strain from South Korea. We characterized the *E. coli* O104:H4 strain isolated in South Korea (EC0417119) in 2004 and compared it with the *E. coli* O104:H4 strain associated with the current EHEC outbreak in Europe.

The serotype EC0417119, isolated from a patient with HUS in 2004, was reconfirmed as *E. coli* O104:H4. The strain was positive for *stx*1 and *stx*2 by PCR (*4*) but negative for *agg*R by PCR (*5*). In the antimicrobial drug susceptibility test using VITEK 2 AST-N169 test kit (bioMérieux, Marcy L'Etoile, France), the strain was resistant to ampicillin, ampicillin/sulbactam, and trimethoprim/sulfamethoxazole but susceptible to ceftriaxone, cefotaxime, nalidixic acid, and tetracycline.

We also performed pulsed-field gel electrophoresis (PFGE) for EC0417119, according to the PulseNet standard protocol (*6*), and compared its PFGE profile with that of the current outbreak strain *E. coli* O104:H4, which was obtained from the PulseNet Asia Pacific network. PFGE profiles resolved by either *XbaI* or *BlnI* did not match each other. The percentage similarity of *XbaI*- and *BlnI*-digested PFGE profiles of the 2 isolates was 75% and 66.7%, respectively, as shown in the Figure.

Infections with the EHEC O104 strain were reported several times worldwide. In Europe, such occurrence was rare, and before the current outbreak, the EHEC O104:H4 strain was documented only once in South Korea. For this reason, it was logical to examine the possible relatedness of the EC0417119 strain and the strain causing the current outbreak. However, the EC0417119 strain has many different characteristics compared with the current outbreak strain: not possessing enteroaggregative *E. coli* determinant, not producing extended-spectrum β-lactamases, and not showing indistinguishable PFGE patterns. In conclusion, there is no evidence that the *E. coli* O104:H4 strain isolated in South Korea in 2004 is related to the strain that has a caused the massive and unprecedented EHEC outbreak in Europe.

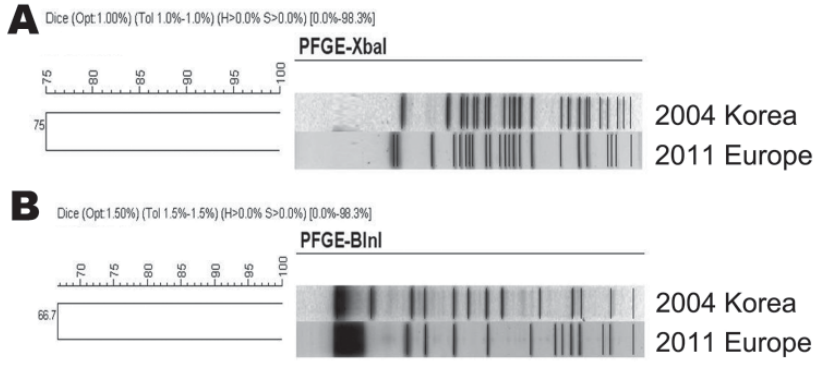

Figure. Clustering of A) *XbaI*- and B) *BlnI*-digested DNA fragments by pulsed-field gel electrophoresis (PFGE) for *Escherichia coli* O104:H4 2011 outbreak strain in Europe and isolate obtained in South Korea in 2004.

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DOI: http://dx.doi.org/10.3201/eid1709.110879

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Respiratory Illness in Households of School-Dismissed Students during Pandemic (H1N1) 2009

To the Editor: In response to the emergence of pandemic (H1N1) 2009 virus (*1*), the Centers for Disease Control and Prevention (CDC) issued interim guidance for preventing spread of the pandemic virus in schools. Initial guidance recommended that dismissal of students be considered for schools with confirmed cases of pandemic (H1N1) 2009 infection. The guidance was subsequently revised to recommend monitoring for respiratory illness and exclusion of ill students until they were noninfectious, rather than dismissal.

In Chicago, Illinois, USA, the first cases of pandemic (H1N1) 2009 infection were identified on April 28, 2009, of which 1 occurred in an elementary school student (*2*). In accordance with CDC guidance at the time, the school (school A) was closed for 1 week, April 29–May 5, 2009. CDC and the Chicago Department of Public Health investigated respiratory illnesses among students and their households during the period surrounding the school closure.

A telephone survey of students' households was conducted during May 15–20, 2009 (*3*). One adult member of each household was asked whether any household members had been "sick with cold or flu symptoms or fever" since April 12. Age, date of illness onset, and symptoms and signs (fever, cough, sore throat, rhinorrhea or nasal congestion [runny or stuffy nose]) were recorded. Acute respiratory illness was defined as ≥1 symptom or sign from the list provided. Influenza-like illness was defined as fever plus cough or sore throat. Reports were excluded if onset date was before April 12 or unknown. Descriptive analysis was performed, and household attack rates were calculated. Dates of onset were used to evaluate timing of illness in relation to school closure and possible transmission within households. The investigation was approved as nonresearch by CDC.

Of 609 eligible households, 439 (72%) had a working telephone number, of which 170 (39%) completed the survey. Thirty-nine (23%) households, representing 181 persons, reported 58 illnesses that met the acute respiratory illness definition, of which 37 (64%) also met the influenza-like illness definition. Median age was 10 years (range <1–48 years). Of 57 household members for whom age and student status were recorded, 42 (74%) were students at school A. Thirty-four (60%) reported onset of symptoms before or on the day of school dismissal (Figure).

Household attack rates ranged from 10% to 100% (median 25%). Five (13%) households reporting illness had no ill students who attended school A. In 4 of 11 households reporting ≥2 illnesses, students became ill before nonstudent household members. In the remaining 7 households, onset dates did not suggest student-to-nonstudent transmission.

Even though the school was closed almost immediately after the first pandemic (H1N1) 2009 case was confirmed in a student, onset of ≈60% of reported illnesses occurred before or on the day of school dismissal, suggesting that unrecognized transmission was already occurring in the school or community. These results are supported by data on confirmed cases of pandemic (H1N1) 2009 in Chicago, which suggest that community transmission was high during the survey period (*2*). Our results also indicated that at least some

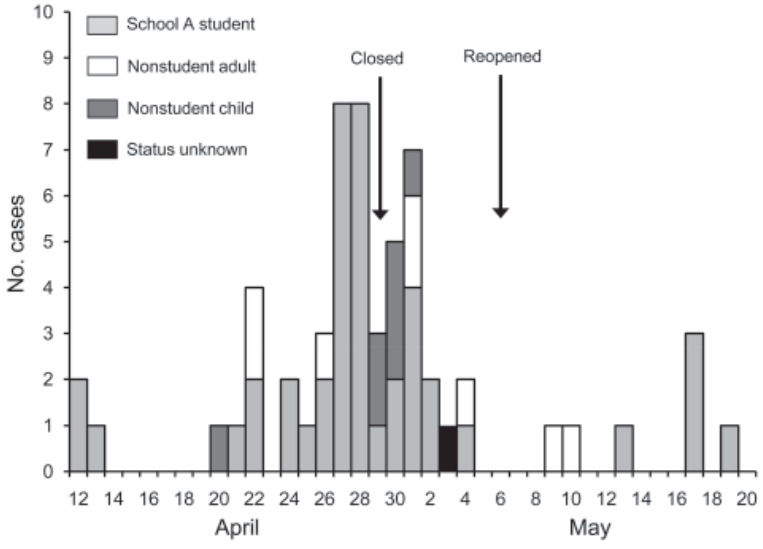

Figure. Respiratory illness in households of school-dismissed students during the pandemic (H1N1) 2009 outbreak, Chicago, Illinois, USA, 2009. Arrows indicate dates when school A closed and reopened.

illness among school A households originated from sources other than the school and support the approach of considering school dismissal only in conjunction with other community mitigation strategies.

In Hong Kong Special Administrative Region, People's Republic of China, where all primary schools, kindergartens, and child care centers were immediately closed for 14 days after identification of the first local case of pandemic (H1N1) 2009, school closures were concluded to have substantially decreased transmission (*4*). The applicability of these findings to communities where such sweeping measures might be less acceptable is unclear.

If school dismissal is considered as a strategy, dismissal early in the pandemic most likely would have the most impact, depending on duration of dismissal, other mitigation measures, and compliance with social distancing recommendations (which was mixed during the 2009 pandemic [*3*,*5*]). Polling of parents whose children experienced school dismissal showed high acceptance of short-term (3–5 days) dismissals and low economic impact, especially on lower income families (*3*,*6*). However, dismissal for longer periods needs to be balanced by the adverse impact on education, loss of student services, and socioeconomic impact on families (*7*–*9*).

This investigation was limited by the relatively low response rate; however, demographics for the sample in our study were similar to those of the school as a whole (*3*). Other limitations included the exclusive use of reported symptoms to document illness, possible unrecognized asymptomatic cases, and absence of similar data from later in the pandemic. The 1-week closure period might not have provided enough information to capture any effect, and comparative data were not available from schools that were not dismissed during the pandemic. Further investigation is needed to evaluate the efficacy and impact of school dismissal, including the timing of dismissal in relation to recognition of cases in a school or community and the impact of school dismissal relative to other community mitigation strategies.

Acknowledgments

We thank Francisco M. Averhoff and Daniel B. Fishbein for their contributions to this investigation. We also thank Susan I. Gerber, Julie Y. Morita, Roderick C. Jones, Cortland Lohff, and others at the Chicago Department of Public Health for their contributions.

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DOI: http://dx.doi.org/10.3201/eid1709.101589

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Pandemic (H1N1) 2009 Virus in Swine Herds, People's Republic of China

To the Editor: During March and early April 2009, a new swine-origin influenza A (H1N1) virus emerged in Mexico and the United States; this virus subsequently spread across the globe by human-to-human transmission at an unprecedented rate. Pandemic (H1N1) 2009 virus also affected pigs. On May 2, 2009, the Canadian Food Inspection Agency notified the World Organisation for Animal Health that the novel influenza A virus had been confirmed on a pig farm in Alberta, Canada. Infection of pigs with pandemic (H1N1) 2009 virus has been observed in multiple

countries (*1*). In this study, we report transmission of pandemic (H1N1) 2009 virus from humans to pigs in the People's Republic of China.

During August 2009–April 2010, swine influenza virus surveillance was conducted in the provinces of central and eastern China, including Henan, Hubei, Hunan, Jiangxi, and Anhui. A total of 1,021 samples, comprising tracheal mucus swabs and lungs, from pigs on 30 farms, were collected, and dozens of swine influenza viruses, H1N1, H1N2, H3N8, H9, and H10 subtypes, were isolated. Eight isolates were subtype H1N1, including 4 novel pandemic (H1N1) 2009 viruses: A/swine/Nanchang/3/2010 (H1N1) (Gen Bank accession nos. JF275917–24), A/swine/Nanchang/5/2010 (H1N1) (GenBank accession nos. JF275933–40) and A/swine/Nanchang/6/2010 (H1N1) (GenBank accession nos. JF275941–48), which were isolated from tracheal mucus, and A/swine/Nanchang/F9/2010 (H1N1) (GenBank accession nos. JF275925–32), isolated from the lung. Pigs from which the novel viruses were isolated showed mild respiratory signs, including depression, cough, and transient increase in body temperature. Compared with the sequence of A/California/04/2009 (H1N1), genomic sequencing of the 4 pandemic viruses showed 15 common point mutations, such as polymerase basic protein 2, T588I; polymerase acidic protein, A70V, P224S, D547E; hemagglutinin, P100S, D103E, S145P, T214A, S220T, I338V; nucleocapsid protein, V100I, H289Y; neuraminidase, V106I, N248D; and nonstructural protein, I123V.

Recent studies have shown that the novel pandemic (H1N1) 2009 human influenza viruses were almost avirulent for mice (50% mouse lethal dose $\geq 10^6$ PFU for A/CA/04/09 [*2*,*3*]). In this study, mice were anesthetized with ketamine/xylazine, as described (*4*) and 50 μL of phosphate-buffered saline containing the indicated doses (10^5 50% egg infectious dose) of the 4 viruses were instilled into anesthetized mice through nostrils. Interestingly, the 4 pandemic (H1N1) 2009 viruses isolated from pigs could cause systemic infection on mice. All mice had extensive loss of body weight, and some of the infected mice died within 2 weeks postinfection (data not shown).

To detect whether the 4 isolated pandemic (H1N1) 2009 viruses could cause clinical diseases in pigs and what kinds of pathologic damage could be induced in infected pigs, pigs were intratracheally challenged with the 4 pandemic (H1N1) 2009 viruses at a dose of 10^7 50% egg infectious dose and monitored for clinical signs and pathologic changes. Clinical signs in pigs were mild, and no deaths occurred. Except for slightly labored breathing, no infected pigs showed dominant signs, such as cough, nasal discharge, facial edema, and dyspnea. Body temperatures of infected pigs started to increase at 2 days postinfection (dpi), peaked ≈3 dpi at 41.5°C, and returned to the initial temperature by 5 dpi.

We observed necrosis of the tracheal wall and pneumonia foci in the 4 pandemic (H1N1) 2009 virus–infected pigs. Hematoxylin and eosin–stained sections of the trachea from the infected pigs showed mild necrotizing tracheitis. The necrotic epithelial cells were present in the lumen, and a mixed inflammatory cell infiltrate was present throughout (Figure). In the lung, we also observed alveolar septal edema and interstitial inflammatory cell infiltrates, as well as histologic changes, including alveolar epithelial hyperplasia, a mixed inflammatory infiltrate, and interstitium broadening (Figure). Additionally, immunohistochemical analysis for the distribution of viral antigens showed positive staining in bronchial epithelial cells and alveolar pneumocytes.

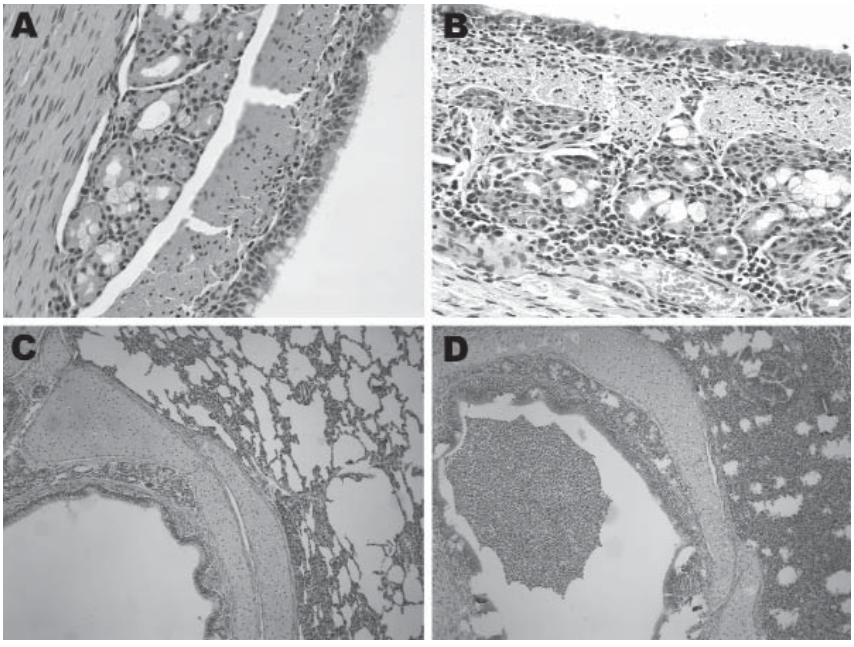

Figure. Hematoxylin and eosin–stained trachea and lung controls and samples from pigs infected with pandemic (H1N1) 2009 virus. A) Control trachea sample; B) mixed inflammatory cell infiltrate present throughout trachea sample from infected pig; C) control lung sample; D) mixed inflammatory infiltrate and interstitium broadening in lung sample from infected pig. Original magnifications: panels A and B, ×40; panels C and D, ×10. A color version of this figure is available online (www.cdc.gov/EID/17/9/101916-F.htm).

