EMERGING INFECTIOUS DISEASES®

July 2011

Parasitic and Tropical Infections

EMERGING INFECTIOUS DISEASES®

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

Salum Kambi (b. 1970)
The Village Hut (2008)
Acrylic on canvas (60.32 cm × 60.32 cm)
Courtesy of U*Space Gallery (www.uspacegallery.com), Atlanta, Georgia, USA

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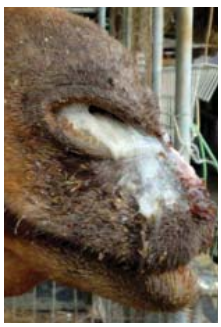

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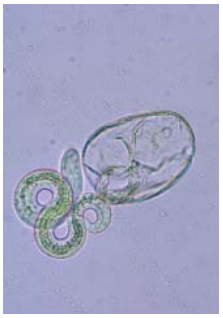

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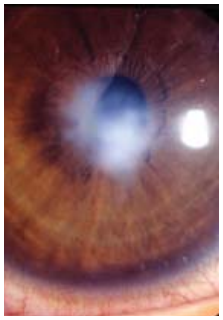

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Understanding the Cholera Epidemic, Haiti

Renaud Piarroux, Robert Barrais, Benoît Faucher, Rachel Haus, Martine Piarroux, Jean Gaudart, Roc Magloire, and Didier Raoult

After onset of a cholera epidemic in Haiti in mid-October 2010, a team of researchers from France and Haiti implemented field investigations and built a database of daily cases to facilitate identification of communes most affected. Several models were used to identify spatiotemporal clusters, assess relative risk associated with the epidemic's spread, and investigate causes of its rapid expansion in Artibonite Department. Spatiotemporal analyses highlighted 5 significant clusters ($p<0.001$): 1 near Mirebalais (October 16–19) next to a United Nations camp with deficient sanitation, 1 along the Artibonite River (October 20–28), and 3 caused by the centrifugal epidemic spread during November. The regression model indicated that cholera more severely affected communes in the coastal plain (risk ratio 4.91) along the Artibonite River downstream of Mirebalais (risk ratio 4.60). Our findings strongly suggest that contamination of the Artibonite and 1 of its tributaries downstream from a military camp triggered the epidemic.

On October 21, 2010, the Haitian Ministry of Public Health and Population (MSPP) reported a cholera epidemic caused by *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor (*1*). This epidemic was surprising as no cholera outbreak had been reported in Haiti for more than a century (*1*,*2*). Numerous media rapidly related the epidemic to the deadly earthquake that Haiti had experienced 9 months earlier. However, simultaneously, a rumor held recently incoming Nepalese soldiers responsible for importing cholera, along with accusations of illegal dumping of waste tank contents (*3*). A cholera outbreak was indeed reported in Nepal's capital city of Kathmandu on September 23, 2010, shortly before troops left for Haiti (*4*,*5*). Two hypotheses then emerged to explain cholera in Haiti.

Some researchers posited the transmission of an environmental strain to humans (*6*). Reasoning by analogy with cholera epidemiology in South Asia, they hypothesized that weather conditions, i.e., the La Niña phenomenon, might have promoted the growth of *V. cholerae* in its environmental reservoir (*6*). The second hypothesis suggested importation of the disease from a cholera-endemic country. The sequencing of 2 isolates of *V. cholerae* supported this second hypothesis by establishing an exogenous origin, probably from southern Asia or eastern Africa (*7*). Responding to a request from Haitian authorities to the French Embassy for the support of epidemiologists, we conducted a joint French–Haitian investigation during November 7–November 27, 2010, to clarify the source of the epidemic and its unusual dynamic.

Morbidity and Mortality Survey

As soon as the epidemic was recognized, a nationwide monitoring program was implemented to register all ambulatory patients, hospital admissions, and deaths (*1*). Each day, all government and nongovernmental health facilities in Haiti reported cases to the Direction of Health in each department, which colligated data before sending them to MSPP. For this study, the departments were asked to provide more precise data corresponding to the 140 Haitian communes from October 16 through November 30. Probable cholera cases were defined as profuse, acute watery diarrhea in persons. In each department, bacteriologic confirmation was obtained only for the first

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DOI: 10.3201/eid1707.110059

cases. Children <5 years of age were included because age was not always reported. Community deaths were additionally reported by local authorities. Comparison with epidemiologic surveys performed by other actors (Doctors without Borders, medical brigades from Cuba) enabled confirmation of the consistency of the database. Cholera incidence was calculated by using population numbers from Haitian authorities and mapped together with environmental settings by using ArcGIS (ESRI, Redlands, CA, USA). Maps of gridded population density (*8*), communes, rivers, roads, altitude, internally displaced persons (IDP) camps, and health facilities were obtained from Haitian authorities and the United Nations Stabilization Mission in Haiti (MINUSTAH) website (http://minustah.org).

Field Surveys

The first team of epidemiologists from Haiti went to Mirebalais during October 19–24. Then, from November 7–27, epidemiology teams from France and Haiti visited the most affected areas, namely Mirebalais, St-Marc, Gonaïves, Cap Haïtien, St-Michel-de-l'Attalaye, Petite-Rivière-de-l'Artibonite, Ennery, Plaisance, and Port-au-Prince. These visits included interviews with health actors and civilian authorities and investigation of environmental risks among inhabitants and patients from cholera treatment centers.

Statistics

To investigate for space–time case clustering, we analyzed the daily case numbers in each Haitian commune from October 16 through November 30 using SaTScan software (Kulldorf, Cambridge, UK). To detect clusters, this software systematically moves a circular scanning window of increasing diameter over the studied region and compares observed case numbers inside the window to the numbers that would be expected under the null hypothesis (random distribution of cases). The maximum allowed cluster size corresponded to 50% of the Haitian population. The statistical significance for each cluster was obtained through Monte Carlo hypothesis testing, i.e., results of the likelihood function were compared with 999 random replications of the dataset generated under the null hypothesis (*9*,*10*).

On the basis of these results, we further analyzed risk factors for spread in Ouest, Centre, and Artibonite Departments during October 20–28 using a regression model with adjustment on spatial variability. The initial focus, Mirebalais, was precluded to better estimate the relationship between the epidemic spread and the distance to the epidemic source. Because data on cholera cases were non-normally distributed and thus violated basic assumptions for linear regression, we used a generalized additive model (GAM) (*11*–*13*). Furthermore, because of the over-dispersion of the data (variance was greater than mean), we used a quasi-Poisson model (variance = $c \times$ mean, where c is an estimated constant) (*14*). The use of a Poisson model would not have been relevant because the main assumption for Poisson models is that variance equals mean. The GAM was allowed to model the count of cases in each commune, analyzing 1 continuous variable (distance to Mirebalais) and 3 binary variables (location downstream of Meille River, presence of camps of IDP, and commune partially or totally located in coastal plain). The models were adjusted on the population and the spatial distribution of communes. Conditions of use were checked by using classical graphic means. The goodness-of-fit was also assessed by the percentage of explained deviance.

In the communes bordering the Artibonite River, namely Mirebalais, St-Marc, Dessalines, Petite-Rivière-de-l'Artibonite, Grande Saline, Verrettes, Desdunes, and L'Estère, during October 16–31, we searched for synchronizations between communal epidemiologic curves by calculating and testing Spearman correlation coefficients. Statistical analyses were performed by using R version 2.10.1 software (www.r-project.org/foundation), particularly with the *mgcv* package (GAM modeling) (*11*). We compared p values to the probability threshold $\alpha = 0.05$.

Initiation

On October 18, the Cuban medical brigades reported an increase of acute watery diarrhea (61 cases treated in Mirebalais during the preceding week) to MSPP. On October 18, the situation worsened, with 28 new admissions and 2 deaths. MSPP immediately sent a Haitian investigation team, which found that the epidemic began October 14. The first hospitalized patients were members of a family living in Meille (also spelled Méyè), a small village 2 km south of Mirebalais (Figure 1). On October 19, the investigators identified 10 other cases in the 16 houses near the index family's house. Five of the 6 samples collected in Meille from these outpatients, who became sick during October 14–19, yielded *V. cholerae* O1, serotype Ogawa, biotype El Tor. Environmental and water source samples proved negative.

Meille village hosted a MINUSTAH camp, which was set up just above a stream flowing into the Artibonite River. Newly incoming Nepalese soldiers arrived there on October 9, 12, and 16. The Haitian epidemiologists observed sanitary deficiencies, including a pipe discharging sewage from the camp into the river. Villagers used water from this stream for cooking and drinking.

On October 21, the epidemic was also investigated in several wards of Mirebalais. Inhabitants of Mirebalais drew water from the rivers because the water supply network was being repaired. Notably, prisoners drank water from the

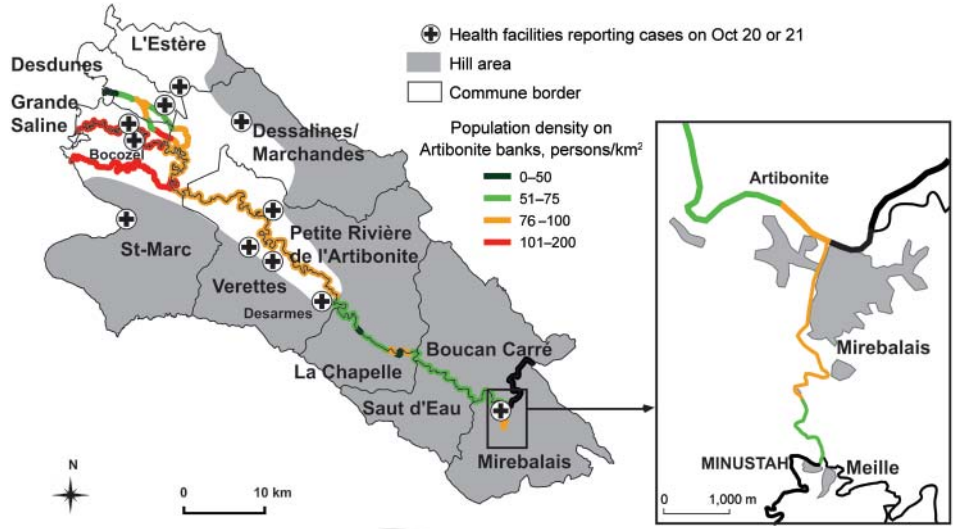

Figure 1. Location of health centers reporting cholera cases in communes along the Artibonite River on October 20, 2010, Haiti. MINUSTAH, United Nations Stabilization Mission in Haiti.

same river, downstream from Meille. No other cause was found for the 34 cases and 4 deaths reported in the prison.