Previous studies showed that pandemic (H1N1) 2009 may have become established in swine populations in Canada, Norway, and Hong Kong (*1*,*5*–*8*). The human-to-pig transmission of pandemic (H1N1) 2009 may substantially affect virus evolution and subsequent epidemiology. Although the pandemic was mild, the virus could develop further reassortment in swine and gain virulence. On the other hand, subtype H5N1 and H9N2 viruses have become established in pigs, so the introduction of pandemic (H1N1) 2009 virus to pigs has provided the possibility for the incorporation of avian virus genes into mammalian-adapted viruses. That transmission could occur from humans to pigs and vice versa is especially troublesome. Given the possible production of novel viruses of potential threat to public health, we should emphasize influenza surveillance in pigs and establishment of the genetic basis of the viral genome for rapidly identifying such reassortment events.

This study was supported by China National Basic Research Program (China "973" Program 2011CB505004 and 2010CB534002), National Natural Science Foundation of China (no. 31072154 and 30800832) and National Transgenic Major Program (2009ZX08009-141B).

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DOI: http://dx.doi.org/10.3201/eid1709.101916

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Pulmonary Disease Associated with Nontuberculous Mycobacteria, Oregon, USA

To the Editor: Nontuberculous mycobacteria (NTM) are environmental organisms ubiquitous in soil and water, including municipal water supplies. When inhaled, these organisms cause chronic, severe lung disease in susceptible persons (*1*). Recent epidemiologic studies suggest NTM pulmonary disease is increasingly prevalent in North America, with annual incidence rates of 13 cases per 100,000 population in persons ≥50 years of age and 2–4-fold higher in older age groups (*2*–*4*). The current distribution of pulmonary NTM disease has been poorly characterized with regard to environment, climate, and other factors.

We recently performed a statewide NTM surveillance project in Oregon, United States, where we documented higher pulmonary disease rates within the moister, temperate western regions of the state. Oregon is bisected north-south by mountains into 2 distinct climate zones. Western Oregon, where 87% of the state's population lives, is temperate and wet; eastern Oregon is primarily rural, with an arid, high desert climate. Our goal was to evaluate whether disease clustering within the state could be explained by population density.

For all Oregon residents who had newly diagnosed and existing pulmonary NTM disease during 2005 and 2006, we used case-patient home ZIP code and county of residence to construct statewide disease maps (*4*). We obtained state ZIP code and county-level census data for 2005 and 2006 from the Portland State University Population Research Center and used Oregon Office of Rural Health criteria to designate

ZIP codes as urban or rural and counties as rural (nonmetropolitan), micropolitan, or metropolitan (*5*,*6*). Unlike ZIP code data, which lacked age information, county census data were age stratified and consisted of population numbers aggregated in 5-year age groups (e.g., 0–4 years, 5–9 years). Because nearly all pulmonary NTM disease occurred in persons ≥50 years of age, we calculated age-adjusted disease prevalence rates (by using 95% Poisson exact confidence intervals) for patients ≥50 years of age in the county census data. We used the Cochran-Armitage test for trend to evaluate differences in rates by rural, micropolitan, and metropolitan county designations.

Statewide, 385 (94%) of 411 NTM cases occurred among residents of western Oregon, and the crude rate of annual disease prevalence was significantly higher in western than in eastern Oregon (6.0 vs. 2.7/100,000; $p<0.05$) (online Appendix Figure, www.cdc.gov/EID/content/17/9/101929-appF.htm). Within the western region, rates were significantly higher in urban than in rural ZIP codes. Using county-level data, we found that age-adjusted prevalence rates in western Oregon strongly correlated with increasing levels of population density (Table). In eastern Oregon, where only 26 cases occurred, age-adjusted rates among residents ≥50 years of age were similar (7.6 cases/100,000 population) to those in rural counties within western Oregon (6.5/100,000).

In Oregon, where most pulmonary NTM disease is caused by *Mycobacterium avium* complex (MAC), our findings suggest that the higher rates of disease in the wet western portion of the state are best explained by differences in population density (*4*). Disease rates there were highly correlated with increasing population density, and in rural areas of western Oregon, disease rates were similar to those in the arid, primarily rural eastern portion of the state.

Humans presumably are exposed to NTM daily through showering, bathing, and other activities where water or soil is aerosolized (*7*). Previous environmental studies suggest that persons living in urban areas could potentially have greater NTM exposure during these activities because NTM is more prevalent in piped networks of municipal water systems than in well-water systems primarily used in rural regions (*8*). A study in Japan in the 1980s found a similar association of pulmonary NTM disease (primarily MAC) with urban and wet environments compared with arid and rural regions in our study but unlike our study was not able to evaluate differences in disease rates between urban and rural areas independent of climate differences (*9*). A 1979 Texas study found an association of pulmonary NTM with rural living, although this result was driven by *M. kansasii* disease, and rates of MAC were actually higher in rural areas (*10*). These and other similar studies were conducted decades ago when the epidemiology of NTM was substantially different

Table. Prevalence of pulmonary nontuberculous mycobacterial disease, by geographic region and population density, Oregon, USA, 2005 and 2006*

Region/population density	Total no. cases	Prevalence† (95% confidence interval)		
		All age groups	Age <50 y	Age ≥50 y
Total	411	5.6 (5.0–6.1)	1.1 (0.8–1.4)	15.2 (13.6–16.9)
County-level analysis				
Western	385	6.0 (5.4–6.6)	1.2 (0.9–1.6)	16.5 (14.8–18.4)
Metropolitan	341	6.3 (5.7–7.0)	1.3 (1.0–1.8)‡	18.0 (16.0–20.2)§¶#
Micropolitan	39	4.5 (3.2–6.1)	0.2 (0.0–1.0)‡	11.1 (7.8–15.2)§¶
Rural	5	3.6 (1.2–8.3)	1.3 (0.0–7.1)	6.5 (1.8–15.9)§#
Eastern	26	2.7 (1.8–4.0)	0.2 (0.0–0.9)	7.6 (4.9–11.2)
Metropolitan	9	3.0 (1.4–5.6)	0.0 (0.0–1.8)	8.6 (3.9–16.3)
Micropolitan	12	2.5 (1.3–4.3)	0.3 (0.0–1.7)	6.8 (3.4–12.2)
Rural	5	2.9 (1.0–6.8)	0.0 (0.0–3.5)	7.8 (2.5–18.2)
ZIP code**				
Western	365	5.7 (5.2–6.4)	–	–
Urban	277	6.4 (5.7–7.2)††	–	–
Rural	88	4.3 (3.5–5.3)††	–	–
Eastern	26	2.8 (1.8–4.0)	–	–
Urban	5	2.7 (0.9–5.7)	–	–
Rural	21	2.8 (1.7–4.1)	–	–

*Annualized 2-year disease period prevalence, Oregon, 2005 and 2006.
†Cases per 100,000 population.
‡Significant differences ($p<0.05$) between western Oregon metropolitan and micropolitan counties, age <50 y.
§West, age ≥50 y, metropolitan, micropolitan, and rural (Cochran-Armitage test for trend, $p<0.01$).
¶Significant differences ($p<0.05$) between western Oregon metropolitan and micropolitan counties, age ≥50 y.
#Significant differences ($p<0.05$) between western Oregon metropolitan and rural counties, age ≥50 y.
**Included only the 391 patients for whom with ZIP code data were available. –, age-level data not available for ZIP codes.
††Significant differences ($p<0.05$) between western Oregon urban and rural ZIP codes.

(i.e., predominantly a disease of male patients) and before the formulation of the 2007 American Thoracic Society/ Infectious Diseases Society of America pulmonary NTM disease criteria (*1*).

We were limited in drawing firm conclusions about why pulmonary NTM is more common in urban areas because we were not able to evaluate patients or regional water systems within our study. Persons living rurally might be less likely to seek medical care and thus have NTM diagnosed, which would account for the differences in our study. However, given the reasonably close proximity of western Oregon's rural regions to major medical centers, we believe this scenario is unlikely.

Our findings suggest that pulmonary NTM disease is closely associated with urban living. We suspect the difference in disease rates between urban and rural areas might reflect differences in host exposure to these pathogens. Further studies should be undertaken to elucidate the environmental exposures associated with pulmonary NTM.

Acknowledgments

This study was presented in part in abstract form at the Infectious Diseases Society of America conference, October 2010, Vancouver, British Columbia, Canada. We acknowledge the assistance of NTMir.org in helping to fund this study.

The work of K.L.W. was funded by an Agency for Healthcare Research and Quality grant (1K08HS017552-01) and a grant from NTMir.org.

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DOI: http://dx.doi.org/10.3201/eid1709.101929

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Carriage of Meningococci by University Students, United Kingdom

To the Editor: *Neisseria meningitidis* causes septicemia and meningitis (*1*). Meningococci usually persist on the nasopharyngeal mucosa of asymptomatic carriers (*2*). Because carriers are the only reservoir of meningococci, carriage in at-risk populations should be monitored. Meningococcal carriage rates have been assessed during 1997–8 for first-year students at the University of Nottingham (*3*) and in autumn during 1999–2001 for >48,000 sixth-form students (pre-university, age range 15–17 years) throughout the United Kingdom (*4*). Serogroup B and nongroupable strains predominated; serogroup Y strains were found in only 1%–2% of participants.

From November 2008 through May 2009, to investigate persistence and spread of meningococcal strains in students living in dormitories, we conducted a longitudinal study in a cohort of 190 first-year students at the University of Nottingham. We found high rates of carriage and prevalence of serogroup Y strains (*5*).

During September 2009 (first week of term) through March 2010, we conducted a large repeated cross-sectional study analyzing pharyngeal swabs from students in all school-year groups at Nottingham University.

The objective of this study was to determine the significance of changes in overall meningococcal and serogroup Y-specific carriage rates among students.

In September, first-year students were recruited on the main campus during registration and subsequently in dormitories and the main library. Undergraduates not in the first year were all recruited in the main library. This September sample of 823 first-year students represents 16.5% of the 5,000 undergraduate students registered each academic year on the main campus. Although not intentional, some overlap occurred when students were resampled during subsequent visits to the same dormitories and library, e.g., among the 557 first-year students from whom swab samples were collected in December, 74 (13%) had previously provided swab samples. Our study was approved by the Nottingham University Medical School Ethics Committee, and written informed consent was obtained from all participants.

Pharyngeal swab samples were spread onto GC selective agar (Oxoid, Basingstoke, UK) and incubated at 37°C in air containing 5% CO_2. After 48 hours, colonies suggestive of *Neisseria* spp. were examined for positive oxidase reaction; single colonies were confirmed as meningococci by amplification of meningococcal genes *crgA* plus *ctrA* and/or *porA* (*6*). PCR-based serogrouping was performed as described (*6*,*7*). Chi-square tests for significance were performed by using STATCALC (Epi Info version 6.04; Centers for Disease Control and Prevention, Atlanta, GA, USA).

Among first-year students, carriage rates increased from 23.2% in late September to 55.7% by mid-December and remained at a similar level in March (Table). Among second- and third-year students, carriage rates were 34.2% and 30.5% in September, respectively, and remained at similar levels throughout the academic year. The increase in carriage among first-year students from September through December was mainly the result of a significant (23%) increase in carriage of serogroup Y strains (Table). In contrast, during the same period, carriage rates of serogroup Y strains did not change significantly among second- and third-year students (Table).

Initial carriage rates were significantly higher for incoming (first-year) students in September 2009 than in 1997 (13.9% [*3*]; $\chi^2 = 14$, 1 df; $p<0.0001$); swabbing and culture protocols and sampling sites were identical in both studies, so the increases are real. Because 83% of students at Nottingham University come from all regions of the United Kingdom and 17% from other countries, the increased rates

Table. Characteristics of meningococci carriage, University of Nottingham students, United Kingdom, 2009–10*

Collection date/year and group	Carriage rate, no. (%) carriers	Serogroup distribution: B, No. isolates (% carried strains)	B, % All participants (95% CI)	Y, No. isolates (% carried strains)	Y, % All participants (95% CI)	Others, No. isolates (% carried strains)	Others, % All participants (95% CI)
September 2009							
First, n = 823	191 (23.2)†	58 (30.3)	7.0 (5.3–8.8)	24 (12.6)	2.9 (1.8–4.1)‡	109 (57.1)	13.2 (10.9–15.6)
Second, n = 441	151 (34.2)†	34 (22.5)	7.7 (5.2–10.2)	46 (30.5)	10.4 (7.6–13.3)§	71 (47.0)	16.1 (12.7–19.5)
Third, n = 321	98 (30.5)†	35 (35.7)	10.9 (7.5–14.3)	20 (20.4)	6.5 (3.6–8.9)¶	43 (43.9)	13.4 (9.7–17.1)
December 2009							
First, n = 557	310 (55.7)#	53 (17.1)	9.5 (7.1–12.0)	142 (45.8)	25.5 (21.9–29.1)‡	115 (37.1)	20.6 (17.3–24.0)
Second, n = 312	123 (39.4)#	33 (26.8)	10.6 (7.2–14.0)	32 (26.0)	10.3 (6.9–13.6)§	58 (47.2)	18.6 (14.3–22.9)
Third, n = 180	52 (28.9)#	12 (23.1)	6.7 (3.0–10.3)	11 (21.2)	6.1 (2.6–9.6)¶	29 (55.8)	16.1 (10.7–21.5)
March 2010							
First, n = 379	224 (59.1)#	44 (19.6)	11.6 (8.4–14.9)	64 (28.6)	16.9 (13.1–20.7)‡	116 (51.8)	30.6 (26.0–35.2)
Second, n = 187	69 (36.9)#	17 (24.6)	9.1 (5.0–13.2)	25 (36.2)	13.4 (8.5–18.3)§	27 (39.1)	14.4 (9.4–19.5)
Third, n = 112	37 (33.0)#	13 (35.1)	11.6 (5.7–17.5)	5 (13.5)	4.5 (0.6–8.3)¶	19 (51.4)	17.0 (10.1–23.9)

*CI, confidence interval.
†Carriage rate significantly lower for first-year students than for other year-group students, $p<7 \times 10^{-2}$.
‡First-year students, $p<8 \times 10^{-7}$.
§Second-year students, not significant.
¶Third-year students, not significant.
#Carriage rate significantly higher for first-year students than for other year-group students in December 2009 and March 2010, $p<10^{-8}$.

of carriage may reflect a nationwide change (*8*).

Furthermore, testing within the first week of term meant that recovered strains were predominately brought into the university. Serogroup Y carriage rates for incoming students (2.9%) were significantly higher than rates detected by identical genotyping methods during 1999–2001 (1.7%–1.8% [*4*]; χ^2 = 4.6%–6.4%, 1 df; $p<0.05$), suggesting that meningococcal carriage by young adults, particularly of serogroup Y strains, has increased across the United Kingdom. The major increase in serogroup Y strains among first-year students during 2009–10 probably resulted from spread of clones within dormitories, as observed in the 2008–9 study (*5*) and may be facilitated by characteristics of the organism, lack of immunity, or a combination of these factors.

The high prevalence of serogroup Y strains in carriers may help explain the recent increased incidence of serogroup Y disease in the United Kingdom: from 20 to 62 laboratory-confirmed cases in England and Wales from 2003 through 2009 (*9*). In the United States during the late 1990s, a similar increase in serogroup Y carriage was linked to a concomitant increase in serogroup Y disease (*10*).

In conclusion, in a representative UK student cohort we detected high rates of carriage and elevated prevalence of serogroup Y strains of meningococci. Any further significant increase in serogroup Y disease should lead to prompt reconsideration of the current vaccine policy in the United Kingdom.

Acknowledgments

We are grateful to all volunteers who participated in this study. We also thank members of the Molecular Bacteriology and Immunology Group and medical staff from the Department of Clinical Microbiology, Queen's Medical Centre, Nottingham, for their assistance with sample collection.

Funding for this study was provided by Sanofi Pasteur and the Healthcare and Biosciences, Innovation Networks. C.D.B. was supported by a fellowship from the Research Councils United Kingdom.

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DOI: http://dx.doi.org/10.3201/eid1709.101762

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Pandemic (H1N1) 2009 in Neonates, Japan

To the Editor: In 2009 in Japan, a medical response to pandemic (H1N1) 2009 infection in neonates was proposed by the Japan Pediatric Society (JPS) (*1*). Few such cases have been reported (*2*–*7*). Because the effects of pandemic (H1N1) 2009 in neonates are unknown, the JPS Committee of Neonatal Medicine conducted a nationwide survey during 2009. Surveys were mailed to neonatal care units in 522 facilities certified by JPS as teaching hospitals, which included almost all tertiary neonatal intensive care units in Japan. The survey asked whether during April 2009–March 2010 any neonates had been born to

mothers with pandemic (H1N1) 2009 onset from 7 days before delivery until obstetric discharge and whether pandemic (H1N1) 2009 developed in any neonates <28 days of age before hospital discharge. The study was approved by the Bioethics Committee and Board of Directors of JPS.

Of the 522 facilities, 327 (62.6%) responded. During the period in question, 52,774 neonates had been hospitalized for any cause except routine care. Pandemic (H1N1) 2009 infection of the mother was reported by 47 (16.1%) facilities. From the 37 of these facilities, detailed information was available for ≈42 mothers with pandemic (H1N1) 2009 infection who gave birth to 43 neonates (29 full-term and 14 preterm births). Of these 42 mothers, a diagnosis of influenza A was made by rapid influenza diagnostic kit for 33 (78.6%) and for pandemic (H1N1) 2009 by reverse transcription PCR (RT-PCR) for 5 (11.9%). Only 1 case of pandemic (H1N1) 2009 in a mother was reported in May 2009, when influenza subtype H3 was dominant in Japan. This infection was confirmed by RT-PCR, and the mother was included in the study.

During the study period, except April and May 2009, almost all influenza A infections were caused by pandemic (H1N1) 2009 virus. Delivery on the day after symptom onset was most frequent (14 [32.6%] births), followed by delivery on the same day as symptom onset (8 [18.6%] births). A similar trend was observed for preterm births. Of the 42 mothers, 40 (95.2%) received antiviral medications. Mixed feeding of breast and formula milk was most common, and 8 neonates were breast-fed only.

Among the 43 neonates, pandemic (H1N1) 2009 infection developed in only 1 (male, gestational age 37 weeks, birthweight 2,665 g). His mother had high fever, and pandemic (H1N1) 2009 was diagnosed by RT-PCR; she received oseltamivir and delivered her son 2 days after illness onset. The neonate became lethargic at 4 days of age, and pandemic (H1N1) 2009 infection was confirmed by a rapid-antigen detection kit. The neonate received oseltamivir and recovered the next day. He received oseltamivir for 5 days and was discharged at 12 days of age with no subsequent medical problems. Because onset occurred 4 days after birth, the possibility of horizontal infection from the mother cannot be excluded. Except for this 1 neonate, prophylactic antiviral drugs were not given to the other 42 neonates, none of whom became infected.

With respect to nosocomial pandemic (H1N1) 2009 infection in hospital wards caring for neonates, no cases of onset within 28 days after birth were reported. However, pandemic (H1N1) 2009 infection before discharge but after 28 days of age was reported for 6 neonates. These diagnoses were made by rapid diagnostic kit, specific RT-PCR, or both. Of these 6 neonates, 1 was born at 29 weeks of gestation and had a low birthweight (1,026 g); symptom onset at 32 days of age; and complications of respiratory distress, pneumothorax, and systemic inflammatory response syndrome. Oseltamivir was given to 5 of these 6 neonates, none had adverse effects and all 6 recovered.

Pandemic (H1N1) 2009 infection may have caused preterm labor. According to our findings, the virus does not seem to be transmitted during breast-feeding, and antiviral drugs, if given to the mothers, may not always be needed by neonates. However, because of the limitations of this observational study, these findings need further support. Dulyachai et al. confirmed vertical transmission of pandemic (H1N1) 2009 virus at 31 weeks of gestation (*5*). Jajoo and Gupta reported a 32-week-old preterm patient with pandemic (H1N1) 2009 who died of pneumonia and multiorgan failure (*6*). Maternal pandemic (H1N1) 2009 infection associated with preterm labor may adversely affect the fetus or neonate (*2*,*3*,*5*,*6*).

Our results show that pandemic (H1N1) 2009 virus infection in mothers seldom occurred in their neonates, i.e., vertical transmission was rare. This finding is consistent with the fact that few such cases have been reported (*8*,*9*). On the basis of the results of this survey, JPS published Guideline for Management of Influenza (including Pandemic [H1N1] 2009) in Neonates during the Early Postnatal Period in 2010–2011 Season (*10*).

Acknowledgments

We extend our deep appreciation to the neonatologists from the JPS training facilities for their survey responses.

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DOI: http://dx.doi.org/10.3201/eid1709.101803

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Social Network as Outbreak Investigation Tool

To the Editor: The recent article by Oh et al. (*1*) discussed the utility of email surveys for the investigation of outbreaks. After they have been created, digital surveys require less time to administer than paper-based or telephone surveys and can produce high-quality and timely data. During an outbreak in Illinois, we used email and a social networking site to distribute a link to a confidential Inquisit (www.millisecond.com) survey and compared characteristics of the groups that responded to each.

In December 2010, the Illinois Department of Public Health received a report of an outbreak of gastrointestinal illness among guests at a wedding reception. Health department staff converted a standard foodborne outbreak questionnaire to a digital format. The survey link was then distributed to guests by 2 methods: email from the reception hosts and the note function on the host's Facebook page. Facebook has 500 million active users, 50% of whom check their Facebook pages every day (*2*). The Facebook note function is a blogging feature through which users can publish content visible to linked friends.

A total of 14 persons responded to the email-distributed survey link and 41 to the Facebook-distributed survey link. For each survey, data quality was high and response rates for questions were >90%. Facebook respondents were younger than email respondents (mean ages 29.8 and 37.4 years, respectively). Information provided by Facebook respondents covered persons 11 months to 80 years of age and by email respondents 1–67 years of age. Parents were asked to complete surveys for any children unable to answer the questions independently. The Facebook-distributed survey had a higher percentage of male respondents (41.5%) than did the email-distributed survey (21.4%).

Facebook-distributed surveys were answered significantly faster than email-distributed surveys ($p<0.05$). The mean number of hours from distribution to response was 42.3 for the email survey and 8.7 for the Facebook survey. The Facebook survey link was distributed at 6:00 PM on a Thursday evening; 34 (82.9%) surveys were completed by 9:00 AM on Friday morning. On the basis of these responses, health department staff were able to identify the implicated foods the day after the questionnaires were distributed.

Distributing foodborne outbreak questionnaires through Facebook generated data that were complete and timely. Facebook-distributed surveys captured a wide range of respondent age groups and more male respondents than did email-distributed surveys. Previous studies of online survey response rates found rates to be significantly higher for women than for men (*3*). In addition to low cost and significantly improved survey response times, social networking distribution holds other advantages for health departments. Recall errors are reduced by distributing the survey to persons simultaneously and immediately. Posting of surveys through a health department's social networking accounts could also enable participation of persons for whom the health department does not have contact information. Given these advantages and the widespread use of social networking, use of these tools should be considered as an option for survey distribution during outbreak investigations.

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DOI: http://dx.doi.org/10.3201/eid1709.110088

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Susceptibility of Health Care Students to Measles, Paris, France

To the Editor: A measles epidemic is currently occurring in several countries in Europe (*1*,*2*). Although most cases concern unvaccinated children and young adults, health care professionals (HCPs) are also affected. Cases occur mostly in unvaccinated persons, but also in those who have received a single dose of vaccine.

In France, the measles vaccine was introduced in the childhood-immunization schedule in 1983. Current guidelines recommend 2 doses: one at 12 months of age and the second between 13 and 24 months of age. For persons born after 1992, one catch-up dose is recommended (*3*). Coverage by ≥1 dose, by the age of 2 years, remained at 83%–87% during 1997–2005. The latest figures show a slight increase to 90% in 2007 (*4*).

The risk for measles in HCPs has been estimated as 13× higher than that for the general population (*5*) and is also higher among students (*6*). Vaccination against measles is recommended, not mandatory, for HCPs and health care students (HCSs) (medicine, nursing, and midwifery) who have no history of measles. The objective is to prevent transmission to a nonimmunized patient or another HCP, and from patients to susceptible HCPs. HCSs are in close and repeated contact with patients and therefore targeted by the recommendations. We conducted a cross-sectional survey in the university hospitals in Paris, France, to assess measles vaccination coverage in HCSs.

The sampling frame included 15 hospitals with an obstetrics department. All midwifery students were selected. Other students were selected through a multistage random sampling. Sampling units were selected at each stage by simple random sampling. We selected 10 hospitals at the first stage, 10 clinical wards by hospital at the second stage, and all nursing students and half the medical students by ward at the third stage. A total of 116 students were required from each profession to estimate 50% coverage with 10% precision.

Students gave oral informed consent. Information was collected by face-to-face interview. Vaccination-status was assessed from a document when available. Measles vaccination coverage was defined as the number of students with no history of measles who had received ≥1 dose of vaccine divided by the total number of students with no history of measles. The study was approved by the French Ethics Board and conducted from March 2009 through July 2009.

Of the 106 selected wards, 10 could not be included (clearance from the head of department was not given). Of the 488 selected students, 432 were enrolled in the study (participation rate 88.5%); 178 (41%) were medical students, 147 (34%) nursing students, and 107 (25%) midwifery students. A document confirming the student's vaccination status was available for 376 (87%) students; 38 (10.1%) had a history of measles (removed from analysis). Median age was 22 years (interquartile range 21–24 years); 74% were female. Measles vaccination was cited by 61.5% (95% confidence interval [CI] 50.0%–71.9%) as a recommended vaccination. Measles vaccination coverage was 79.3% (95% CI 71.0%–75.8%) for ≥1 dose and 49.6% (95% CI 40.3%–59.1%) for 2 doses (Table). When considering only the students' accounts (without written confirmation), 1- and 2-dose vaccination coverage was 93.3% (95% CI 88.0%–96.3%) and 83.6% (95% CI 68.0%–92.4%), respectively. In multivariate analysis, younger students (<22 years of age) were more likely to have had 1 dose than older students ($p \leq 0.001$).

In the context of measles epidemics affecting France, and considering that the World Health Organization recommends 95% coverage of the population with 2 doses of a measles vaccine, our study

Table. Age, gender ratio, and rates of measles vaccination coverage for health care students, Paris, France*

Characteristic	Medical students, n = 178	Nursing students, n = 147	Midwifery students, n = 107	Total, n = 432
Median age, y	23	22	22	22
Gender ratio, M:F	0.68	0.09	0.05	0.26
One dose, % (95% CI)	79.9 (67.1–88.6)	85.7 (67.1–88.6)	76.8 (63.1–86.5)	79.3 (71.0–85.8)
Two doses, % (95% CI)	46.3 (31.2–62.2)	66.9 (55.2–76.8)	55.7 (41.1–69.4)	49.6 (40.3–59.1)

*CI, confidence interval.

shows insufficient coverage among students currently being trained as HCPs in university hospitals within the Paris area. Thus, all unvaccinated students (20.7%) and ≈5% of the 50% who have received 1 dose could be susceptible to measles. Moreover, a rather low proportion of students knew that measles vaccination was recommended, as described for HCPs (*7*), which may explain the insufficient coverage.

In France, this situation has resulted in several measles outbreaks within hospitals in recent years (*8*). Particular efforts should be made in certain units, such as pediatrics, because an early waning of maternal antibodies has been demonstrated (*9*). Measures should be taken to reinforce the vaccination policy in this well-defined group; information can be obtained and follow-up vaccinations can be provided easily during their training period. A mandatory health check could contribute to increasing the vaccination coverage (*10*).

Our study provides original data for measles vaccination coverage in HCSs in France. A similar conclusion applies to HCS-recommended vaccines for which we found insufficient coverage (<50% had received a pertussis booster in the past 15 years and only 6/27 without a history of varicella have been vaccinated against varicella). Such evaluations should be performed regularly. Although our data were collected from a representative sample of students in Paris, it is likely that the situation is qualitatively similar in other regions and therefore could contribute to future nosocomial epidemics. Mandatory vaccination of HCPs against vaccine-preventable diseases protects not only the HCP and his/her family, but also protects the patient. Increased vaccination of this group should contribute to a better control of measles outbreaks in France.

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DOI: http://dx.doi.org/10.3201/eid1709.110141

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Toxigenic *Corynebacterium ulcerans* in Woman and Cat

To the Editor: Diphtheria and diphtheria-like illness are caused by *Corynebacterium* spp. that harbor the diphtheria toxin–encoding *tox* gene. Recently in many industrialized countries, cases of diphtheria-like infection caused by toxigenic *C. ulcerans* have outnumbered those caused by toxigenic *C. diphtheriae* (*1*,*2*). *C. ulcerans* infection was originally associated with consumption of raw milk and dairy products or contact with cattle, but *C. ulcerans* has increasingly been isolated from domestic animals such as pet dogs and cats (*3*–*5*). So far, isolation of an identical toxigenic *C. ulcerans* strain from an animal and its owner has been documented only for dogs (*3*,*4*) and a pig (*6*). We report the isolation of an identical toxigenic *C. ulcerans* strain from an asymptomtic pet cat and a person with pharyngeal diphtheria-like illness; therefore, it might be speculated that the woman

has acquired her infection from the cat.

In November 2010, an 86-year-old woman with arterial hypertension and rheumatoid arthritis was admitted to an ear, nose, and throat clinic in Dresden, Germany, with a 3-day history of sore throat, hoarseness, and nasal respiratory obstruction. Fever was not reported. Because the patient had visible fibrinous rhinitis, a nasal and pharyngeal swab was obtained before treatment with amoxicillin was begun. The patient had no history of recent travel abroad or contact with livestock. Her complete vaccination status against diphtheria was unknown, but she had received a vaccination booster in 2006.