On October 31, it was observed that sanitary deficiencies in the camp had been corrected. At the same time, daily incidence of cholera tended to decrease. Afterwards, incidence rose again to reach a second peak on November 10 (Figure 2).

Spatiotemporal Modeling

By using SaTScan (Kulldorf), several spatiotemporal clusters were identified (Figure 3): Mirebalais, October 16–19 ($p<0.001$), and in the Artibonite delta, October 20–28 ($p<0.001$). Overlapping staggered clusters occurred in the North-West (November 11–29; $p<0.001$); Port-au-Prince area (November 14–30; $p<0.001$); and North (November 21–30; $p<0.001$).

Epidemic in Lower Artibonite

The start of the cholera epidemic was explosive in Lower Artibonite (communes of Grande Saline, St-Marc, Desdunes, Petite-Rivière-de-l'Artibonite, Dessalines, and Verrettes). It peaked within 2 days and then decreased drastically until October 31 (Figure 2). On October 19, the departmental Direction of Health received a first alert from Bocozel (commune of St-Marc) where 3 children had died from acute watery diarrhea at school. The same day, clusters of patients with severe acute diarrhea and vomiting were admitted to a hospital in Dessalines, and deaths caused by severe diarrhea and vomiting were concomitantly reported in the community. During the next 24 hours, new alerts were registered from ≥10 health centers and hospitals located in each commune covering the lower course of the Artibonite River, from Desarmes (a locality 30 km from the sea) to the seashore (Figure 1). On October 21 at noon, <48 hours after the first alert, 3,020 cholera cases (including 1,766 hospitalizations) and 129 deaths were reported. No cholera cases had been reported in the Lower Artibonite area before October 19. In contrast, almost no cholera cases were recorded in the communes of Saut d'Eau (no case), Boucan Carre (no case), and La Chapelle (2 cases) on October 20 and 21. Only a few hamlets of these 3 communes located between Mirebalais and the Artibonite delta are crossed by the Artibonite River, so population density on its banks is low (Figure 1). Similarly, only 1 case, imported from Lower Artibonite, was reported in Gonaïve on October 20. Gonaïve is built in a floodplain adjacent to the Artibonite delta but watered by a different river running from the north.

The quasi-Poisson GAM model provided a fair goodness-of-fit with deviance explained of 89.4%. Adjusted for population and spatial location, location downstream of the Meille River and commune location in coastal plain were significant risk factors (risk ratios [RRs] 4.91 and 4.60, respectively) but the closeness to Mirebalais was not (Table 1).

A strong correlation was found between the epidemic curves of the communes of the delta but not with that of Mirebalais (Table 2). The correlation was maximum (0.934) between St-Marc and Grande Saline, the 2 seashore communes bordering the main branch of the Artibonite River.

Spread Out of Artibonite Basin

On October 22, cholera cases were notified in 14 additional communes, most of them in the mountainous regions bordering the Artibonite plain and in Port-au-Prince. We visited several of these communes (Gonaïve, Ennery, Plaisance, Saint-Michel-de-l'Attalaye, and Port-au-Prince) and investigated the circumstances of the onset of cholera outbreaks. In each case, cholera started after the

arrival of patients who fled from the ravaging epidemic in the Artibonite delta. There, numerous persons from bordering communes worked in rice fields, salt marshes, or road construction. The deadly epidemic provoked a panic that made them flee back home. Soon after, their communes of origin were experiencing outbreaks. In contrast, the southern half of Haiti remained relatively free of cholera after 6 weeks of epidemics (Figure 3). Spatiotemporal analysis identified slightly staggered clusters occurring from November 11, in North-West, Port-au-Prince, and North Departments, which are roughly equidistant from Artibonite delta. In the North, the largest epidemics occurred in the main cities located in floodplains, especially Cap Haitien and Gonaïve, but numerous deaths were recorded in the mountainous areas between Artibonite plain and northern coast. On November 20, almost 1 month after the first cases had been notified in Saint-Michel-de-l'Attalaye (139,000 inhabitants), we observed several small ongoing cholera outbreaks, striking 1 hamlet after another, leading to 941 cases (including 366 hospitalizations). Forty-one patients died in the hospital, and 110 died in the community. After 1 month, the death rate reached 1.08% in Saint-Michel-de-l'Attalaye.

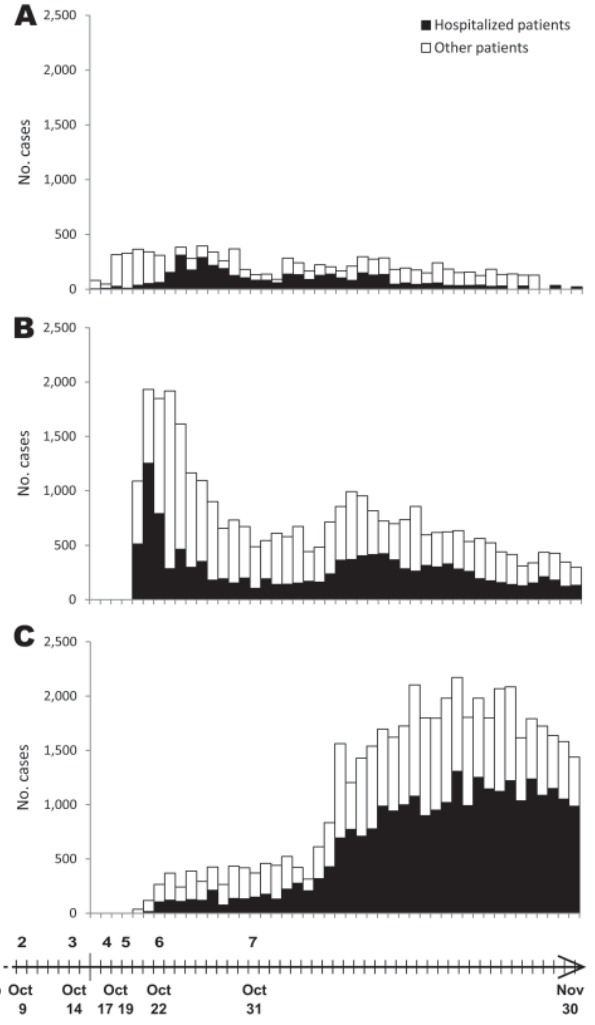

Figure 2. Cholera cases by date of onset of the epidemics and major related events, Haiti. A) Cases in Mirebalais, commune hosting the first cases of cholera; B) cases in seven communes simultaneously struck on October 20 (St-Marc, Dessalines, Desdunes, Grande Saline, Lestere, Petite-Rivière-de-l'Artibonite, Verrettes); C) cases in other communes. Timeline at bottom indicates 1) cholera outbreak in Kathmandu, Nepal; 2) first arrival of newly incoming Nepalese soldiers in Meille; 3) first cases in Meille; 4) first death registered in Mirebalais hospital (patient from Meille); 5) initiation of epidemic investigations and spread into the Artibonite delta; 6) epidemiologic confirmation of cholera cases in Meille; 7) United Nations camp sanitary dysfunction no longer observed.

In Port-au-Prince, the epidemic had 2 phases. For 15 days after the first patients arrived from Artibonite, the epidemic remained moderate with 76 daily cases on average from October 22 through November 5, causing only 77 hospitalizations. Then, the epidemic exploded in Cite-Soleil, a slum located in a floodplain close to the sea. However, after 6 weeks of epidemic, IDP camps were still relatively free of cholera. Despite the earthquake-related damages and the presence of many IDP camps, cholera struck less severely in Port-au-Prince, as demonstrated by incidence rate (0.51% until November 30, compared with 2.67% in Artibonite, 1.86% in Centre, 1.4% in North-West, and 0.89% in North) and cholera-related mortality rate (0.8 deaths/10,000 persons in Port-au-Prince, compared with 5.6/10,000 in Artibonite, 2/10,000 in Centre, 3.2/10,000 in North, and 2.8/10,000 in North-West). Living in the Port-au-Prince metropolitan area was associated with lower incidence (RR 0.51, 95% confidence interval 0.50–0.52; $p<10^{-7}$) and lower mortality rates (RR 0.32, 95% confidence interval 0.28–0.37; $p<10^{-7}$) than overall Haiti, even when considering unaffected departments.

Discussion

Determining the origin and the means of spread of the cholera epidemic in Haiti was necessary to direct the cholera response, including lasting control of an indigenous bacterium and the fight for elimination of an accidentally imported disease, even if we acknowledge that the latter might secondarily become endemic. Putting an end to the controversy over the cholera origin could ease prevention and treatment by decreasing the distrust associated with the widespread suspicions of a cover-up of a deliberate importation of cholera (*15*,*16*). Demonstrating an imported origin would additionally compel international organizations to reappraise their procedures. Furthermore, it could help to contain disproportionate fear toward rice culture in the future, a phenomenon responsible for important crop losses this year (*17*). Notably, recent publications supporting an imported origin (*7*) did not worsen social unrest, contrary to what some dreaded (*18*–*20*).