Toxigenic *C. ulcerans* grew from culture of the nasal swab specimen; it was identified by biochemical differentiation (API Coryne code 0111326; bioMèrieux, Nürtingen, Germany), *rpoB* sequencing (*6*), and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Bremen, Germany) (*7*). Toxigenicity was verified by real-time PCR (*8*) and a modified Elek test (*6*).

Because the microbiological result suggested diphtheria-like illness, the patient was transferred to an infectious diseases department in an academic hospital, where she was isolated and treated with amoxicillin for 12 days. Because the patient's condition was stable and no severe complications occurred during her hospital stay, she was not given diphtheria antitoxin. Her predominant symptoms, such as sore throat and earache, improved after antimicrobial drug therapy, and she recovered quickly. Electrocardiogram performed before discharge from hospital showed no signs of myocarditis or other toxin-related effects, such as neurologic disorder.

Although person-to-person transmission of *C. ulcerans* has not yet convincingly been demonstrated, an outbreak investigation involving the patient's close contacts (6 family members, the physician, and 19 nurses and other health care workers) was conducted. Although all close contacts had completed the series of diphtheria toxoid vaccinations, they were all given postexposure prophylaxis with erythromycin.

Because of the zoonotic potential of human *C. ulcerans* infections, nasal and pharyngeal swab samples were collected from the patient's asymptomatic pet cat. Strains of *tox*-positive *C. ulcerans* (which we named KL251 and KL252) grew on culture; the API Coryne code was identical to that of the human isolate KL246. In contrast to the human isolate, which yielded a weakly positive Elek result, both isolates from the cat showed Elek-negative results.

Antimicrobial drug susceptibility testing of the 3 isolates was performed on Mueller-Hinton blood agar (supplemented with 5% sheep blood) by using the Etest system after overnight incubation at 37°C and in 5% CO_2. In the absence of standardized breakpoints for *C. ulcerans*, susceptibility was determined by using the Clinical Laboratory Standards Institute criteria for broth microbouillon dilution susceptibility testing for *Corynebacterium* spp. (*9*). All *C. ulcerans* strains were susceptible to amoxicillin, benzyl penicillin, ceftriaxone, erythromycin, and tetracycline (MICs 0.19–0.5 μg/mL) but less susceptible to clindamycin in vitro (MIC 2 μg/mL).

Sequencing of *rpoB* and *tox* showed 100% homology between the strains from the woman and the cat. Ribotyping revealing a U3-like ribotype (*5*), and multilocus sequence typing (*10*) confirmed the clonal identity of the strains.

The cat was given a combined preparation of benzyl penicillin and streptomycin. After completion of therapy, *C. ulcerans* no longer grew from nasal swab specimens from the woman or the cat.

Our findings of transmission of toxigenic *C. ulcerans* between a woman and her cat underline the zoonotic potential of this organism and highlight the need for more studies investigating the carrier status of companion animals such as cats and dogs. Although clindamycin is not a first-line drug for diphtheria therapy, the intermediate susceptibility of *C. ulcerans* against clindamycin underscores the necessity of standardized susceptibility testing for diphtheria cases because clindamycin-resistant toxigenic *C. ulcerans* strains in human infections have been recently reported (*6*). Toxigenic *C. ulcerans* strains are rare, but the numbers of human wound infections or diphtheria-like disease caused by *C. ulcerans* have increased in the past few years. However, detection of toxigenic *C. ulcerans* is often still incidental, often resulting in delayed specific therapy, including patient isolation or contact tracing.

Acknowledgments

We thank Wolfgang Schmidt, Karola Grünwald, Marzena Maggipinto, and Daniela Sebah for cultivation and microbiological and molecular characterization of the *Corynebacteria*.

The study was partially supported by the Bavarian State Ministry of the Environment and Public Health, by the European Commission's Directorate General for Health and Consumer Policy through the Diphtheria Surveillance Network, and by the German Federal Ministry of Health through the Robert Koch-Institute and its National Reference Laboratories Network.

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DOI: http://dx.doi.org/10.3201/eid1709.110391

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Isoniazid-Resistant Tuberculosis, Taiwan, 2000–2010

To the Editor: Vinnard et al. (*1*) reported that the risk factors associated with initial isoniazid resistance among patients with tuberculous meningitis in the United States during 1993–2005 included young age (25–34 years) and foreign birth (*1*). In a previous survey, conducted in Taiwan during 2000–2008, we found the rate of antituberculosis drug resistance to be lower for older patients than for younger patients (*2*); however, current information about the patient characteristics associated with isoniazid-resistant tuberculosis (TB) in Taiwan is lacking. Therefore, to determine the risk factors associated with initial isoniazid resistance among patients with TB in Taiwan, we conducted a retrospective study.

The study was conducted at the National Taiwan University Hospital, a 2,500-bed tertiary care center in northern Taiwan. We analyzed culture-confirmed *Mycobacterium tuberculosis* isolates obtained from hospitalized patients during January 2000–December 2010. A nonduplicate isolate was defined as 1 isolate collected for evaluation from 1 patient who visited the hospital (as inpatient or outpatient). If multiple isolates were available from a patient, only the one first isolated was analyzed. All specimens were processed and pretreated as described elsewhere (*3*). Patients with multidrug-resistant TB were excluded on the basis of evidence for differences in the epidemiology of isoniazid-resistant (rifampin-susceptible) TB and multidrug-resistant TB (*4*). Immigrant populations in Taiwan are limited; therefore, we did not analyze the origin of the patients.

After excluding patients with multidrug-resistant TB, we analyzed 4,289 nonduplicate isolates, of which 3,842 (89.6%) were susceptible to isoniazid and the other 447 (10.4%) were resistant to isoniazid. In terms of demographic associations, patients 34–<44 years of age were more likely than those ≥74 years of age to have an isoniazid-resistant strain (Table). In addition, patients with extrapulmonary TB were less likely than patients with pulmonary TB to be infected with isoniazid-resistant TB. We also identified 34 patients with TB meningitis. After excluding 2 patients with multidrug-resistant TB, we found that 31 patients (mean age 56.6 years) had isoniazid-susceptible TB meningitis and a 50-year-old man had meningitis caused by isoniazid-resistant TB.

Our results are in agreement with those reported in a previous study in the United States, which found that the rate of isoniazid resistance was lower for isolates from elderly patients (*1*,*4*). This phenomenon may be attributable to the reactivation of a dormant infection. Because isoniazid was introduced to Taiwan for the treatment of TB in 1952, elderly persons in Taiwan probably did not

Table. Factors associated with isoniazid resistance among *Mycobacterium tuberculosis* isolates, National Taiwan University Hospital, Taiwan, 2000–2010*

Factor	No. infections	No. isoniazid-resistant infections	Resistance rate	OR (95% CI)
Patient age, y				
<14	42	2	4.76	0.32 (0.08–1.23)
14 to <24	241	22	9.13	0.64 (0.42–1.07)
24 to <34	342	36	10.53	0.75 (0.48–1.18)
34 to <44	384	52	13.54	Reference
44 to <54	490	56	11.43	0.82 (0.55–1.23)
54 to <64	609	70	11.49	0.83 (0.57–1.22)
64 to <74	845	96	11.36	0.82 (0.57–1.17)
74 to <84	986	85	8.62	0.60 (0.42–0.87)
84	350	28	8.00	0.56 (0.34–0.90)
Patient sex				
F	1,356	128	9.44	0.85 (0.69–1.06)
M	2,933	319	10.88	Reference
Pulmonary tuberculosis				
No	772	56	7.25	0.63 (0.47–0.84)
Yes	3,517	391	11.12	Reference

*OR, odds ratio; CI, confidence interval.

receive isoniazid if their TB developed when they were young. In the present study, the resistant rate was lower for *M. tuberculosis* strains isolated from elderly persons than from younger adults. These findings suggest that first-line anti-TB medications still have good in vitro activity against *M. tuberculosis* strains in elderly patients.

In contrast to the study by Vinnard et al. (*1*), our results showed that isoniazid-resistant *M. tuberculosis* was significantly less likely to be isolated from nonrespiratory than from respiratory specimens. The reasons for this finding are unclear. Continuous monitoring of antimicrobial drug resistance among *M. tuberculosis* isolates isolated from various body sites needs to be incorporated into any TB surveillance program.

Gathering data on drug resistance rates is a major aspect of the global TB control program. Clinicians must have knowledge of local epidemiology, and mycobacteriology laboratories should maintain up-to-date information on drug susceptibility test profiles of local *M. tuberculosis* isolates.

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DOI: http://dx.doi.org/10.3201/eid1709.110447

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Novel *Mycobacterium* Species in Seahorses with Tail Rot

To the Editor: Seahorses (*Hippocampus guttulatus* and *H. hippocampus*) with signs of tail rot disease (lethargy, lack of appetite, white spots on the skin, and necrotic tail lesions) were collected from aquaria at the Institute of Marine Research, Spain, during March 2007 through May 2009 (online Appendix Figure, www.cdc.gov/EID/content/17/9/101289-appF.htm). Microscopic examination of cutaneous lesions after Ziehl-Neelsen staining disclosed acid-fast bacilli. Microbiologic analysis showed unidentified *Mycobacterium* strains. Subsequently, we used PCR amplification of repetitive bacterial DNA elements to group the strains (*1*). The results showed an identical PCR pattern for the strains; thus, we selected strain BFLP-6^T for analysis. On the basis of phenotypic and genotypic data, we consider the unknown acid-fast bacillus to represent a novel species of the genus *Mycobacterium*, for which the name *M. hippocampi* sp. nov. is proposed.

Extraction and amplification of genomic DNA for 16S rRNA sequence analysis were conducted as described (*2*), and the RNA polymerase B (*rpoB*) gene was amplified and sequenced as described by Adékambi et al. (*3*).

Sequences obtained were compared against the sequences available in the GenBank, EMBL, and DDBJ databases obtained from the National Center for Biotechnology Information by using the BLAST program (*4*). Phylogenetic analysis were performed by using MEGA version 4.0 (*5*) after multiple alignments of data by ClustalX (*6*). Distances (distance options according to the Kimura 2-parameter model) and clustering with the neighbor-joining method were determined by using bootstrap values for 1,000 replications.

The 16S rRNA sequence of strain BFLP-6^T was a continuous stretch of 1,473 bp (GenBank accession no. FN430736). Sequence similarity calculations after a neighbor-joining analysis indicated that the closest relatives of strain BFLP-6^T were *M. flavescens* (98.26%), *M. goodii* (98.01%), *M. duvalii* (97.94%), *M. smegmatis* (97.92%), and *M. novocastrense* (97.86%) (Figure). Similar results were obtained for strain BFLP-6^T when the maximum-parsimony algorithm was used. The *rpoB* gene has also been proposed as a useful marker for inferring bacterial phylogeny (*7*,*8*). A pairwise analysis of the *rpoB* sequence of strain BFLP-6^T (GenBank accession no. FR775976) showed low levels of similarity (<89.8%) with other species of the genus *Mycobacterium*. The G + C content of DNA, as measured by the thermal denaturation method, was 66.7 mol%.

Strain BFLP-6^T was found to consist of gram-positive–staining, aerobic, acid-alcohol–fast, nonmotile, and nonsporulating cells. A scanning electron micrograph showed that strain BFLP-6^T is irregular, rod-shaped, ≈1.2–1.4 μm in length, and 0.4 μm in diameter. Colonies on Lowenstein-Jensen medium supplemented with 1.5% (wt/vol) sodium chloride were orange after incubation at 25°C for 5 days. The colonies were positive for catalase, glucose fermentation, arginine dihydrolase, urease, and aesculin, and assimilation of glucose, mannitol, potassium gluconate, and malate. The colonies were negative for nitrate reduction to nitrite, oxidase, indole production, gelatin hydrolysis, *N*-acetyl-D-glucosamine; and assimilation of arabinose, mannose, maltose, caprate, adipate, citrate, and phenylacetate. The major fatty acids were C18:1ω9*c*, C16:0, and C16:1ω6*c*. Mycolic acids included α-mycolates, keto-mycolates, and nonhydroxylated fatty acid methyl esters.

In addition, strain BFLP-6^T showed resistance to isoniazid, thiophene-2-carboxylic hydrazide, hydroxylamine, thiacetazone, and picrate. However, the strain exhibited susceptibility to ciprofloxacin, clarithromycin, and rifampin. The type strain BFLP-6^T has been deposited in the German Collection of Microorganisms and Cell Cultures, under reference DSM 45391^T; and in the Belgian Coordinated Collections of Microorganisms under reference LMG 25372^T.

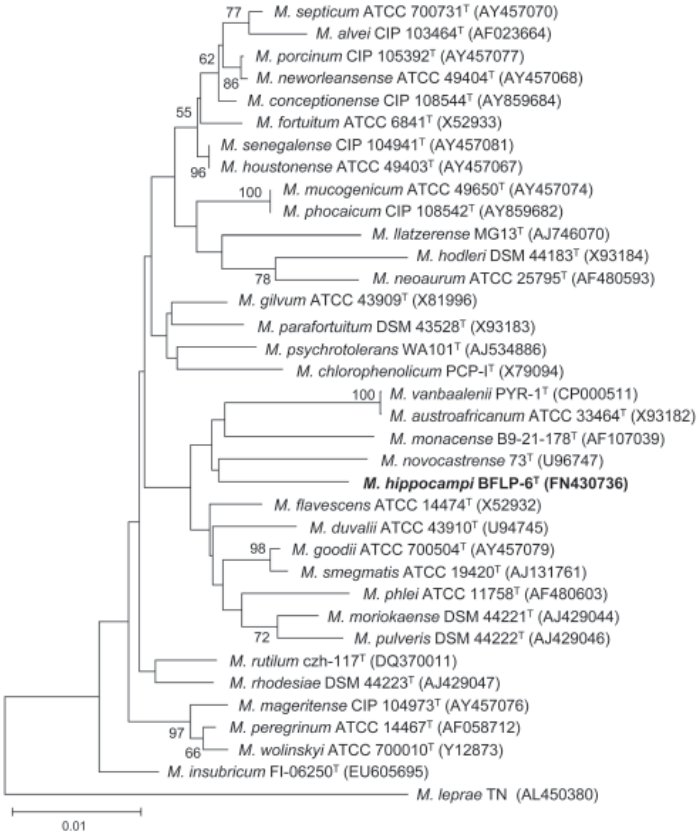

Figure. Neighbor-joining phylogenetic tree constructed from 16S rRNA gene sequences, showing the position of strain BFLP-6^T (in **boldface**) among other *Mycobacterium* species. Numbers at node indicate bootstrap values (expressed as percentages of 1,000 replications); only values >50% are given. *Mycobacterium leprae* TN was used as an outgroup. Scale bar indicates 0.01 substitutions per nucleotide position. GenBank accession numbers are in parentheses.

Acknowledgments

We thank P. Quintas, A. Chamorro, M. Cueto, S. Otero, and P. Ruiz for skillful technical assistance.

This study was supported by the Spanish Ministry of Science and Technology (*Hippocampus* CGL2005-05927-C03-01 and Hipporearing CGL2009-08386). J.L.B. was supported by a postdoctoral I3P contract from the Spanish Council for Scientific Research.

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DOI: http://dx.doi.org/10.3201/eid1709.101289

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Mycoplasma leachii sp. nov. in Calves, China

To the Editor: *Mycoplasma leachii* sp. nov., a new species designation for *Mycoplasma* sp. bovine group 7 (*1*), was initially isolated from joint fluids of arthritic calves in southern Queensland, Australia, and its pathogenicity was established by experimental infection (*2*). It was represented by the type strain PG50. Subsequently, *M. leachii* was reported infrequently as a cause of polyarthritis in calves and mastitis in cows; the pathogen was also isolated from aborted fetuses and pneumonic bovine lungs (*3*–*6*) and from small ruminant hosts (*7*).

M. leachii is one of 5 recognized members of the *M. mycoides* cluster, which comprises 3 species (*1*). Most notable are *M. mycoides* subsp. *mycoides* small colony and *M. capricolum* subsp. *capripneumoniae*, the etiologic agents of contagious bovine and caprine pleuropneumonia, which are listed by the World Organisation for Animal Health as notifiable animal diseases. The *M. mycoides* subsp. *capri* and *M. capricolum* subsp. *capricolum* cause various symptoms in small ruminants (*8*). Strains of *M. leachii* that cause mastitis and polyarthritis in cattle are serologically distinct from other bovine *Mycoplasma* spp. (*9*). Most reported isolates of *M. leachii* were detected in Australia. We report the isolation of *M. leachii* in cattle in China.

During January–May 2009, severe polyarthritis was observed in ≈100% of ≈350 female calves at the central calf rearing unit of a farm in Helongjiang Province, People's Republic of China. Clinical signs were noticed at ≈3–5 days of age, with severity gradually increasing over the next 2 days. At that time, the carpal and tarsal joints were greatly enlarged because of accumulation of intraarticular fluid. Ampicillin, sulfonamide, and streptomycin antimicrobial drug regimens for polyarthritis were ineffective. Approximately 100 calves died during the outbreak; the remaining calves recovered irrespective of treatment, but permanent disfigurement of the appendicular skeleton was evident. The disfigurement led to the calves being culled.

Necropsy was conducted on the calves that died during the outbreak, and gross and histopathologic findings similar to those described (*2*,*3*) were observed. Nearly all diarthroidal joints were enlarged and contained yellow-gray turbid synovial fluid and large yellow fibrin clots. The synovial membranes were slightly thickened, congested, and had some villous proliferation. Histologic examination of the affected articulations found severe, diffuse, subacute arthrosynovitis and bursitis.

Routine bacterial culture of 2 joint fluid samples collected aseptically from different animals showed no bacterial growth. *Mycoplasma* spp. infection was suspected, and the samples were forwarded to the laboratory for specific culture; 2 were positive for *Mycoplasma* spp. These isolates were designated GN407 and GN408.

The presence of *M. leachii* in joint fluids and *Mycoplasma* spp.–positive cultures was detected by PCR with the partial *lppA* gene amplified with a protocol modified from the method described by Frey et al. (*10*) and amplification of the complete 16S rRNA gene was performed by using the primers 16S-upper 5′-AAAATGAGAGTTTGATCCTGG-3′ and 16S-lower 5′-AGAAAGGAGGTGATCCATCCG-3′. The primers were designed on the basis of the 16S rRNA gene sequence of *M. leachii* PG50 (U26054). PCR products were sequenced directly in both directions. Sequence analyses

were conducted by using MEGA version 4.1 (www.megasoftware.net). The partial *lppA* gene nucleotide sequences of isolates GN407 and GN408 were submitted to GenBank under accession nos. HQ699892 and HQ699893, respectively.

PCR amplifications of the 2 joint fluids and their cultures were positive for *M. leachii*. When we compared the complete 16S rRNA gene and the partial *lppA* gene, the 2 isolates from the same epizootic shared 100% nt identity. For 16S rRNA gene, the isolates shared 99.9%, 99.9%, and 99.7% nt identities to *M. leachii* PG50, *M. capricolum* subsp. *capricolum*, and *M. mycoides* subsp. *mycoides* small colony, respectively. For partial *lppA* gene, the isolates shared 99.6%, 95.1%, and 69.6% nt identities to *M. leachii* PG50, *M. mycoides* subsp. *mycoides* small colony, and *M. capricolum* subsp. *capricolum*, respectively.

Intraarticular inoculation of the passage cultures successfully reproduced the polyarthritis in calves 1 month of age. Thus, there are notable similarities between our findings and those reported in Australia (*3*). Multidisciplinary procedures, including clinical assessment and comprehensive laboratory investigations of affected calves, were used to identify the etiologic agent. The results showed that the outbreak of the serious polyarthritis in calves was caused by *M. leachii*.

Our detection of *M. leachii* in China confirms a wider geographic presence of this type of *Mycoplasma* spp. in cattle and suggests *M. leachii* is common and potentially distributed worldwide. Currently, the source of *M. leachii* infection and its means of spread have not been established. However, our epidemiologic and clinical investigations indicated clear evidence of seminal infection because all calves with arthritis were from dams that were fertilized by using the same batch of semen, and cows in the same herd that were fertilized by using a different batch of semen delivered healthy calves. More epidemiologic, molecular, and pathogenic studies are required to determine the relevance, distribution, importance, and diversity of *M. leachii* in cattle.

This work was supported by Special Fund for Agro-scientific Research in the Public Interest of China (No. 200803018).

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DOI: http://dx.doi.org/10.3201/eid1709.101891

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Bartonella clarridgeiae in Fleas, Tahiti, French Polynesia

To the Editor: *Bartonella* species are small, gram-negative, fastidious, and hemotropic emerging pathogens that cause various human diseases and circulate between a large variety of mammalian and arthropod vectors. More than 30 *Bartonella* species have been isolated from humans as well as from wild and domestic animals worldwide (*1*). *B. clarridgeiae* was suggested to be a minor causative agent of cat-scratch disease (CSD) in humans, however, this suggestion remains controversial. Usually, the agent of CSD is *B. henselae* and its principal reservoir is domestic cats (*Felis catus*) (*1*,*2*). The principal vector of these 2 species is the cat flea (*Ctenocephalides felis*) (*3*,*4*).

We report *Bartonella* species in fleas collected from cats and dogs in Tahiti, French Polynesia.

In October 2009, fleas were collected from 1 cat and 9 dogs in Papeete, capital of Tahiti Island, French Polynesia. Fleas collected were kept in 70% ethanol and sent to the military veterinary service in Marseille, France; these fleas were later sent to Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes in Marseilles. The fleas were identified phenotypically by using current taxonomic criteria. DNA from fleas and negative control DNA from noninfected laboratory lice were extracted by using a QIAamp Tissue Kit (QIAGEN, Hilden, Germany), as described (*3*).

Flea samples were tested for *Bartonella* spp. DNA by using the 7900 HT Fast Quantitative Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and primers and Taqman probes specific for the 16S–23S rRNA gene intergenic spacer region as described (*5*). Fleas were considered positive when cycle threshold was ≤30. All positive fleas at screening were confirmed by using standard *Bartonella* PCR and sequencing of partial internal transcribed spacer gene fragments by using primers URBarto1 and URBarto2, as described (*3*). *B. elizabethae* DNA was used as positive control. DNA sequencing reagents were obtained with BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI PRISM; Applied Biosystems). The sequences were assembled in Sequencher 4.2 (GeneCodes 2003; www.genecodes.com) and were compared with *Bartonella* sequences available in GenBank.

Overall, 81 fleas were collected from 1 cat (13 fleas) and 9 dogs (68 fleas). All 81 fleas collected were morphologically identified as *C. felis*.

Sample fleas were collected from animals visiting a veterinary clinic for neutering or vaccinations. The overall rate of *Bartonella*-positive fleas by molecular screening with real-time PCR was 7.4% (6/81): 6 fleas from the cat (6/13) and none from a dog (0/68). These positive samples were confirmed after intergenic spacer PCR amplification and sequencing with sequences at 100% identity with *B. clarridgeiae* (GenBank accession no. EU589237).

B. clarridgeiae was first isolated from the pet cat of an HIV-positive patient in the United States (*6*). However, *B. clarridgeiae* has never been isolated or detected by molecular methods in humans, and thus its implication as a human pathogen remains controversial. The presence of *B. clarridgeiae* antibodies has been reported in a suspected case of CSD and in a patient with a chest-wall abscess (*4*). However, *B. clarridgeiae* has been detected on fleas from various continents, including Europe, Asia, North America (*1*), Africa; New Zealand, and recently from New Caledonia (*7*).

In France, several studies have reported the molecular detection of *B. clarridgeiae* in the blood of a cat or in cat fleas (*C. felis*), indicating the potential role of fleas as vectors of this organism (*8*,*9*). Prevalence of this bacterium in cat fleas may vary and be as high as 67.9% in cat fleas from France (*3*). Moreover, DNA of *B. henselae* and *B. clarridgeiae* has been reported from cat fleas from New Zealand (*10*). Similarly, co-infection with *B. clarridgeiae* and *B. henselae* has been reported in domestic cats from Europe and Asia (*1*). In our study, all *Bartonella* spp.–positive fleas harbored *B. clarridgeiae* only; all were obtained from cats and none from dogs, similar to findings reported from New Zealand (*10*), although *B. clarridgeiae* has been reported from a flea on a dog in Taiwan (*2*).

Papeete, the capital of Tahiti, is located in the South Pacific Ocean, and remains one of the most visited areas by tourists from all over the world. There are many stray cats and dogs in Tahiti that may be infected with *Bartonella* species and thus serve as a reservoir for these pathogens. Our result confirms the presence of *B. clarridgeiae* in Tahiti and is a warning of the presence of flea-borne bartonellosis and the potential risk of *B. clarridgeiae* or other flea-borne diseases for humans exposed to cat fleas.

Acknowledgment

We thank Vincent Perrot for his help in collecting the fleas from Tahiti.

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DOI: http://dx.doi.org/10.3201/eid1709.102063

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Bocavirus in Children with Respiratory Tract Infections

To the Editor: Four species of human bocavirus (HBoV1–4) have been identified since 2005 (*1*–*4*). Several reports have documented that HBoV1 are prevalent in respiratory tract samples. Although there may be many asymptomatic carriers, HBoV1 has been shown to cause respiratory tract diseases (*1*,*5*,*6*). HBoV2 has mainly been detected in fecal samples and has been linked to gastroenteritis (*3*,*7*,*8*). HBoV3 and HBoV4 have recently also been detected in fecal samples (*3*,*4*), although no link to disease has been established for these 2 species.

In this study, we identified and characterized 3 HBoV species (HBoV1–3) detected in respiratory samples. Nasopharyngeal aspirates were collected from 1,238 children (784 boys and 454 girls) with acute lower respiratory tract infections hospitalized in Beijing Children's Hospital from March 2008 through July 2010. Patients' ages ranged from 1 month to 9 years (median 10.0 months, mean 32.1 months).

Viral nucleic acid was extracted from nasopharyngeal aspirates by using the NucliSens easyMAG system (bioMérieux, Marcy l'Etoile, France). We screened for HBoV1–4 by nested PCR with touch-down procedure using primers targeting the viral proteins (VP) 1/2 region (*4*). For HBoV-positive samples, we then quantified viral loads by real-time PCR (online Technical Appendix, www.cdc.gov/EID/content/17/9/110078-Techapp.pdf). Additional viral infections were identified in all screened specimens as described (*9*). To avoid contamination, the PCR process (including master mixture preparation, nucleic acid extraction, reaction installation, and DNA amplifications) were performed in separate dedicated areas. Strict controls were used during the process of nucleic acid extraction and PCR analyses to monitor contamination. All PCR products were verified by sequence analysis.

We found 141 positive samples for HBoVs (11.4%) from patients ranging in age from 1 to 132 months (median 12.0 months, mean 19.8 months) (GenBank accession nos. HQ871520–HQ871650 and HQ871664–HQ871673). Among these samples, 131 (10.6%; patient ages 1–132 months, median 12 months, mean 19 months) were positive for HBoV1, 5 (0.4%; patients 1–113 months of age, median 7.2 months, mean 29.2 months) for HBoV2, and 5 (0.4%; 1–108 months of age, median 12.0 months, mean 30.4 months) for HBoV3 on the basis of sequence alignment and phylogenetic analysis of PCR amplicons. No specimens were positive for HBoV4. The number of samples positive for HBoV1, -2, and -3 in children ≤5 years old was 124/131 (94.7%), 4/5 (80%), and 4/5 (80%), respectively. Additional respiratory viruses were co-detected in 120/141 (85.1%) HBoV-positive patients (Table). An unanticipated finding was that real-time PCR only detected 1/5 positive sample for HBoV2 (viral load 4.87×10^9 copies/mL) and 2/5 positive samples for HBoV3 (viral load 2.59×10^4 and 4.1×10^2 copies/mL). In contrast, we detected viral loads of 8.35×10^4 to 1.28×10^9 copies/mL in 5 randomly selected HBoV-1–positive samples.

The clinical diagnoses of patients providing HBoV-positive samples included pneumonia (63.1%), bronchitis (14.9%), bronchopneumonia (12.8%), and acute asthmatic bronchopneumonia (9.2%). No clinically significant differences were found between the signs and symptoms of patients with HBoV1, -2, and -3 (cough, sputum production, fever, runny nose, wheezing, and diarrhea). For patients positive for HBoV3, the major diagnoses were pneumonia (2/5), bronchopneumonia (2/5), and acute asthmatic bronchopneumonia (1/5). Main signs and symptoms included cough (5/5), wheezing (4/5), sputum production (3/5), fever (3/5), runny nose (1/5), and diarrhea (1/5). For patients positive for HBoV2, the diagnoses were pneumonia (4/5) and bronchopneumonia (1/5), and main signs and symptoms included cough (5/5), sputum production (4/5), wheezing (3/5), fever (3/5), and runny nose (2/5) (Table).

HBoV2 and HBoV3 were detected sporadically during the study. HBoV3 was detected in samples collected in

Table. Distribution and clinical characteristics of HBoV species in 1,238 children with acute lower respiratory tract infections, Beijing, China, 2008–2010*

Parameters	HBoV1	HBoV2	HBoV3
No. (%) positive	131 (10.6)	5 (0.4)	5 (0.4)
Age range, mo	1–132	1–113	1–108
No. (%) patients ≤5 y of age	124 (94.7)	4 (80)	4 (80)
Age mean/median, mo	19/12	29.2/7.2	30.4/12
M/F	72/59	3/2	4/1
Clinical manifestations, no. (%)			
Fever	78 (59.5)	3 (60)	3 (60)
Cough	131 (100)	5 (100)	5 (100)
Sputum production	115 (87.8)	4 (80)	3 (60)
Wheezing	65 (49.6)	3 (60)	4 (80)
Runny nose	46 (35.1)	2 (40)	1 (20)
Diarrhea	9 (6.9)	0 (0)	1 (20)
Mean ± SD leukocyte count, $\times 10^9$/L	9.4 ± 5.3	7.56 ± 1.4	13.45 ± 6.1
Co-detection in samples, no. (%)			
Rhinovirus	58 (44.3)	1 (20)	2 (40)
Respiratory syncytial virus	42 (32.1)	2 (40)	2 (40)
Parainfluenza virus	24 (18.3)	0 (0)	1 (20)
Influenza virus	11 (8.4)	1 (20)	2 (40)
Conorovirus	15 (11.5)	0	0
Adenovirus	14 (10.7)	0	1 (20)
Human metapneumovirus	16 (12.2)	0	0
Enterovirus	2 (1.5)	1 (20)	0

*Detection rate for HBoV species, $\chi^2 = 234.1$, $p < 0.01$. No HBoV4 was found. HBoV, human bocavirus.