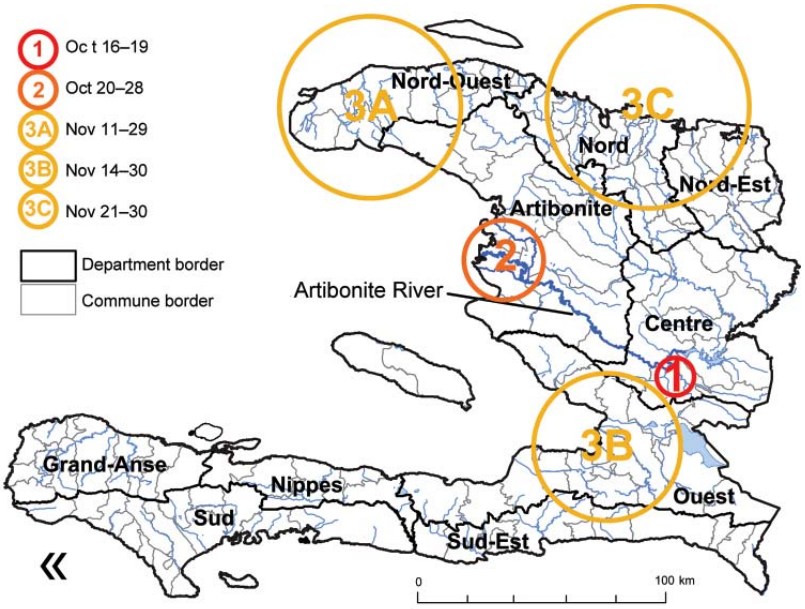

Figure 3. Spatiotemporal clusters of cholera cases, Haiti (results of SaTScan [Kulldorf, Cambridge, UK] analysis). The first cluster covered 1 commune, Mirebalais, October 16–19; the second cluster covered a few communes in or near the Artibonite delta during October 20–28; the next 3 clusters appeared in the North-West Department (A) during November 11–29, in the West Department (B) during November 14–30, and in the North and North-East Departments (C) during November 21–30. Other departments were affected later.

Our epidemiologic study provides several additional arguments confirming an importation of cholera in Haiti. There was an exact correlation in time and places between the arrival of a Nepalese battalion from an area experiencing a cholera outbreak and the appearance of the first cases in Meille a few days after. The remoteness of Meille in central Haiti and the absence of report of other incomers make it unlikely that a cholera strain might have been brought there another way. DNA fingerprinting of *V. cholerae* isolates in Haiti (*1*) and genotyping (*7*,*21*) corroborate our findings because the fingerprinting and genotyping suggest an introduction from a distant source in a single event (*22*).

At the beginning, importation of the strain might have involved asymptomatic carriage by departing soldiers whose stools were not tested for the presence of *V. cholerae*, as the Nepalese army's chief medical officer told the British Broadcasting Corporation (*23*). The risk for transmission associated with asymptomatic carriage has been known for decades (*24*), but asymptomatic patients typically shed bacteria in their stool at ≈10^3 *V. cholerae* bacteria per gram of stool (*25*) and, by definition, have no diarrhea. This small level of shedding would be unlikely to cause interhuman contamination of persons outside the military camp having few contacts, if any, with MINUSTAH peacekeepers. By contrast, considering the presence of pipes pouring sewage from the MINUSTAH camp to the stream, the rapid dissemination of the disease in Meille and downstream, and the probable contamination of prisoners by the stream water, we believe that Meille River acted as the vector of cholera during the first days of the epidemic by carrying sufficient concentrations of the bacterium to induce cholera in persons who drank it. To our knowledge, only infectious doses >10^4 bacteria were shown to produce mild patent infection in healthy volunteers, and higher doses are required to provoke severe infections (*26*,*27*). Reaching such doses in the Meille River is hardly compatible with the amount of bacteria excreted by asymptomatic carriers, whereas if 1 or several arriving soldiers were incubating the disease, they would have subsequently excreted diarrheal stools containing 10^{10}–10^{12} bacteria per liter (*25*). We therefore believe that symptomatic cases occurred inside the MINUSTAH camp. The negativity of the repeated water samples disfavors the hypothesis of an environmental growth of the bacterium in the Meille stream even if the lack of use of molecular approaches precludes detection of low-level bacterial contamination. Alternatively, a contamination related to sewage discharge could have resulted in transient presence of the bacterium in the water, which could be easily missed by punctual samplings.

Our field investigations, as well as statistical analyses, showed that the contamination occurred simultaneously in the 7 communes of the lower course of the Artibonite River, an area covering 1,500 km^2, >25 km from Meille. The abrupt upward epidemic curve in the communes bordering Artibonite dramatically contrasts with the progressive epidemic curve in the other communes of Haiti (Figure 2, panel B). In the latter, it took 19 days before the daily number of cases exceeded 1,000 (Figure 2, panel C). Suspected cholera was diagnosed in 7,232 patients during these 19 days. If the transmission in the communes bordering Artibonite had been similar to that of other communes, a comparable number of cases would have occurred in the days preceding the alert on October 20. So

Table 1. Adjusted risk ratio of cholera in each commune estimated by the generalized additive model, adjusted for population and spatial variability, Haiti, 2010*

Covariate	RR (95% CI)	p value
Location downstream of Meille River	4.91 (1.47–16.47)	0.012
Distance to Mirebalais, km	0.99 (0.94–1.04)	0.594
Presence of IDP camp	0.10 (0.01–1.12)	0.063
Commune located in coastal plain	4.60 (2.28–9.30)	0.0001

*RR, adjusted risk ratio; CI, confidence interval; IDP, internally displaced persons.

many cholera cases would not have remained unnoticed, all the more so as several health facilities of these communes were participating in the MSPP epidemiologic watch. The regression model indicates that the spread of cholera during the peak that occurred from October 20–28 was strongly linked to the Artibonite River and not to the proximity to Mirebalais, as would be expected for road-dependent propagation. This result, as well as the simultaneity of the outbreak onset in 7 communes of Lower Artibonite on October 19, is in accordance with contamination of the Artibonite River in a way that could infect thousands, and kill hundreds, of persons within a few days.

This hypothesis is also sustained by another early investigation during October 21–23 that showed that most affected persons worked or resided in rice fields alongside a stretch of the Artibonite River and that 67% drank untreated water from the river or canals (*1*). Cholera incubation varies from a few hours to 6 days (*26*), and the epidemic curve strongly suggests a rapid decrease of the contamination level in the river because the number of new cases and deaths dropped dramatically after only 2 days. A lasting phenomenon would have induced a continuing increase of incidence and a later peak. However, even for a few hours, contamination of a river such as the Artibonite requires a large amount of bacteria. For instance, to reach concentrations of 10^5 *V. cholerae* bacteria per liter during only 3 hours in the Artibonite River, which usually flows >100 m^3/s in October (*28*), $>10^{14}$ bacteria are required. This level corresponds to the amount of bacteria in 1 m^3 of rice-water stools harboring 10^{11} *V. cholerae* bacteria per liter. Notably, the fact that the peak in Mirebalais occurred later, on October 26, when daily incidence was dropping dramatically in Lower Artibonite also indicates that a specific mechanism was responsible for the onset of cholera in Lower Artibonite distinct from continuous spread from the primary focus.

Besides the particular circumstance that provoked the Artibonite's outbreak, other factors may have played a role in the severity of the epidemic in Haiti: the absence of immunity among the population, the higher infectivity of epidemic strains shed in human rice-water stools than of environmental strains, and the role of hypervirulent variant strains in provoking epidemics (*24*,*29*,*30*). The recent sequencing of isolates from Haiti exhibited several structural variations that are hallmarks of the particularly virulent variant strains that have emerged in southern Asia (*7*).

Whatever its cause, this violent outbreak in Lower Artibonite provoked the flight of persons and resulted in a wave of epidemics that spread centrifugally and overwhelmed the nascent sanitarian response. This wave explains the difference between the delayed and progressive starting of epidemics in the south and the immediate impact of cholera in the north. Furthermore, after 6 weeks of epidemics, the IDP camps were still relatively free of cholera. Because the January earthquake led to population displacement, formation of camps, and overcrowding, numerous field actors considered that it was a favorable circumstance for a cholera epidemic. However, in most IDP camps, access to food, safe water, and sanitation was better than in neighboring wards (*2*,*31*). This low risk for epidemics after geophysical disaster was already reported in a study summarizing the epidemiologic consequences of >600 disasters (*32*).

Overall, this report highlights the importance of an accurate field investigation, especially when an epidemic strikes a previously unscathed area or evolves with unusual speed, to ensure an adequate targeting of the response by providing a feedback to the main field actors. Obviously, we have to be cautious with the interpretation that could be made from our results. Although they are compatible with

Table 2. Spearman rank correlation between the number of cases in the 8 communes of the Artibonite delta and corresponding p values, Haiti, October 16–31, 2010

	Spearman correlation (p value)						
Commune	L'Estère	Des Dunes	Verrettes	Grande Saline	Petite Rivière de l'Artibonite	Des Salines	St-Marc
Mirebalais	0.231(0.389)	0.527 (0.036)	0.276 (0.301)	0.480 (0.0.059)	0.563 (0.023)	0.361 (0.169)	0.459 (0.074)
St Marc	0.678 (0.004)	0.782 (<0.001)	0.652 (0.006)	0.934 (<0.001)	0.704 (0.002)	0.887 (<0.001)	
Des Salines	0.872 (<0.001)	0.713 (0.002)	0.465 (0.069)	0.818(<0.0001)	0.606 (0.013)		
Petite Rivière de l'Artibonite	0.672 (0.004)	0.675 (0.004)	0.586 (0.017)	0.648 (0.007)			
Grande Saline	0.600 (0.014)	0.848 (<0.001)	0.783 (<0.001)				
Verrettes	0.380 (0.147)	0.600 (0.014)					
Des Dunes	0.537 (0.032)						

the reports of several journalists who linked the epidemic with the dumping of a septic tank (*3*), the exact event that provoked the massive contamination of Lower Artibonite cannot be definitively deduced from an epidemiologic study. Rather, identifying the source and the responsibilities falls within the scope and competence of legal authorities. Nonetheless, this epidemic reminds us how critical the management of water and sewage is to prevent cholera spread. To avoid actual contamination or suspicion happening again, it will be important to rigorously ensure that the sewage of military camps is handled properly. Above all else, aid organizations should indeed avoid adding epidemic risk factors to those already existing and respect the fundamental principle of all assistance, which is initially not to harm—*primum non nocere*.

Acknowledgments

We are grateful to the Haitian Ministry of Public Health and Population authorities, to the Haitian medical teams in each Haitian department, and to the Cuban medical teams in Haiti for collecting the data, and to the French Embassy in Port-au-Prince for supporting this study.

Dr Piarroux is a specialist in infectious diseases and parasitology at Université de la Méditerranée. He began studying cholera in various African countries in 1994, when he first experienced a cholera outbreak in a refugee camp in Goma, Democratic Republic of the Congo.