July and October 2008, December 2009, and March and April 2010, whereas HBoV2 was detected in May, June, and October 2008, March 2009, and January 2010. The prevalence of HBoV1, which was responsible for 92.9% of the HBoV cases, was highest in January 2009 and April 2010.

Multiple-alignment analysis of sequences obtained in this study and reference sequences from GenBank (accession nos. FJ948861, NC_012564, EU918736, HM132056, and HQ152935) by using MEGA 4.0 (*10*) showed that amino acids in HBoV VP1/2 regions were 94.8%–100% identical among the strains of same species and 76.3%–100% identical among strains of different species. No obvious nucleotide and amino acid differences were found for the HBoV3 strains detected in respiratory samples and those in stool samples.

In summary, we report detection of genomic DNA of HBoV1, -2, and -3 in children with lower respiratory tract infections in China. The predominant HBoV species identified in our study was HBoV1. HBoV2 and HBoV3 appear to be present in much fewer positively identified cases and their viral load seems very low. For these latter viruses, however, low level mucosal contamination from the gastrointestinal tract cannot be entirely excluded in all cases. Further investigations are needed to confirm potential associations of HBoV2 and HBoV3 with acute lower respiratory tract infections, to determine their replication in the respiratory tract, and the viruses' role in human disease.

Acknowledgments

We thank Yaowu Yang for his assistance with designing the real-time PCR.

This study was supported in part by grants from the International Science and Technology Cooperation Program of China (2010DFB33270) and National Major Science & Technology Project for Control and Prevention of Major Infectious Diseases of China (2009ZX10004-206).

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DOI: http://dx.doi.org/10.3201/eid1709.110078

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Highly Virulent *Escherichia coli* O26, Scotland

To the Editor: Hemolytic uremic syndrome (HUS) is a rare disorder characterized by microangiopathic hemolytic anemia, microthrombi, and multiorgan injury. HUS is one of the commonest causes of acute renal failure in children worldwide and is most frequently precipitated by infection with verotoxin-producing *Escherichia coli* (VTEC) such as *E. coli* O157 (*1*). However, non-O157 VTEC serotypes have been increasingly found in the development of HUS (*2*–*4*).

Although previous surveillance of childhood HUS in Scotland identified *E. coli* O157 in >90% of cases, non-O157 serotypes have also been associated with HUS (*5*). In 2010, several particularly severe cases of HUS were reported to Health Protection Scotland by a consultant pediatric nephrologist. Subsequent tests identified the pathogen in these cases as *E. coli* O26. However, in a recent study of pediatric HUS cases in Europe, children infected with *E. coli* O26 did not exhibit different clinical signs and symptoms from patients infected with other VTEC serotypes (*6*). To establish whether the host pathophysiologic responses to *E. coli* O157 and *E. coli* O26 strains differed, we analyzed a cohort of children with HUS who were infected with these VTEC serotypes.

In Scotland, most patients with pediatric thrombotic microangiopathy are referred to a specialist pediatric hospital, which immediately reports cases of HUS to Health Protection Scotland as part of national surveillance. To test the hypothesis that *E. coli* O26 was more virulent than *E. coli* O157, we performed an age-matched, nested case–case study of HUS patients and compared host clinical markers, treatment, and outcomes from pediatric cases in 2010. Data collection has been described elsewhere (*5*). The statistical significances of associations between categorical variables were investigated by using χ^2, Fisher exact, Mann-Whitney, or *t* tests. All analyses were performed by using SPSS version 11 (SPSS Inc., Chicago, IL, USA) with a significance level of 5%.

Although initial signs and symptoms were similar for both sets of cases, i.e., bloody diarrhea and abdominal pain, statistical analysis showed that children with O26-HUS were more likely to have neurologic complications and diabetes mellitus and require admission to the intensive care unit than O157-HUS patients (p = 0.02 for neurologic complications and diabetes and p = 0.04 for admission to an intensive treatment unit; Table).

All patients with HUS were oligoanuric, and the 2 groups did not differ with respect to this parameter. However, O26-HUS patients had significantly longer periods of anuria than O157-HUS patients (p = 0.04; Table) and were more likely to require treatment with hemofiltration than with peritoneal or hemodialysis (p = 0.001; Table). One patient with O26-HUS also experienced cardiomyopathy resulting in reduced left ventricular function.

Our study illustrates the potential for increased severity of *E. coli* O26 infection in children. In Scotland, HUS is more commonly associated with *E. coli* O157 infection, and the outcome for children infected with this pathogen is much better than that reported in other studies (*7*,*8*). In this study, the clinical severity and outcomes for the children with O26-HUS were worse than for children requiring treatment for O157-HUS. We investigated the prehospital management of *E. coli* O157 and O26 patients in this cohort and found no difference in pharmacologic intervention or duration of delay in admission to hospital.

In our cohort, vtx_1 and vtx_2 genes were detected in isolates from 2 of 3 patients. A diagnosis was made in the third patient by detection of *E. coli* O26 lipopolysaccharide–specific immunoglobulin M in serum; it was therefore not possible to confirm the presence of *vtx* genes. However, it is not unusual for VTEC to be undetectable in stool samples from patients with HUS, most likely because of intrahost bacteriophage lysis. Therefore, serodiagnosis of VTEC is considered a necessary adjunct to bacteriological confirmation of infection (*9*). A recent study suggests *E. coli* O26 exists as a highly dynamic group of organisms that can undergo verotoxin gene loss and be transferred during infection in humans, resulting in new pathogenic clones (*10*). Therefore, vtx_2 gene acquisition by *E. coli* O26 may have contributed to increased virulence.

Table. Characteristics of infection in children with *Escherichia coli* O157 versus *E. coli* O26, Scotland, 2010*

Variable	O157 HUS, n = 12	O26 HUS, n = 3	p value
Age, y	4.5 ± 1.0	3.7 ± 1.3	
Clinical signs and symptoms			
Diarrhea	12	3	
Bloody diarrhea	8 (67)	2 (67)	NS
Abdominal pain	8 (67)	3 (75)	NS
Fever	2 (17)	1 (33)	NS
Neurologic involvement	3 (25)	3 (100)	0.02
Diabetes	1 (12)	2 (67)	0.02
Cardiomyopathy	0	1 (33)	0.04
Anuria, d	7.7 ± 2.4	15.7 ± 1.3	0.04
Clinical parameters			
Leukocyte count × 10^9/L	42.1 ± 14.4	25.9 ± 4.8	NS
C-reactive protein, mg/L	93.5 ± 28.4	151 ± 74	NS
Serum albumin, g/L	22.5 ± 1.3	22.3 ± 2.4	NS
Lactate dehydrogenase, IU/L	2,521 ± 362	1,991 ± 642	NS
Admission to ITU	3 (25)	3 (100)	0.04
Duration of hospitalization, d	13.2 ± 2.2	25 ± 3.8	0.03
Treatment			
Peritoneal dialysis, d	7.4 ± 1.9	10	NS
Hemodialysis, d	8.5 ± 1.5	8.3 ± 3.0	NS
Hemofiltration	0	3 (100)	0.001
Laparotomy	0	1 (33)	0.04
Postdischarge hypertension	0	2 (67)	0.01

*Values are mean ± SE or no. (%). Significance relates to difference between groups. HUS, hemolytic uremic syndrome; NS, not significant, ITU, intensive treatment unit.

Our study was limited by the small number of patients with pediatric O26-HUS. However, given the severity of the complications experienced by the children in this cohort, we believe it is necessary to communicate these findings promptly to the international community.

We suggest that infection with *E. coli* O26 in children can result in more severe and complicated forms of HUS than those caused by *E. coli* O157. In contrast to the findings of Gerber et al., we found that there was a significant difference in neurologic complications between the 2 groups (*2*). Epidemiologic investigations found that 2 of the 3 children lived on farms and may have acquired infection while playing near their homes (the other was acquired through foreign travel). Risk communication of VTEC infection to parents of young children who live in farming communities remains problematic, perhaps because of the perception that immunity has been acquired. Although this suggestion may be true for adults, children are likely to be immunologically naive. Salient public health messages on simple precautionary behavior need to be regularly reinforced because prevention of VTEC infection prevents HUS.

Acknowledgments

We thank the staff of the renal unit, Royal Sick Children's Hospital, Yorkhill, UK, for their ongoing contributions to the clinical surveillance program and also staff at the Scottish *E. coli* O157/VTEC Reference Laboratory and Gastrointestinal Infections Reference Unit, Health Protection Agency, Colindale.

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DOI: http://dx.doi.org/10.3201/eid1709.110199

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Perinatal Transmission of Yellow Fever, Brazil, 2009

To the Editor: Although urban cases of yellow fever have not been reported in Brazil since 1942, sylvatic yellow fever is still endemic to the northern and middle-western states. In the past decade, the endemic area has spread southward and eastward, approaching most populated states (*1*). In 2009, there was an outbreak of sylvatic yellow fever in São Paulo State that caused 28 cases and 11 deaths. In the affected area, there had been no reports of yellow fever since the 1930s (*2*). In the outbreak setting, a case of perinatal yellow fever transmission was diagnosed.

The mother was a 30-year-old woman exposed to yellow fever in late pregnancy in a sylvatic area near Piraju (23°11′44″S, 49°22′54″W), a city 100 km from Botucatu. The patient had not received yellow fever vaccine and had not traveled to yellow fever–endemic regions in the previous months. The exposure to yellow fever occurred during regular walks in a sylvatic area, a habit that continued until late pregnancy. She had fever, headache, and jaundice on March 14, 2009. Three days later, on March 17, she delivered a female infant through vaginal partum in a hospital in her hometown.

The mother´s symptoms were mild. She was admitted to Botucatu Medical School Hospital 7 days after delivery; she had fever, jaundice, and conjunctival suffusion. Liver enzymes were elevated (aspartate aminotransferase [AST] 246 U/L, alanine transaminase [ALT] 324 U/L, γ-glutamyl transpeptidase 221 U/L, and alkaline phosphatase 338 U/L). She was mildly anemic (hemoglobin level 10.2 g/dL), but leukocyte and platelet counts were within reference ranges. There were no other laboratory abnormalities. She was discharged after 7 days with complete recovery.

The infant girl was born asymptomatic on March 17, with a birthweight of 3,800 g and Apgar scores of 9–10. She was discharged from the hospital after 2 days of exclusive breast-feeding. On the third day of life, she had fever and cyanosis and was readmitted to the local hospital with suspected pneumonia. She received antimicrobial drugs but showed no improvement. On the 8th day of life, she had hematemesis, melena, bleeding at venipuncture sites, hypoglycemia, and oliguria.

The newborn was transferred to the Neonatal Intensive Care Unit at Botucatu Medical School Hospital. At admission, she had hypotension, tachycardia, cutaneous paleness, jaundice, hepatomegaly, and melena. The initial diagnostic hypothesis was congenital or hospital-acquired sepsis, but the mother´s diagnosis prompted doctors to investigate possible yellow fever. The infant was intubated for ventilatory support and received volume expansion, vasoactive amines, antimicrobial drugs, blood components (erythrocytes, platelets, fresh frozen plasma, cryoprecipitate), and drugs to control bleeding. Liver enzymes values were initially high (AST 4,072 U/L, ALT 1,420 U/L) but fell abruptly within 3 days (AST 150 U/L, ALT 114 U/L).

Despite the therapy, the newborn experienced liver and renal failure, disseminated intravascular coagulation, seizures, and finally coma. She died on the 12th day of life (4th day of hospitalization in the neonatal intensive care unit). Autopsy specimens showed massive liver necrosis, pulmonary hemorrhage, and acute tubular necrosis (Figure).

The mother had serologic tests (immunoglobulin M antibody capture ELISA) done on the 11th day of disease. Test results were positive for yellow fever and negative for dengue fever. The newborn had similar results from serum samples collected 5 days after onset of symptoms with confirmation by reverse transcription PCR (RT-PCR). RT-PCR was performed as described by Deubel et al. (*3*). Nucleotide sequencing showed a wild yellow fever virus belonging type I of South American genotype 1E, according to the classification proposed by Vasconcelos et al. (*4*). RT-PCR performed with samples of the mother´s serum did not amplify yellow fever virus sequences. However, the serum was collected on the 11th day post symptoms when the sensitivity of the test is low.

The vertical transmission of arboviruses has been documented. Pouliot et al. reviewed direct and indirect evidence for vertical transmission of dengue virus (*5*). Vertical transmission has also been reported for West Nile encephalitis and western equine encephalitis (*6*). This is not the case for yellow fever. Reports of yellow fever during pregnancy are scarce (*7*), and we found none that describe vertical transmission to newborns. However, vaccine virus was isolated from asymptomatic newborns from pregnant women who

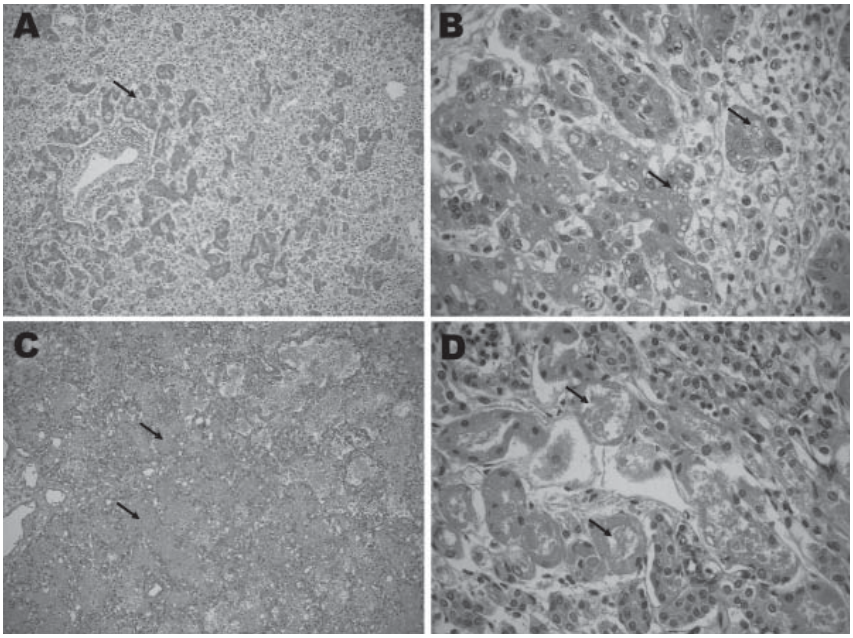

Figure. Microscopy findings of autopsy specimens from a 12-day-old girl with yellow fever, Brazil, 2009. A) Massive liver necrosis, with proliferation of ductular-like structures around portal tracts (arrow); B) hepatocytes with microvesicular fatty changes (arrows); C) intra-alveolar lung hemorrhage (arrows); D) renal tissue showing acute tubular necrosis (arrows). Panels A and C, original magnification ×200; panels B and D, original magnification ×400. A color version of this figure is available online (www.cdc.gov/EID/content/17/9/110242-F.htm).

were inadvertently administered 17D vaccines (*8*). Still, mother-to-child transmission in late pregnancy or during delivery is a likely explanation for this newborn infection. The possibility of acquiring the virus through a mosquito bite is unlikely because urban cases of yellow fever have not occurred in Brazil in the past 50 years and were not reported in the present outbreak.

The timing of the newborn's symptoms also argues against the possibility of transmission from a mosquito. We cannot rule out transmission through breast-feeding, which has been reported for the yellow fever vaccine virus (*9*). However, this would presuppose a short incubation period. In conclusion, this case points out to the possibility of vertical transmission of yellow fever. We imagine that similar cases can occur in yellow fever–endemic settings and may be identified by improved surveillance.

Acknowledgments

We thank Ligia Maria Suppo de Souza Rugolo and Thamy Yamashita for special collaboration during preparation of the manuscript.

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DOI: http://dx.doi.org/10.3201/eid1709.110242

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Pathogenic *Leptospira* spp. in Wild Rodents, Canary Islands, Spain

To the Editor: Leptospirosis is a major emerging infectious disease with a worldwide distribution (*1*). It is a systemic disease of humans and domestic animals (*2*). Regarded globally as a zoonosis because it is acquired by humans from contact with animals or from water contaminated with the urine of infected animals, it is presumed to be the most widespread zoonotic disease in the world (*1*,*2*). Species such as mice (*Mus* spp.) and rats (mainly *Rattus norvegicus* and *R. rattus*) serve as reservoirs for their host-related serovars (*3*).

Human patients usually exhibit a nonspecific self-limiting febrile illness; however, in 5%–10% of cases, severe forms of the disease develop, including Weil disease and severe pulmonary hemorrhagic syndrome. Case-fatality rates for Weil disease and severe pulmonary hemorrhagic syndrome are >10% and >74%, respectively (*4*,*5*).

Because leptospirosis has been found in humans in the Canary Islands (J. Alcoba-Flores, pers. comm.), detecting carrier animals is vital to the understanding of enzootic and epizootic leptospirosis in this environment. We examined the possible role of the rodent species found in the Canary Islands in the transmission of this pathogen to determine the risk for humans in these islands.

A total of 149 wild rodents (74 *R. rattus* and 75 *Mus domesticus*) were captured during 20009–2010 from 4 of the Canary Islands (Tenerife, El Hierro, La Gomera, Lanzarote). Urinary bladders of the animals were collected and preserved in 100% ethanol. Genomic DNA was extracted by using the Fast DNA Kit (BIO 101 Systems: MP Biomedicals, Santa Ana, CA, USA).

The *lipL32* fragment (497 bp), which is present only in pathogenic leptospires, was amplified according to the method of Bomfim et al. (*6*) by using a MyCycler thermocycler (Bio-Rad, Hercules, CA, USA). *L. interrogans* serovar Icterohemorragiae (RGA strain) was used as a positive control.

Twenty-two samples were positive for *Leptospira* spp., indicating a general prevalence of 14.8% in the rodents. Although the prevalence was higher in rats (20.3%) than in mice (9.3%) (Table), the difference was not significant (χ^2 test). Positive samples were obtained from all the studied islands and for both host species in all of them (Table), without significant differences in the prevalences between host species or between islands.

To confirm the amplified products belonged to pathogenic *Leptospira* spp., we sequenced some amplicons. Sequencing reactions were performed for both strands at the University of La Laguna Genomic Service. When the sequences were compared, 2 different sequences were obtained. The first sequence, L19 (GenBank accession no. HQ231747), from rats, clustered with *L. interrogans* serovar Copenhageni (GenBank accession no. AE016823) and different serovars of the same species by BLAST (99% identity). Previous results associate *L. interrogans* serovar Copenhageni with *Rattus* spp. (*7*). However, the sequence obtained from mice (GenBank accession no. HQ231748), L47, showed a 100% BLAST identity with *L. borgpetersenii* (GenBank accession nos. DQ320625.1 and DQ286415.1).

New and published *Leptospira* sequences were aligned with the multiple alignment program ClustalW in MEGA3.1 (*8*), and minor corrections were made manually. The alignment for the 497-bp fragment starts at nt position 208, with respect to the complete sequence of the *lipL32* (AY609332), and ends at nt position 705.

Phylogenetic relationships were inferred by using the neighbor-joining distance method with MEGA3.1. At least 1,000 bootstrap replicates were used to infer statistical support at branch nodes. The consensus tree yielded 3 monophyletic groups clearly separated by high bootstrap values. The first clade was formed by *L. interrogans, L. kirschneri,* and *L. noguchii* (93% bootstrap value). The sequence L19 was included in the *L. interrogans* node (92% bootstrap value). The second clade included *L. borgpetersenii* and *L. weilii* as a monophyletic group

Table. Prevalence of pathogenic *Leptospira* spp. in rodents, by island, Canary Islands, 2009–2010*

Island and host species	No. (% positive)	% Prevalence (95% CI)
Tenerife	11 (49)	22.4 (10.7–34.1)
Mus domesticus	2 (12)	16.6 (0–37.6)
Rattus rattus	9 (37)	24.3 (10.5–38.1)
La Gomera	4 (16)	25.0 (3.8–46.2)
M. domesticus	3 (10)	30.0 (1.6–58.4)
R. rattus	1 (6)	16.7 (0–46.5)
El Hierro	2 (29)	6.9 (0–16.1)
M. domesticus	1 (16)	6.20 (0–18.11)
R. rattus	1 (13)	7.7 (0–22.2)
Lanzarote	5 (55)	9.1 (1.5–16.7)
M. domesticus	1 (37)	2.7 (0–7.9)
R. rattus	4 (18)	22.2 (3–41.4)
Total	22 (149)	14.8 (11.9–17.7)
M. domesticus	7 (75)	9.3 (2.7–15.9)
R. rattus	15 (74)	20.3 (11.1–29.5)

*No., number of rodents studied; positive, samples positive for pathogenic *Leptospira* spp.; CI, confidence interval.

(97% bootstrap value). The sequence L47 clustered with *L. borgpetersenii* DQ286415 with a high bootstrap value (82%). These results are in accordance with those obtained by Haake et al. (*9*) based on *lipL32*. Finally, *L. santarosai* sequences formed the third separate clade (100% bootstrap) (data not shown).

Although the method we used does not enable specific identification, determining the most similar species by BLAST is needed for control programs. *L. interrogans* serovar Copenhageni is the predominant infecting serovar among patients with severe leptospirosis (*7*), and *L. borgpetersenii* is also commonly acquired from mice.

On the basis of these findings, the global distribution of *Leptospira* spp. must be revised to include the Canary Islands, with rodents as natural hosts. Because pathogenic *Leptospira* spp. were detected on every island studied and in both analyzed species, *R. rattus* and *M. domesticus*, the distribution of this pathogen likely extends to even the islands not studied. The high incidence found suggests that rodents play a role in transmission of human leptospirosis. Further studies are needed to identify other possible reservoir hosts and to determine the risk areas for acquiring pathogenic leptospires in the Canary Islands.

Acknowledgments

We thank the Excmos Cabildos Insulares of Tenerife, La Gomera, El Hierro, and Lanzarote.

This work was supported by projects Red de Investigación Colaborativa de Centros de Enfermedades Tropicales (RD06/0021/0005) and CGL 2009-07759BOS.

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DOI: http://dx.doi.org/10.3201/eid1709.101470

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Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus, Asia

To the Editor: Recently, the novel and highly virulent variant of porcine reproductive and respiratory syndrome virus (PRRSV), which first emerged in the People's Republic of China and Vietnam in 2006 (*1*), has rapidly spread in pigs in Southeast Asia. The affected countries include Bhutan, Cambodia, Laos, Malaysia, Myanmar, the Philippines, Thailand, and Singapore. In eastern and northern Asia, South Korea and Russia were also reported to be affected (*2*) (Figure). The epidemic affected not only large commercial farms but also the backyard industry, which created a serious problem for the global swine industry and for food safety. In February 2011, the Veterinary and Animal Breeding Agency in Ulaanbaatar, Mongolia, confirmed an outbreak of porcine reproductive and respiratory syndrome (PRRS) (*3*). Nearby neighbors, such as Japan, North Korea, Indonesia, and other Asia-Pacific countries, are also at risk.

PRRS was first reported in the United States in 1987. The disease causes reproductive failure during late-term gestation in sows and respiratory disease in pigs of all ages. In 2006, a new, highly pathogenic PRRS emerged, characterized by high fever (41°C–42°C), skin discoloration/reddening, high incidence of illness (50%–100%), and high proportion of deaths (20%–100%) in pigs of all ages. This new PRRS has spread throughout the swine industry in China, resulting in the culling of an estimated 20 million pigs annually in 2006–2007 in China (*4*). PRRSV is a member of the family *Arteriviridae* in the order Nidovirales, which also

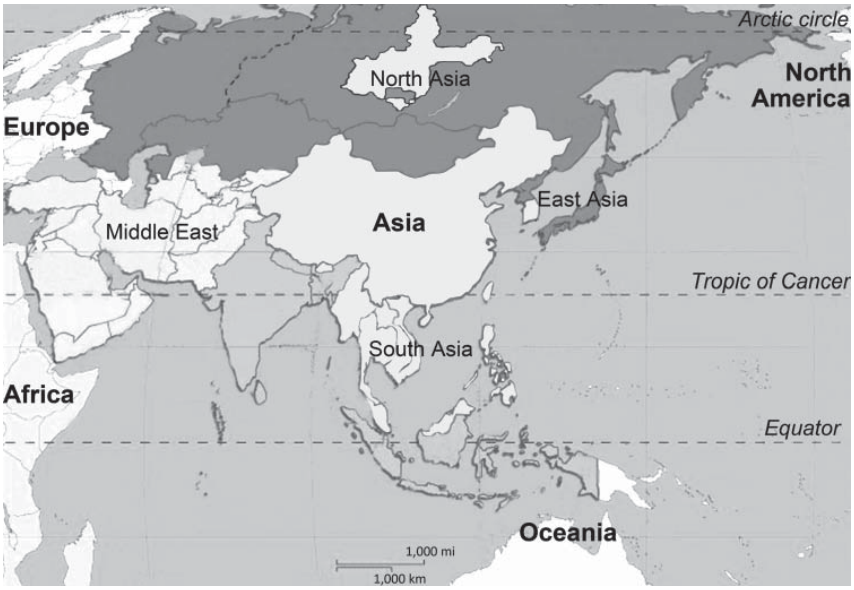

Figure. Areas in Asia where outbreaks of highly pathogenic porcine reproductive and respiratory virus syndrome occurred. The countries or regions affected (North Asia, East Asia, Asia, and South Asia) are indicated. A color version of this figure is available online (www.cdc.gov/EID/content/17/9/110411-F.htm).

includes severe acute respiratory syndrome coronavirus.

PRRSV is a single-stranded positive sense RNA virus that shows high rates of genetic diversity. In the genome of the novel highly pathogenic PRRSV mutant, 4 deletions (2 deletions in nonstructural protein 2, one deletion in the 5′ untranslated region, and one deletion in the 3′ untranslated region), and some other point mutations, have occurred, which were markedly different from those found in any other previous virus isolate.

After a surveillance study of the epidemic and an analysis of >300 novel highly pathogenic PRRSVs were conducted, the highly pathogenic PRRSV from China was considered to have gradually evolved from CH-1a, a local PRRSV isolate. The evolutionary path could be traced through intermediate PRRSV strains (*5*). Moreover, we found that highly pathogenic PRRSV has a further enlarged deletion in nonstructural protein 2.

Highly pathogenic PRRSV first emerged in China and Vietnam almost simultaneously in 2006, and the epidemic focus was in the area between southern China and northern Vietnam (*6*,*7*). Although no evidence has shown that the highly pathogenic PRRSV isolate from China or Vietnam has spread in other areas, highly pathogenic PRRS has spread throughout the Malaysian Peninsula to southern Russia.

In addition, all highly pathogenic PRRSV isolates share high sequence identity and have the same deletions as the highly pathogenic PRRSV isolated from China or Vietnam. PRRSV can spread through a variety of routes, including direct contact between pigs, droplet contact through nasal secretions, direct contact with saliva and feces, and indirect contact.

PRRS has spread rapidly around the world through pig sales, semen, and airborne transmission, including from airline passengers who carry the virus on their clothing, shoes, or equipment while traveling (*8*). In the global market, any virus emerging in the highly pathogenic form is a threat. The risk of highly pathogenic PRRS spreading to other countries is increasing.

This study was supported by grants from the National Natural Science Foundation of China (nos. 30901080, 31001050) and National Postdoctoral Science Foundation (nos. 20090451026, 201003467).

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DOI: http://dx.doi.org/10.3201/eid1709.110411

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Etymologia: *Pseudoterranova azarasi*

To the Editor: Regarding the March 2011 Etymologia on *Pseudoterranova azarasi* (*1*), we think that someone literally missed the boat on the derivation of *Pseudoterranova*. Although the Greco-Latin amalgam, *Pseudoterranova*, translates to "false new earth," the generic name of the organism refers to the ship, the *Terra Nova*, which Robert Falcon Scott captained en route to Antarctica exactly 100 years ago in his ill-fated attempt to be the first person to reach the South Pole.

During the Antarctic summer of 1911–12, while Scott and 4 companions trudged toward the South Pole, the ship's surgeon, Edward Leicester Atkinson, who remained with the *Terra Nova*, dissected polar fish, birds, and sea mammals, looking for parasites. Atkinson found an unusual nematode in a shark, and in 1914, he, along with parasitologist Robert Thomson Leiper of the London School of Tropical Medicine, commemorated the ship by conferring the name *Terranova antarctica* upon this newly discovered creature (*2*).

The genus *Pseudoterranova* was established by Aleksei Mozgovoi in 1951 for a somewhat similar nematode obtained from a pygmy sperm whale. *Pseudoterranova azarasi*, the subject of the Etymologia, was originally described in 1942 as *Porrocecum azarasi*, but recent molecular work, as described by Arizono et al. (*3*) and Mattiucci and Nascetti (*4*), showed that this nematode is part of a large species complex within *Pseudoterranova*. Thus, it has been transferred to this genus as part of the *P. decipiens* species complex.

The nomenclatural specifics are complex and arcane. However, in this centennial year of the *Terra Nova* expedition, we think it is worthwhile to remember the historic origins of these names.

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DOI: http://dx.doi.org/10.3201/eid1709.110541

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Corrections

Vol. 16, No. 2

Some data were listed incorrectly in Table 1 and the text in the article Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999–2007 (E. Galanis et al.). The article has been corrected online (www.cdc.gov/eid/content/16/2/251.htm).

DOI: http://dx.doi.org/10.3201/eid1709.C11709

Vol. 17, No. 3

References were misnumbered in the Appendix Table of the article *Mycobacterium lentiflavum* in Drinking Water Supplies, Australia (H.M. Marshall et al.). The article has been corrected online (www.cdc.gov/eid/content/17/3/395.htm).