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Rickettsia parkeri Rickettsiosis, Argentina

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Rickettsia parkeri, a recently identified cause of spotted fever rickettsiosis in the United States, has been found in *Amblyomma triste* ticks in several countries of South America, including Argentina, where it is believed to cause disease in humans. We describe the clinical and epidemiologic characteristics of 2 patients in Argentina with confirmed *R. parkeri* infection and 7 additional patients with suspected *R. parkeri* rickettsiosis identified at 1 hospital during 2004–2009. The frequency and character of clinical signs and symptoms among these 9 patients closely resembled those described for patients in the United States (presence of an inoculation eschar, maculopapular rash often associated with pustules or vesicles, infrequent gastrointestinal manifestations, and relatively benign clinical course). Many *R. parkeri* infections in South America are likely to be misdiagnosed as other infectious diseases, including Rocky Mountain spotted fever, dengue, or leptospirosis.

Rickettsia parkeri, a tick-borne bacterium discovered in 1937, was considered nonpathogenic until 2004. Since 2004, >25 cases of *R. parkeri* rickettsiosis have been reported in persons living within the recognized range of the tick vector, *Amblyomma maculatum*, in the United States (*1–4*; Centers for Disease Control and Prevention, unpub. data). The clinical features of this newly recognized disease appear less severe than those produced by *R. rickettsii* bacteria, the agent of Rocky Mountain spotted fever (RMSF). For many years, investigators in several countries of South America, including Argentina, Brazil, and Uruguay, have recognized eschar-associated infections that clinically resemble *R. parkeri* rickettsiosis (*5–7*). These reports, and the discoveries of *R. parkeri* in *A. triste* ticks collected from these same countries, suggest that human infections with *R. parkeri* also occur in South America (*8–10*); to our knowledge, no confirmed cases of disease caused by this *Rickettsia* species have been reported from this continent.

The Paraná Delta, situated in the provinces of Buenos Aires and Entre Ríos in Argentina, represents the terminus of the Paraná River as it approaches and drains into the Uruguay River and subsequently into the Río de la Plata. This alluvial ecosystem, where braided river branches create a network of islands and wetlands, covers ≈14,000 km^2 (5,405 mi^2) and extends for ≈320 km (200 mi). This region also contains abundant populations of *A. triste* ticks (*10*). The Paraná Delta has always been a major agricultural and farming region. Recently, this area has become increasingly developed; roads have been built to allow greater access for tourism and recreational activities by many of the ≈14 million inhabitants of nearby Buenos Aires. In 2005, an eschar-associated febrile infection was diagnosed in a male beekeeper from the Paraná Delta; the infection was later confirmed as a spotted fever group (SFG) rickettsiosis by serology and immunohistochemistry (*5*). He had been bitten by a tick not far from several sites where *R. parkeri* was subsequently detected in *A. triste* ticks (*10*). We report confirmed cases of *R. parkeri* rickettsiosis in 2 patients in Argentina and describe additional suspected cases of this disease, or similar infections, in patients from the provinces of Buenos Aires, Chaco, and Entre Ríos.

Materials and Methods

Patients were identified after referral to the Zoonosis Service of Hospital F.J. Muñiz in Buenos Aires Province,

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DOI: 10.3201/eid1707.101857

Argentina. In each case, a rickettsial disease was considered from specific clinical signs and symptoms, including fever, rash, and an eschar, accompanying a history of recent tick bite. Serum and skin biopsy specimens were collected from these patients and evaluated by various assays to confirm infection with an SFG *Rickettsia* species. Serum samples were tested for immunoglobulin (Ig) G reactive to antigens of *R. parkeri* and *R. rickettsii* by using indirect immunofluorescence antibody assays, as described (*1*,*3*). Reciprocal antibody titers ≥64, or a 4-fold rise in titer, to either antigen were considered evidence of infection with an SFG *Rickettsia* species. When available, skin biopsy specimens were tested by using an immunoalkaline phosphatase technique to detect SFG rickettsiae in formalin-fixed, paraffin-embedded tissues, as described (*1*,*11*), or by use of PCR.

For molecular evaluations, DNA was extracted from eschar biopsy specimens by using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA). A segment of the rickettsial outer membrane protein B gene (*ompB*) was amplified by using primers 120–2,788 and 120–3,599 (*12*) in a 50-μL reaction mixture containing 5 μL of DNA template. A segment of the citrate synthase gene (*gltA*) was also amplified by using primers CS78 and CS323 (*13*) in a 40-μL reaction mixture containing 8 μL of DNA template. Amplified gene segments, excluding primers, were compared with sequences in the GenBank database by using the Basic Local Alignment Search Tool (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov). Gene sequences were aligned by using ClustalX software (*14*).

A suspected case was defined as a clinically and epidemiologically compatible illness, with ≥1 supportive serologic or immunohistochemical test results through use of group-specific assays for SFG *Rickettsia* spp. (*11*). A confirmed case of *R. parkeri* rickettsiosis was defined by PCR amplification of gene sequences specifically matching that of *R. parkeri*.

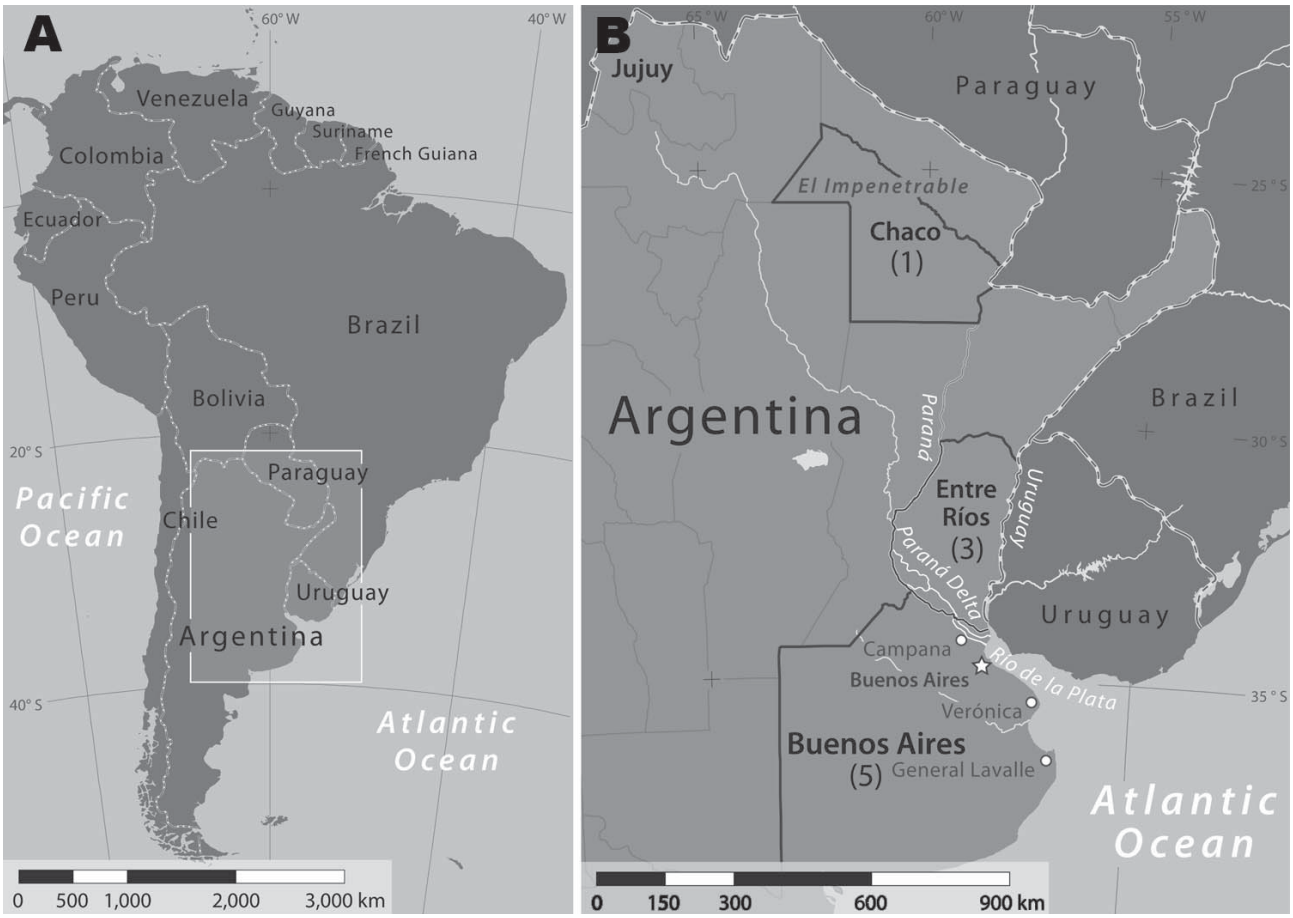

Figure 1. Confirmed and suspected cases of *Rickettsia parkeri* rickettsiosis, Argentina. The box (A) enlarged in panel (B) shows the extent of the area in which Argentinean provinces, representing patient exposure locations to ticks, are labeled and highlighted. A previous study (*10*) identified ticks collected from the Paraná Delta near the city of Campana. Numbers of suspected and confirmed cases of *R. parkeri* rickettsiosis, by province during 2004–2009, are shown in parentheses. The national capital city of Buenos Aires continues to experience rapid population growth into adjacent lands in and near the Paraná Delta. Rocky Mountain spotted fever, a more severe tick-borne rickettsiosis, has been described in the province of Jujuy in the northwestern corner of Argentina (*15*,*16*).

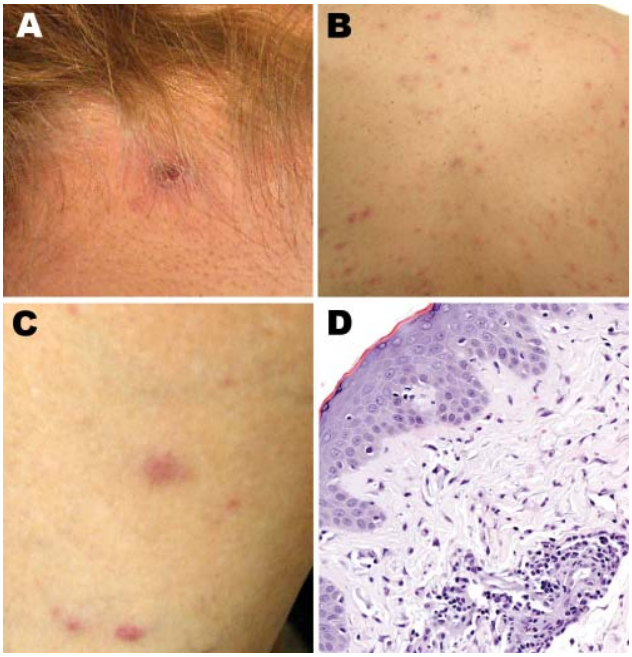

Figure 2. Cutaneous lesions of patients with suspected and confirmed *Rickettsia parkeri* rickettsiosis in Argentina. A) Eschar at the nape of the neck at the site of recent tick bite. B, C) Papulovesicular rash involving the back and lower extremities. D) Histopathologic appearance of a papule biopsy specimen, showing perivascular mononuclear inflammatory cell infiltrates and edema of the adjacent superficial dermis and an intact epidermis (hematoxylin and eosin stain; original magnification ×100).

Results

During 2004–2009, nine patients in Argentina with an SFG rickettsiosis were identified at Hospital F.J. Muñiz. Three were women. Median age of patients was 53 years (range 38–76 years). All patients reported tick bites that occurred during August–January and preceded fever onset by a median of 6 days (range 4–15 days). Exposures to ticks were associated with recreational activities for 7 patients and outdoor labor for 2. Six patients sustained tick bites in the Paraná Delta; 2 other patients were bitten in rural areas of the province of Buenos Aires near the towns of Verónica and General Lavalle; and 1 was bitten in a rural area of the province of Chaco, known as "El Impenetrable" (Figure 1, panel B).

A painless inoculation eschar, ranging from 1 cm to 1.5 cm, developed in 8 patients at the site of the tick bite (Table; Figure 2). These lesions were located in the following regions: head (3 patients); back (2); and leg, hand, and abdomen (1 each). Multiple eschars were not identified on any patient. Nonpruritic rashes developed in all patients and involved predominantly the trunk and extremities, represented by maculopapules on 8 patients, papulovesicles on 5 patients, and petechiae on 2 patients (Figure 2, panels B, C). Other commonly reported manifestations included headache and myalgias in 8 and 6 patients, respectively. Infrequently reported findings included arthralgias (3 patients); sore throat (2); and diarrhea, photophobia, and bilaterally injected conjunctivae (1 each). No patients required hospitalization, and all recovered rapidly after oral therapy with doxycycline.

Laboratory identification of cases included serology for 7 patients, immunohistochemistry for 1, and PCR for 2. Seroconversion, defined as a 4-fold rise in titer, was identified for 5 patients submitting paired serum samples, and 1 serum sample was positive in 2 patients; IgG titers to *R. parkeri* or *R. rickettsii* antigens ranged from 64 to 2,048. SFG *Rickettsia* spp. antigens were detected in formalin-fixed, paraffin-embedded sections of eschar and papule biopsy specimens that showed histopathologic features (Figure 2, panel D) compatible with those described for *R. parkeri* rickettsiosis (*3*,*11*). Infection with *R. parkeri* was confirmed specifically by molecular analyses of eschar biopsy specimens from 2 patients whose histories are provided below. Amplicons of the expected sizes were obtained from both specimens by using the primers for the rickettsial *ompB* and *gltA* genes. Sequence analyses of amplified *ompB* segments showed 100% identity with only the corresponding sequence of *R. parkeri* (GenBank accession no. AF123717). The next closest similarities were 711/714 (99%) with *Rickettsia* sp. BJ-90 (accession no. AY331393), 711/714 (99%) with *R. sibirica* (accession no. AF123722), and 710/714 with *R. africae* (accession no. CP001612). Sequencing of 332 bp of the *gltA* gene showed 100% identity with *R. parkeri* (accession no. U59732) and with *Rickettsia* sp. strain S (accession no. U59735), *R. sibirica* (accession no. U59734), and *Rickettsia* sp. BJ-90 (accession no. AF178035). Complete identity was also seen with a smaller (309-bp) overlapping segment of the *gltA* gene of *Rickettsia* sp. Atlantic rainforest (accession no. GQ855235), *Rickettsia* sp. NOD (accession no. EU567177), and *Rickettsia* sp. COOPERI (accession no. AY362704).

Case Histories

Patient 1, a 48-year-old man, was referred to F.J. Muñiz Hospital in November 2008 with recurring fever (temperatures to 40ºC) associated with headache, neck pain, and rash. Six days before onset of these symptoms, he removed a tick attached to his abdomen, ≈24 hours after a fishing expedition in the Paraná Delta in the province of Entre Ríos (Figure 1, panel B). A painless eschar developed at the bite site 6 days after the tick was removed. Physical examination indicated a febrile patient in otherwise good condition with a generalized, nonpruritic, maculopapular, and papulovesicular rash that involved predominantly his trunk and upper and lower limbs; his face, palms, or soles were not affected. His serum alanine aminotransferase level was 43 U/L (reference <31 U/L). All other hematologic and biochemistry measurements were within reference ranges.

A serum sample and eschar and papule biopsy specimens were obtained, and the patient was treated empirically with doxycycline 200 mg/day for 6 days.

Patient 2, a previously healthy 55-year-old-man, was referred to F.J. Muñiz Hospital in January 2009 with fever (temperature 38.5°C), rash, headache, photophobia, conjunctival injection, myalgias, and arthralgias. Seven days before onset of symptoms, he had removed a tick from his back while camping in the Paraná Delta in the province of Buenos Aires (Figure 1, panel B), and a painless black eschar appeared subsequently at the bite site. Three days later, he visited a community clinic and received treatment (cephalexin, 500 mg every 6 hours) for presumed cellulitis, without improvement. The patient did not appear severely ill on physical examination. He had a generalized, nonpruritic maculopapular rash that involved predominantly his trunk and extremities but not his face, palms, or soles. Hepatomegaly or splenomegaly, and nuchal rigidity were not found. Laboratory tests showed a serum aspartate aminotransferase level of 67 U/L (reference range <31 U/L), and alkaline phosphatase level of 270 U/L (reference range 40–129 U/L). All other hematologic and biochemical results were within reference ranges. A serum sample and an eschar biopsy specimen were obtained, and the patient was treated empirically with doxycycline (200 mg/d for 6 days). His fever disappeared within 48 hours after starting treatment, and complete remission of symptoms occurred within 6 days.

Discussion

We confirmed infection with *R. parkeri* in 2 patients bitten by ticks in the Paraná Delta region of Argentina and eschar-associated rickettsioses in 7 other patients from this region and other areas within Argentina (Figure 1, panel B). Because awareness of *R. parkeri* rickettsiosis by clinical practitioners in Argentina is low, and because it appears to be a self-limiting infection, it is likely that many, if not most, cases remain undiagnosed. All case-patients we studied had relatively mild illnesses similar to cases of *R. parkeri* rickettsiosis described in the United States. Indeed, the frequency and character of the clinical features of this disease among case-patients in Argentina closely resemble the signs and symptoms documented in patients in the United States (Table), including the occurrence of an eschar, a maculopapular rash often associated with pustules or vesicles, and the infrequency of gastrointestinal manifestations.

For several years, investigators suspected *R. parkeri* as a cause of at least some of the eschar-associated rickettsioses described in patients from several countries of South America (*5*,*7*–*10*). Our identification of *R. parkeri* DNA in cutaneous lesions of 2 persons in Argentina provides definitive evidence for this *Rickettsia* species as a cause of disease on this continent. During a previous investigation, *R. parkeri* was detected in ≈8% of questing adult *A. triste* ticks collected in the lower Paraná Delta, near the city of Campana (Figure 1, panel B) (*10*). No ticks were saved by patients described in the present report for species identification; however, most patients were bitten while at work or during leisure time in the Paraná Delta during August–November when adult *A. triste* ticks are relatively abundant and actively seeking hosts (*10*). Three cases originated far beyond the boundaries of the Paraná Delta, which suggests that infections with *R. parkeri* might occur in other regions of Argentina. It is also possible that other tick vectors may be involved in the transmission of *R. parkeri* or that other *Rickettsia* species are responsible for these distant cases. Tick- and flea-borne SFG *Rickettsia* spp. identified recently in patients in Argentina and Brazil include *R. felis*, *R. massiliae*, and a *Rickettsia* sp. related closely to, but distinct from, *R. parkeri* (*17*–*20*).

Table. Comparison of selected clinical characteristics reported for suspected and confirmed cases of *Rickettsia parkeri* rickettsiosis in patients from Argentina and the United States, 2004–2009*

Clinical characteristic	% Argentina patients, n = 10	% United States patients, n = 15
Fever	100	100
Inoculation eschar	90	93
Rash		
Any type	100	87
Macules or papules	90	87
Vesicles or pustules	50	40
Petechiae	20	13
On palms or soles	20	43
Headache	90	80
Myalgias	70	80
Sore throat	30	NR†
Injected conjunctivae	10	NR
Lymphadenopathy	10	27
Diarrhea	10	0
Nausea or vomiting	0	7
Hospitalization	0	33
Death	0	0

*See (*3*,*5*,*11*).
†NR, not reported.

Because the patients in this series typically had fever, rash, and myalgias, a diagnosis of leptospirosis or dengue was often considered during initial evaluation. These diseases are endemic across many provinces of northern Argentina, and some cases of *R. parkeri* rickettsiosis are likely to be misdiagnosed as dengue, leptospirosis, or other infectious diseases. Investigations in Mexico and Colombia have identified SFG rickettsioses as common causes of fever among patients believed initially to be infected with dengue virus (*15*,*16*). RMSF, endemic in the province of Jujuy (Figure 1, panel B) and possibly other areas of Argentina, shares several clinical features with *R. parkeri* rickettsiosis. However, it is characteristically a more severe

infection that may result in death. By comparison, 6 of 10 persons with confirmed or probable RMSF in Jujuy during 1993–2004 died from this infection, and the surviving patients were hospitalized (*21*,*22*). However, no deaths or hospitalizations were identified among the patients with *R. parkeri* rickettsiosis reported here. Serologic and immunohistochemical tests cannot readily distinguish between these diseases because of cross-reactive epitopes; molecular methods are necessary to identify the specific etiologic agent.