DOI: http://dx.doi.org/10.3201/eid1709.C21709

Vol. 17, No. 6

The abstract of the article Wild Birds and Increased Transmission of Highly Pathogenic Avian Influenza (H5N1) among Poultry, Thailand (Juthatip Keawcharoen et al.) incorrectly referred to the swab samples collected. The article has been corrected online (www.cdc.gov/eid/content/17/6/1016.htm).

DOI: http://dx.doi.org/10.3201/eid1709.C31709

Vol. 17, No. 7

In the article Hansen Disease among Micronesian and Marshallese Persons Living in the United States (P. Woodall et al.), the first paragraph of the Results section should reference 686 total cases of Hansen disease in the United States. The article has been corrected online (www.cdc.gov/eid/content/17/7/1202.htm).

DOI: http://dx.doi.org/10.3201/eid1709.C41709

Frans Snyders (1579–1657). *Still Life with Fighting Monkeys* (1635) Oil on canvas (74.9 cm × 108 cm). Bequest of John Ringling, 1936, The John and Mable Ringling Museum of Art, the State Art Museum of Florida, a division of Florida State University

The Monkey's Paw[1]

Polyxeni Potter

Unexpected consequences fuel the creative mind. The stuff of adventure in literature, their twists and turns wreak havoc in the science laboratory and the art studio alike. Frans Snyders, old master and founder of Baroque still life with animals, understood the unexpected and its power to surprise and used it to animate his work. A thriving arts market in 17th-century Flanders, supported by growing prosperity and curiosity about the natural world, provided a rich environment for still life painting, known since antiquity but always relegated low down the ladder of genres.

As he expressed the exuberance of his age in lifeless objects made dynamic and relevant, Snyders elevated the genre. His compositions often contained live animals as well as carcasses at the butcher shop or game fresh from the hunt. He so loved this part of the work that he abandoned still lifes altogether to become one of the first animaliers. He collected specimens of local and exotic animals to observe their behavior and physical characteristics and improve his specialized portrayal of them.

Snyders was born in Antwerp, center of the arts during the Counter Reformation and playground of such luminaries as the Brueghel family of painters, Peter Paul Rubens, and Anthony van Dyck. A student of Pieter Brueghel the Younger, Snyders managed to convey the local culture not just with accuracy but with humor and commentary in compositions later considered best of the genre. He joined the Antwerp painters Guild of St. Luke and, like all serious artists of his time, visited Italy to study the masters. He was competent and prolific and attracted royal patronage and great popularity at home and abroad.

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DOI: http://dx.doi.org/10.3201/eid1709.AC1709

[1]Short story by W.W. Jacobs.

Snyders enjoyed the friendship of the best artists of his day rather than being overshadowed by them. Rubens, whose star eclipsed all those around him, admired his skills and commissioned him to paint animals and still life elements in many of his works. Once, when a patron could not tell their work apart, Rubens consented that no one could depict dead animals better than Snyders, though when it came to live ones, he, Rubens, was the best. Van Dyck painted several portraits of Snyders, who was related to the Connelis de Vos family of painters by marriage and counted among his students and associates Jan Fyt, a still life master in his own right.

Animal still life—hunting or market scenes, butcher stalls, kitchen pantries—were transformed from static displays to vibrant collections of specimens shown to best advantage, colored with symbolism, and injected with humanity and interest. In his more than 50-year career, Snyders developed and refined his skills, leaving behind many paintings and drawings, hundreds of which survive. *Still Life with Fighting Monkeys*, on this month's cover, contains many of the artist's finest features.

Middle-class folks were not allowed to hunt in Snyders' Flanders, only the nobility. And though this painting does not show large trophy game, the row of colorful finches secured on a willow branch and small birds lying pathetically on the edge of the table hint at the status of this household—also home of such exotic pets as the mischievous monkeys in the center of the action. In the artist's style, what might have been a sedate tabletop scene is enlivened by altercating primates, themselves joined by hostile feline intruders.

Having toppled the basket, upsetting the fruit and scattering the arrangement, the monkeys tugged nervously on the branch of rainier cherries already manhandled and jutting off to nowhere. The finch display collapsed in a heap, china overturned and worse, and two angry cats ready to pounce from opposite ends complete the picture. Snyders' skills shine in the fur of the live animals and the texture of the game birds, which far from rigid or damaged by the hunt are soft and languid as they rest on their backs human-like. The fruit is plump and enticing, even rolled to the edge of the cloth. Small branches with crinkly leaves add to the natural feel of the original arrangement.

Monkeys were frequent visitors in Flemish paintings of this era, often linked to excess and greed, their troublesome anthropomorphic features mimicking the foolish aspects of human behavior. Shameless and unruly, they invite symbolism in this scene: the best choreographed arrangements could be instantly ruined by the slightest intrusion. This not only in still life painting but anywhere the law of unintended consequences applies, and no less in public health, where each day nature's basket is toppled by unexpected ecologic, social, and biologic paws.

In this issue of Emerging Infectious Diseases alone, diverse offerings from around the world attest to the immense influence of the monkey's paw, particularly when another creature inflames an already dangerous situation. Such is the case with influenza. In the past century, three pandemics swept the globe in which viruses from birds likely played a role. A new strain, influenza A virus (H5N1), spread through bird populations across Asia, Africa, and Europe, infecting domesticated birds, including ducks and chickens, and long-range migratory birds. Its first recorded appearance in humans was in Hong Kong in 1997.

Each time a new element of uncertainty is thrown into the mix, what will come out and how it will behave become more difficult to predict. Snyders knew this when he painted the fighting monkeys and the cats in his still life in Flanders. But the monkey's paw in pandemic influenza remains to be seen.

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EMERGING INFECTIOUS DISEASES

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Humans Infected with *Borrelia miyamotoi*

Azole Resistance in *Aspergillus fumigatus*, the Netherlands

Plasmodium knowlesi Malaria in Humans and Macaques, Thailand

Outpatient *Clostridium difficile* Infection, Baltimore, Maryland, and New Haven, Connecticut

Unexpected Rift Valley Fever Outbreak, Northern Mauritania

Seroconversion to Pandemic (H1N1) 2009 Virus

Pandemic (H1N1) 2009 and Encephalitis in Adults

Timeliness of Surveillance for *Escherichia coli* O104:H4 During Outbreak in Germany, 2011

Novel Arenavirus, Zambia

Household Transmission of Pandemic (H1N1) 2009 Virus, Taiwan

Group B Streptococcus and HIV Infection in Pregnant Women, Malawi

Isolation and Phylogenetic Grouping of Equine Encephalosis Virus, Israel

Complete list of articles in the October issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

September 17–20, 2011
51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)
McCormick Place Chicago
Chicago, IL, USA
http://www.icaac.org

September 19–22, 2011
VIIth Meeting of the International Leptospirosis Society
Universidad Autonoma de Yucatan Auditorium
Merida, Yucatan, Mexico
http://www.fmvz.unam.mx/leptospirosis.html

October 3–5, 2011
1st Global Forum on Bacterial Infections: Balancing Treatment Access and Antibiotic Resistance
India Habitat Centre
New Delhi, India
http://www.globalbacteria.org/home

October 12–15, 2011
The Denver TB Course
Denver, CO, USA
http://www.njhealth.org/TBCourse

October 20–23, 2011
49th Annual Meeting of the Infectious Diseases Society of America
Boston, MA, USA
http://www.idsociety.org/idsa2011.htm

November 6–8, 2011
2011 European Scientific Conference on Applied infectious Diseases Epidemiology (ESCAIDE)
Stockholm, Sweden
http://www.escaide.eu, or email escaide.conference@ecdc.europa.eu

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to **www.medscape.org/journal/eid**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Inpatient Capacity at Children's Hospitals during Pandemic (H1N1) 2009 Outbreak, United States

CME Questions

1. You sit on a planning commission for children's healthcare in your region, and the commission is reviewing health system performance during the 2009 H1N1 influenza pandemic. Overall, how did this pandemic compare with prior influenza pandemics among children in the United States?

A. H1N1 had a lower attack rate and a lower case-hospitalization rate
B. H1N1 had a lower attack rate but a higher case-hospitalization rate
C. H1N1 had a higher attack rate but a lower case-hospitalization rate
D. H1N1 had a higher attack rate and a higher case-hospitalization rate

2. On the basis of the current study, what can you tell the commission in regard to the inpatient occupancy rate among children's hospitals during the 2009 H1N1 influenza pandemic?

A. It never exceeded 85%
B. It was lower than that of the 2008-2009 influenza season
C. It surged higher compared with occupancy rates immediately before and after the pandemic
D. It could have accommodated 50% more admissions before going over 100% of capacity

3. What should your commission consider in regard to the virulence of influenza and hospital occupancy?

A. The 2009 H1N1 influenza pandemic affected inpatient occupancy more than emergency department capacity
B. The 2009 H1N1 influenza pandemic affected inpatient occupancy and emergency department capacity equally
C. The emergency department-to-hospital admission rate for influenza-related illness patients was slightly more than 5% in 2009
D. Higher acuity of influenza cases will probably have little effect on hospital occupancy rates

4. Approximately how many additional admissions per 10 hospital beds would have raised the overall hospital occupancy to 100% during the 2009 H1N1 influenza pandemic?

A. 1
B. 4
C. 7
D. 9

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

Earning CME Credit

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Article Title

Mycobacterium chelonae-abscessus Complex Associated with Sinopulmonary Disease, Northeastern USA

CME Questions

1. Which of the following statements regarding the members of the *Mycobacterium chelonae-abscessus* complex is most accurate?

A. There are 22 known members of the *M. chelonae-abscessus* complex
B. These mycobacteria can promote skin infections, pneumonia, and abscesses
C. Infections occur only among immunocompromised hosts
D. There are clear taxonomical relationships between members of the *M. chelonae-abscessus* complex

2. "*Mycobacterium franklinii*" shared which of the following gene sequences with *M. chelonae* in the current study?

A. *rpoB*
B. *sodA*
C. *hsp65*
D. 16S rRNA

3. What was the most common source of "*M. franklinii*" in the current study?

A. Skin
B. Liver lesion
C. Central line
D. Respiratory

4. Which of the following statements regarding the antimicrobial susceptibility of "*M. franklinii*" is most accurate?

A. "*M. franklinii*" has the same susceptibility profile as other members of the *M. chelonae-abscessus* complex
B. "*M. franklinii* " is more likely to be susceptible to minocycline compared with other members of the *M. chelonae-abscessus* complex
C. "*M. franklinii* " is more likely to be susceptible to cefoxitin compared with other members of the *M. chelonae-abscessus* complex
D. "*M. franklinii* " is more likely to be resistant to cefoxitin compared with other members of the *M. chelonae-abscessus* complex

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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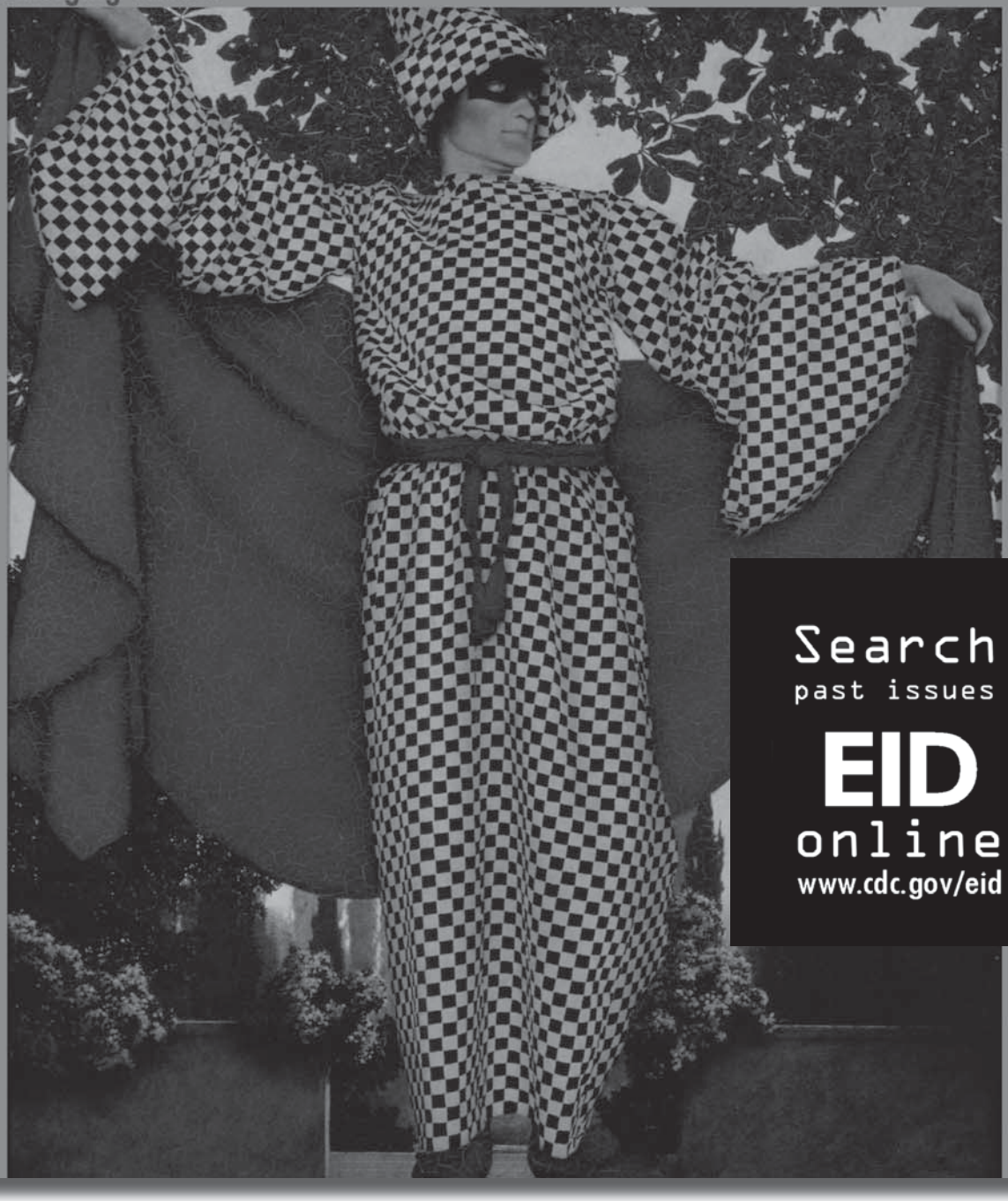
August 2011

Emerging Viruses



High Museum of Art, Atlanta, Georgia, USA. Gift of Ruth Jarmgon McGinty

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The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles, see below and visit www.cdc.gov/eid/ncidod/ EID/instruct.htm.

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Instructions to Authors

Manuscript Submission. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

Manuscript Preparation. For word processing, use MS Word. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, and figure legends. Appendix materials and figures should be in separate files.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

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

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit figures as separate files, in the native format when possible (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpg or .tif files. Other file formats may be acceptable; contact fue7@cdc.gov for guidance. Figures should not be embedded in the manuscript file. Use color only as needed. Use Arial font for figure lettering. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced to print size. Large figures may be made available online only. Place figure keys within the figure; figure legends should be provided at the end of the manuscript file.

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Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may 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.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. 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.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that 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.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

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. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only and should contain 500–1,000 words. They should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.