The leading edge of the Paraná Delta is advancing steadily from the deposition of sediments and is expected to reach or surpass the boundaries of the city of Buenos Aires within the next 100 years (*23*). The long-term effects of this process on the distribution and frequency of *R. parkeri* rickettsiosis are difficult to predict; however, as humans continue to encroach on the Paraná Delta through urbanization, agriculture, and tourism, recognized cases of this disease are likely to escalate.

Dr Romer is an infectious disease clinician and microbiologist at Hospital F.J. Muñiz in Buenos Aires, Argentina. Her current research interests include arthropod-borne diseases, particularly those caused by *Rickettsia* spp.

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Neurognathostomiasis, a Neglected Parasitosis of the Central Nervous System

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Release date: June 27, 2011; Expiration date: June 27, 2012

Learning Objectives

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

- Describe food sources of gnathostomiasis parasitic infection
- Describe different clinical manifestations of neurognathostomiasis infection
- Identify methods of diagnosis of neurognathostomiasis
- Describe treatment options for gnathostomiasis
- Describe outcomes of gnathostomiasis infection

Editor

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

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Gnathostomiasis is a foodborne zoonotic helminthic infection caused by the third-stage larvae of *Gnathostoma* spp. nematodes. The most severe manifestation involves infection of the central nervous system, neurognathostomiasis. Although gnathostomiasis is endemic to Asia and Latin America, almost all neurognathostomiasis cases are reported from Thailand. Despite high rates of illness and death, neurognathostomiasis has received less attention than the more common cutaneous form of gnathostomiasis, possibly because of the apparent geographic confinement of the neurologic infection to 1 country. Recently, however, the disease has been reported in returned travelers in Europe. We reviewed the English-language literature on neurognathostomiasis and analyzed epidemiology and geographic distribution, mode of central nervous system invasion, pathophysiology, clinical features, neuroimaging data, and treatment options. On the basis of epidemiologic data, clinical signs, neuroimaging, and laboratory findings, we propose diagnostic criteria for neurognathostomiasis.

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DOI: 10.3201/eid1707.101433

Foodborne parasitic infections are common in the tropics, where many foodborne parasites are endemic and ingestion of raw shellfish and freshwater fish, as well as undercooked meat, is frequent among local populations (*1*). Increased international travel to areas endemic for these foodborne parasites and migration from tropical areas have led to the emergence of these diseases in temperate climates (*2*), where such infections are rarely seen by physicians and thus may not be considered in differential diagnoses. Gnathostomiasis is a foodborne zoonotic helminthic infection caused by the third-stage larva of *Gnathostoma* spp. nematodes (Figure 1, panels A, B). At least 13 species have been identified (*3*), with 5 recorded in humans. *G. spinigerum* is the most common of these nematodes in Asia. Human infection with *G. hispidum*, *G. doloresi*, and *G. nipponicum* were found only in Japan (*4*). In the Americas, *G. binucleatum* is the only proven pathogenic *Gnathostoma* nematode in humans. Humans are infected primarily by eating raw or undercooked freshwater fish (Figure 1, panel C), frogs, and chicken. Humans are accidental unsuitable hosts; the parasite rarely develops to an adult worm, and the disease in humans is caused by the migrating larva.

Gnathostomiasis can be divided into cutaneous, visceral, and ocular forms, depending on the site of larval migration and subsequent signs and symptoms (*2*). The most common clinical presentation is the cutaneous one (Figure 1, panel D), which is characterized by localized, intermittent, migratory swellings of the skin and is often associated with localized pain, pruritus, and erythema (*5*,*6*). Visceral involvement can manifest in virtually any organ and any part of the body (*3*). The most severe manifestation of the visceral disease is involvement of the central nervous system (CNS), i.e., neurognathostomiasis. Neurognathostomiasis has been reported only in *G. spinigerum* infections (*3*).

We found 24 reports describing a total of 248 patients with neurognathostomiasis published in English-language literature. In this article, we review epidemiology, mode of CNS invasion, pathophysiology, clinical features, neuroimaging data, and treatment options, and we propose diagnostic criteria for this emerging disease.

History of Neurognathostomiasis

The first clinical case of *G. spinigerum* meningoencephalitis was reported by Daensvang in 1949 (*7*). Eighteen years later, Chitanondh and Rosen (*8*) documented the first pathologic evidence of *G. spinigerum* invasion of the CNS from the autopsy of a 34-year-old Thai woman. In their seminal study, Boongird et al. (*9*)

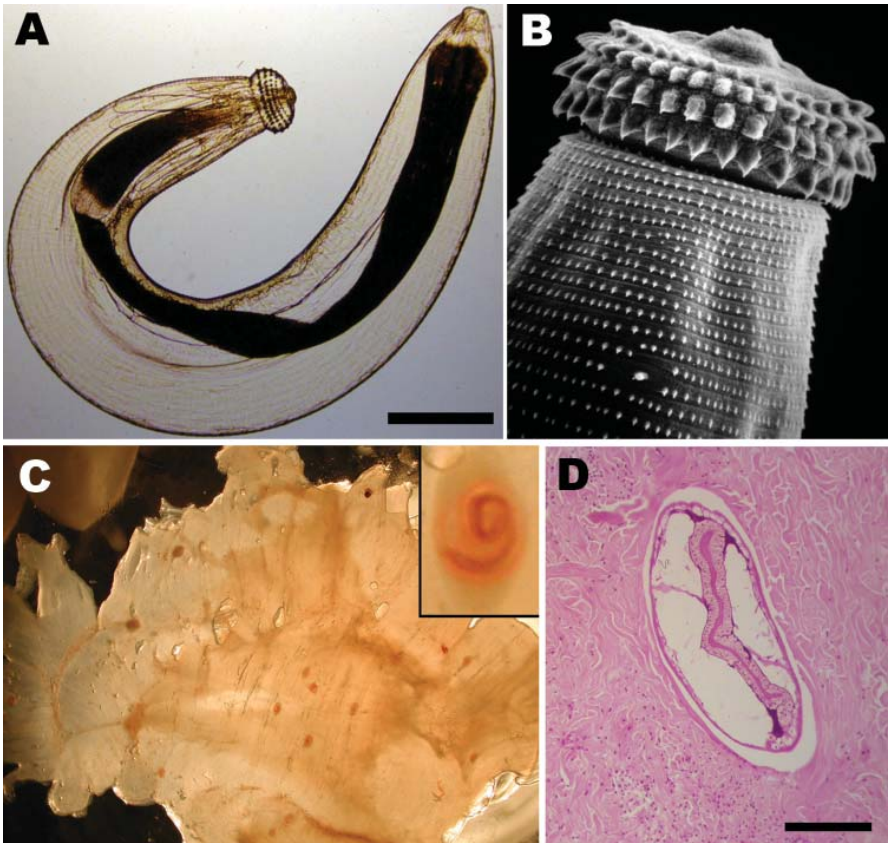

Figure 1. A) Third-stage larva of the nematode *Gnathostoma* sp. Scale bar = 250 μm. B) Scanning electronic microscopy image depicting head bulb with 4 cephalic hooklet rows. Original magnification ×500. C) *Gnathostoma* sp. larvae in the flesh of their intermediate host, *Eleotris picta* fish. Original magnification ×4. Inset: Higher magnification of an encysted larva; original magnification ×100. Larvae photographs courtesy of Dr Diaz-Camacho, Universidad Autónoma de Sinaloa, Sinaloa, Mexico. D) Cross-section of a *Gnathostoma* sp. larva in human skin biopsy sample (hematoxylin and eosin stain). Scale bar = 250 μm.

delineated the clinical syndromes of neurognathostomiasis, which facilitated further prospective and retrospective case identification (*10*). With the advance of high-resolution neuroimaging, the parasitic tracks of *Gnathostoma* could be demonstrated on magnetic resonance imaging (MRI), aiding the diagnosis of neurognathostomiasis in the absence of worm recovery (*11*).

Epidemiology of Neurognathostomiasis

Most (241/248) identified cases of neurognathostomiasis reported in the English-language literature were from Thailand. Two patients were infected in Laos (*12*,*13*), 1 in Japan (*14*), 1 in Myanmar (*13*), and 2 in South Korea (*15*,*16*). One patient had traveled to Southeast Asia and Japan (*17*), so the geographic origin of his infection could not be determined. The incubation period of neurognathostomiasis can be as long as 10 years because of the long persistence of the nematode larvae in the soft tissues before CNS invasion (*2*,*15*), which means it can occur in immigrants in a country in which the parasite is not endemic (*16*,*18*). All case-patients were reported to be infected with *G. spinigerum*. Although gnathostomiasis has been increasingly detected outside of Asia, particularly in Mexico, no cases of neurognathostomiasis have been reported in other regions. The occurrence of another *Gnathostoma* species in Latin America, namely *G. binucleatum*, is a possible explanation for this phenomenon.

Neurognathostomiasis in Travelers

The first case of neurognathostomiasis in a traveler was reported in 2003 (*19*). Since then, 4 patients in Europe returning from gnathostomiasis-endemic countries have been published (*13*,*20*,*21*). Two patients traveled to Thailand (*19*,*20*), 1 patient to Lao People's Democratic Republic (*13*) and 1 patient to Myanmar (*13*). The patients were reported to have indulged in local customs of eating raw fish (*19*) and shrimp (*20*). Gnathostomiasis has been recently reported in travelers returning from southern Africa (*22*,*23*). However, the patients had cutaneous manifestation of *Gnathostoma* spp. infection without neurologic involvement.

Pathophysiology of Neurognathostomiasis

Gnathostoma spp. nematodes are highly invasive parasites. After being ingested, the third-stage larvae penetrate the mucosal wall of the gastrointestinal tract. Larvae can enter the CNS by invading directly through the loose connective tissues of the neural foramina of the skull base along the cranial nerves and vessels or through intervertebral foramina along the spinal nerves and vessels (*24*). The larvae release a variety of molecules into their surrounding environment that facilitate tissue penetration and invasion (*25*). Proteases are the major molecular types among various components of these so-called excretor–secretory products, both quantitatively and qualitatively (*26*). In *Gnathostoma* spp., matrix metalloproteinases might play a key role in invasion of host tissues (*27*).

Gnathostoma spp. nematodes typically enter the spinal cord along the nerve roots, causing radiculomyelitis (*9*,*28*). The parasite is then able to ascend the spinal cord and reach the brain (*3*,*29*). This journey may take as long as several years and has been documented in a Thai patient through sequential imaging (*29*).

Given the length of the migrating larva, which averages 3.0 mm (*30*), the migration of the worm in the CNS causes direct mechanical injury because of tearing of nerve tissue. The hallmark sign of neurognathostomiasis is the evidence of multiple hemorrhagic tracks; these tracks have been documented throughout the spinal cord and brain tissue by postmortem examination (*9*), as well as through neuroimaging (*31*). Subarachnoid hemorrhage can result from larvae burrowing through a cerebral arteriole (*32*,*33*). Eosinophilic meningitis caused by *Gnathostoma* spp. is characterized by erythrocytes in cerebrospinal fluid (CSF) (*33*), which suggests mechanical damage by the worm. Apart from the structural damage, the inflammatory response to the migrating larvae might further contribute to tissue destruction (*34*).

Clinical Signs of Neurologic Involvement

The main clinical syndromes of neurognathostomiasis are radiculomyelitis/ radiculomyeloencephalitis, meningitis/meningoencephalitis, and subarachnoid and intracerebral hemorrhage (Table 1). Each syndrome reflects the tissue injury by the migrating parasite. Because the larvae migrate, the same patient can show sequentially different neurologic syndromes. For example, in 1 Laotian patient, meningomyelitis developed, followed by subarachnoid hemorrhage (*12*). Meningitis, subdural hemorrhage, and then intraparenchymal lesions developed sequentially in 1 patient from northeastern Thailand (*29*). The most common symptom reported virtually by every patient was pain: radicular pain in cases of spinal involvement (often lasting 1–5 days) or headache in cases of meningoencephalitis. The most common manifestation, spinal cord disease, accounted for 55% of all clinical syndromes (Table 1).

Myelopathy in gnathostomiasis is characterized by radicular pain followed by an ascending paresis of lower extremities or quadriparesis with bladder dysfunction (*9*,*10*,*33*,*35*–*38*). A sensory level for all modalities including vibration and proprioception was a frequent finding, commonly localized in the thoracic region (*9*). Cerebral and meningeal involvement is characterized by meningeal signs and decreased consciousness. Abducens nerve palsy was the most common cranial nerve impairment

Table 1. Clinical presentation of 248 patients with neurognathostomiasis*

Syndrome	Probable entry portal entry	Clinical signs and symptoms	No. (%) cases
Radiculomyelitis/myelitis/ myeloencephalitis	Intervertebral foramina along the spinal nerves and vessels	Sharp radicular pain and a spinal syndrome (paraplegia, monoplegia, quadriplegia, bladder dysfunction, sensory disturbances), can progress to cerebral involvement (myeloencephalitis)	140 (55)
Meningitis/ meningoencephalitis	Neural foramina of the skull base along the cranial nerves and vessels	Severe headache, stiffness of the neck, cranial nerve palsies, disturbance of consciousness, focal neurologic signs	77 (30)
Intracerebral hemorrhage	Intervertebral or neural foramina	Headache, sudden-onset focal neurologic signs	21 (8)
Subarachnoid hemorrhage	Intervertebral or neural foramina	Thunderclap headache, meningeal signs	16 (7)

*Because the larvae migrate, patients can have sequential signs and symptoms; thus, the total number of clinical syndromes shown exceeds the number of reported patients.

(*9*,*33*). Intracranial hemorrhages were seen with sudden-onset focal neurologic deficits such as hemiparesis and hemihypaesthesia (*9*,*10*,*18*,*33*).

Isolated Neurologic Disease

The most common clinical feature of gnathostomiasis is intermittent migratory cutaneous swelling (*5*). However, in the largest published series of neurognathostomiasis in 162 patients, only 11 (7%) had a history of cutaneous lesions (*10*). Unilateral eyelid swelling, another typical sign of gnathostomiasis, occurred in only 6 patients (4%) (*10*). Boongird et al. reported only 3 patients with subcutaneous swelling out of 24 patients with neurognathostomiasis; cutaneous gnathostomiasis developed in all of these patients after the onset of neurologic symptoms (*9*).

CSF Studies

Eosinophilic pleocytosis is a hallmark laboratory finding. CSF eosinophilia in patients is usually prominent; median values were 40% (n = 24) and 54% (n = 39) in the studies by Boongird et al (*9*) and Schmutzhard et al (*33*), respectively. In 109 (67%) of patients reported by Punyagupta et al. (*10*), the eosinophil count in CSF was >30%. CSF has been reported as xanthochromic or bloody in 134 patients (64% of all published cases). CSF glucose is usually only mildly reduced but was reported as low as 11 mg/dL (9% of the plasma value) in 1 patient (*37*).

Neuroradiologic Features

Neuroimaging of 11 patients with neurognathostomiasis was reported in English-language literature. On cranial computed tomography, subarachnoid hemorrhage and intracerebral hemorrhage were the most common findings (*12*,*18*,*33*). In 1 patient a subdural hemorrhage was detected (*29*). The hallmark of cerebral gnathostomiasis on MRI was the detection of hemorrhagic tracks (Figure 2, panels A, C, D). The magnetic resonance signal of a hemorrhagic lesion varies in acute, subacute, and chronic lesions. Hence, on T1 and T2 weighted imaging both hypointensities and hyperintensities have been documented. No gradient-echo T2-weighted sequences have been reported in neurognathostomiasis. However, gradient-echo T2-weighted sequences are exquisitely sensitive to local field inhomogeneities of hematoma and could become a useful sequence in radiologic assessment of cerebral gnathostomiasis.

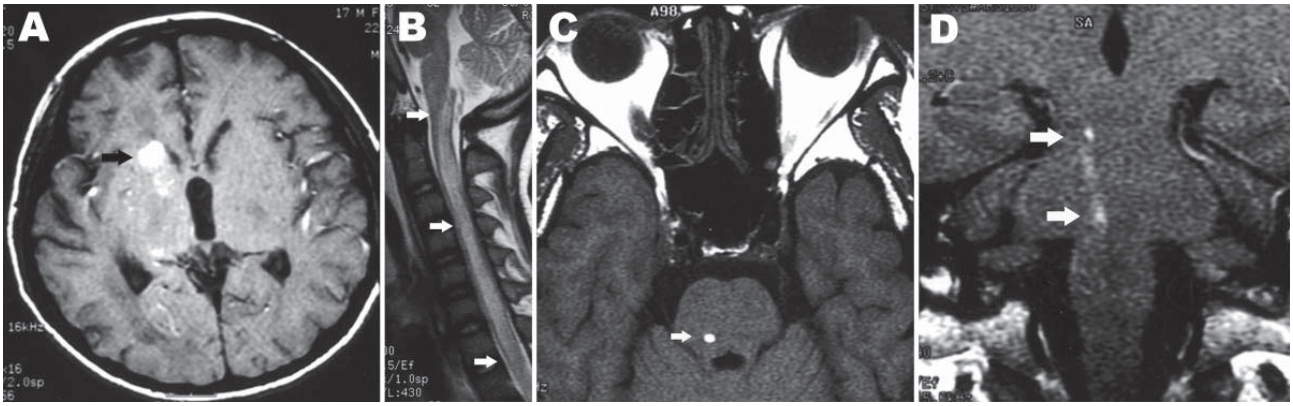

Figure 2. Images of the brains of patients with cerebral gnathostomiasis. A) Axial T1-weighted image showing small hemorrhage in the right basal ganglia (arrow). B) Sagittal T2-weighted images showing diffuse cord enlargement with longitudinal T2 hyperintensity (arrows). C) Axial T1-weighted image showing a hemorrhagic track in the tegmentum of the pons (arrow). D) Coronal T1-weighted postgadolinium image, showing the longitudinal extension of the same hemorrhagic track as in panel C (arrows). Images from K. Sawanyawisuth et al. (*11*), used with permission.

Diffuse multisegmental spinal cord swelling with corresponding T2 hyperintensities was the most common finding on spinal imaging (*11*,*17*) (Figure 2, panel B). Gadolinium enhancement of the lesions was reported in cranial and spinal MRI of 4 patients as slight, nodular, and ill-defined (*11*,*17*,*35*). Table 2 summarizes typical findings on neuroimaging in neurognathostomiasis.

Table 2. Neuroradiologic features of neurognathostomiasis*

Site	Procedure	Findings
Brain	CT	Parenchymal (single or multiple), subdural or subarachnoid hyperdensities corresponding to intracranial hemorrhage
	MRI	Multiple (worm-like) T2-weighted hyperintensities or hypointensities in both hemispheres and the cerebellum of ≥3 mm diameter (hemorrhagic tracks) with or without gadolinium enhancement
Spinal cord	MRI	Dilatation/swelling of the spinal cord with multisegmental T2-weighted hyperintensities, frequent gadolinium enhancement (slight to moderate) on T1 postcontrast images

*CT, computed tomography; MRI, magnetic resonance imaging.

Immunodiagnosis

The detection of antibodies in serum is the main pillar of immunodiagnosis of gnathostomiasis. Two methods have been established for clinical routine: ELISA and Western blot by using crude *Gnathostoma* spp. antigens from the larval extract. The 24-kD band on Western blot was shown to have nearly 100% specificity for gnathostomiasis (*39*). The current practice for serologic diagnosis is to use the ELISA (e.g., multiple-dot ELISA) as the first step (*40*) and to confirm the results by Western blot. At least 2 patients seroconverted during their neurologic illness (*19*,*20*), which emphasizes the need for repeated serologic examination in cases with strong clinical suspicion. The serologic testing can be done in several laboratories, including those in Thailand (Bangkok, Khon Kaen), Japan (Miayzaki), and Europe (Basel, Switzerland). Additional information about laboratories providing this service is available from the corresponding author.

Differential Diagnosis

The main differential diagnosis of neurognathostomiasis is an infection with *Angiostrongylus cantonensis*. However, *Angiostrongylus* spp. infection is usually seen as self-limited eosinophilic meningitis and only rarely causes severe disease with prominent spinal or cerebral involvement (*1*). Immunodiagnostically, positive 24-kD band against *G. spinigerum* antigen and negative 29-kD and 31-kD bands on immunoblot against *A. cantonensis* antigen make a diagnosis of an *Angiostrongylus* spp. infection highly unlikely (*40*). Other causes of a parasitic CNS disease, such as cysticercosis, toxocariasis, schistosomiasis, baylisascariasis, or paragonimiasis, can be distinguished by epidemiologic distribution, clinical signs and symptoms, radiologic appearance, and serologic testing.

Treatment

No randomized trials of antihelminthic therapy have been conducted for neurognathostomiasis. We have identified 9 reports of treatment of neurognathostomiasis with albendazole (*11*,*13*,*17*–*21*,*29*); 5 patients fully recovered, 2 partially recovered, and 2 patients did not recover. Outcomes might reflect the natural course of the disease rather than the effect of treatment. In several reports, antihelminthic treatment was considered as potentially detrimental and was withheld on the basis of its possible induction of brain edema due to sudden helminthic death (*31*,*38*). Three returned travelers from Thailand were treated with albendazole (800 mg/d for 1 month [*19*], 800 mg/d for 3 weeks [*20*], and 400 mg 2×/d for 4 weeks [*21*]) and fully recovered (*19*–*21*). In all these patients, dexamethasone (oral or intravenous) or predinsolone was added to the treatment regimen. A recent retrospective evaluation of long-term treatment efficacy in 13 patients from France with imported gnathostomiasis reported 2 patients with neurologic involvement (*13*), 1 with myelitis, and 1 with encephalitis (C. Strady, pers. comm.). The patient with myelitis fully recovered after albendazole therapy, and the patient with encephalitis was reported to have had 4 relapses despite therapy with ivermectin and albendazole (*13*; C. Strady, pers. comm.).

Corticosteroids have been used in neurognathostomiasis to treat cerebral and spinal edema (*19*–*21*). They also might prevent or alleviate paradoxical worsening after initiation of antihelminthic treatment. However, no conclusion about their efficacy can be drawn from the current data because no randomized control trials have been conducted and the retrospective data are too limited to allow any comparison between the groups. At the moment, no evidence-based recommendations can be issued.

Outcome

The case-fatality rate in the first series of 24 patients was 25% (*9*). Later studies reported mortality rates of 7%–12% (*10*,*33*). However, as the authors remark, these case-fatality rates are almost certainly an underestimate because many patients in critical condition were discharged home to die (*10*). In total, the outcome of 247 patients has been reported in the literature. An unfavorable outcome, e.g., death or severe persistent disability, was reported in 78 patients (32%). However, the recent case reports indicate that the current prognosis might be better. Of 15 patients in whom neurognathostomiasis was diagnosed after 2001, poor outcomes were reported in 3 (20%) patients. In all 4

clinical cases of returned travelers with myelitis, recovery was good after antihelminthic therapy (*13*,*19*–*21*).

Conclusions

Definitive diagnosis of helminthic CNS infections is notoriously difficult. The recovery rate of worms from the CSF is low, and the invasive procedures for diagnosis are seldom justified (*33*,*35*). Neurognathostomiasis is no exception; of 248 published cases, only 27 (11%) were confirmed by recovery of larvae. Moreover, an intermittent subcutaneous swelling, the hallmark of gnathostomiasis, is found only in the minority of neurognathostomiasis cases. Given these difficulties, clinical criteria are useful for practice and research. On the basis of the analysis of published cases, we propose diagnostic criteria that should guide a clinician if a pathologic diagnosis is not possible (Table 3).

Neurognathostomiasis is a parasitic CNS disease endemic to Southeast Asia, particularly Thailand. Because of growing international travel to disease-endemic areas, as well as migration from the tropics, an increasing number of patients will seek medical attention in Europe and North America. Neurognathostomiasis should be suspected in patients who have eosinophilic radiculomyelitis, myeloencephalitis or meningoencephalitis, and a history of travel to gnathostomiasis-endemic areas. Ingestion of raw or inadequately cooked shellfish, freshwater fish, or poultry is a prerequisite for infection; however, because such information might not be volunteered by the patient, clinicians should ask directly about foods eaten during travel. Serologic testing, including Western blot, shows high specificity and can contribute to the diagnosis in an appropriate clinical and epidemiologic setting. No evidence-based protocol is available for treatment of neurognathostomiasis; however, a 3- to 4-week course of albendazole with prednisolone or dexamethasone is 1 of the most frequently used regimens. Clinicians should advise patients traveling to or living in disease-endemic areas to avoid eating undercooked freshwater fish, frogs, poultry, and shellfish.

Table 3. Proposed diagnostic criteria for neurognathostomiasis*

Epidemiologic criteria
Travel or residence in the disease-endemic area AND exposure to undercooked freshwater fish, frogs, poultry, and shellfish
Clinical syndrome
Painful radiculomyelitis/radiculomyeloencephalitis OR meningitis/meningoencephalitis OR intracerebral hemorrhage OR subarachnoid hemorrhage
Indirect evidence of CNS invasion
CSF studies: eosinophilic pleocytosis OR neuroimaging: parenchymal hemorrhagic tracks (≥3 mm)
Immunodiagnosis
Positive detection of reactivity against the 24-kDa component of *Gnathostoma spinigerum* in immunoblot test† Absence of reactivity against the 29–31-kDa components of *Angiostrongylus cantonensis* in immunoblot test

*All criteria should be fullfiled for clinical diagnosis of neurognathostomiasis. CNS, central nervous system; CSF, cerebrospinal fluid.
†If negative, a follow-up examination 4 weeks later is recommended.

Dr Katchanov is a neurologist and a member of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. He has a special interest in neuroinfectious disorders, particularly bacterial meningitis, and neurohelminthiases.

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Effectiveness of Seasonal Influenza Vaccine against Pandemic (H1N1) 2009 Virus, Australia, 2010

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To estimate effectiveness of seasonal trivalent and monovalent influenza vaccines against pandemic influenza A (H1N1) 2009 virus, we conducted a test-negative case–control study in Victoria, Australia, in 2010. Patients seen for influenza-like illness by general practitioners in a sentinel surveillance network during 2010 were tested for influenza; vaccination status was recorded. Case-patients had positive PCRs for pandemic (H1N1) 2009 virus, and controls had negative influenza test results. Of 319 eligible patients, test results for 139 (44%) were pandemic (H1N1) 2009 virus positive. Adjusted effectiveness of seasonal vaccine against pandemic (H1N1) 2009 virus was 79% (95% confidence interval 33%–93%); effectiveness of monovalent vaccine was 47% and not statistically significant. Vaccine effectiveness was higher among adults. Despite some limitations, this study indicates that the first seasonal trivalent influenza vaccine to include the pandemic (H1N1) 2009 virus strain provided significant protection against laboratory-confirmed pandemic (H1N1) 2009 infection.

After the emergence and rapid global spread of pandemic influenza A (H1N1) 2009 virus, development of a pandemic (H1N1) 2009–specific vaccine began (*1*). A candidate reassortant vaccine virus, derived from the A/California/7/2009 (H1N1)v virus as recommended by the World Health Organization, was used to produce a monovalent, unadjuvanted, inactivated, split-virus vaccine for Australia (*2*,*3*). The national monovalent pandemic (H1N1) 2009 vaccination program in Australia ran from September 30, 2009, through December 31, 2010, and vaccination was publicly funded for all persons in Australia ≥6 months of age (*4*,*5*).

In September 2009, the World Health Organization recommended that trivalent influenza vaccines for use in the 2010 influenza season (Southern Hemisphere winter) contain A/California/7/2009 (H1N1)–like virus, A/Perth/16/2009 (H3N2)–like virus, and B/Brisbane/60/2008 (of the B/Victoria/2/87 lineage) virus (*6*). Since March 2010, the Australian Government has provided free seasonal influenza vaccination to all Australia residents ≥65 years of age, all Aboriginal and Torres Strait Islander persons ≥50 years, all Aboriginal and Torres Strait Islander persons 15–49 years with medical risk factors, persons ≥6 months with conditions that predispose them to severe influenza, and pregnant women (*7*). Influenza vaccination is also recommended, but not funded, for persons who might transmit influenza to those at high risk for complications from influenza, persons who provide essential services, travelers, and anyone ≥6 months of age for whom reducing the likelihood of becoming ill with influenza is desired. Individual industries are also advised to consider the benefits of offering influenza vaccine in the workplace (*8*). Because pandemic (H1N1) 2009 was expected to be the dominant strain in 2010, the monovalent vaccine continued to be used despite the availability of the seasonal vaccine, particularly by persons who were not eligible for funded vaccine (M. Batchelor, pers. comm.). However, in 2010, there were no published data on the relative use of monovalent and seasonal vaccines at that time.

The need for rapid implementation of programs results in initial studies using immunogenicity, rather than efficacy, to assess performance of influenza vaccines. After 1 dose of monovalent pandemic (H1N1) 2009 vaccine containing 15 μg hemagglutinin without adjuvant, seroprotection was

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DOI: 10.3201/eid1707.101959

estimated to be 94%–97% in working-age adults (*3*,*9*,*10*) and 75% in children (*10*). Observational studies provide a practical way to calculate vaccine effectiveness under field conditions (*11*,*12*). Effectiveness of monovalent pandemic (H1N1) 2009 was estimated to be 72%–97% by 3 studies in general practice and community-based settings in Europe (*13*–*15*), 90% in a hospital-based study in Spain (*16*), and 100% in a community-based study of children in Canada (*17*). These studies were conducted in populations for which the respective local or national pandemic vaccination program primarily used vaccine without adjuvant.

We assessed effectiveness of the 2010 seasonal influenza vaccine against laboratory-confirmed pandemic (H1N1) 2009 influenza infection in Victoria, Australia. Data came from an established test-negative case–control study in a general practitioner sentinel surveillance network (*18*,*19*).

Methods

Sentinel Surveillance

Victoria is the second most populous state in Australia; it has a temperate climate, and the annual influenza season usually occurs during May–September. Each season, on behalf of the Victorian Government Department of Health, the Victorian Infectious Diseases Reference Laboratory conducts surveillance for influenza-like illness (ILI; defined as history of fever, cough, and fatigue/malaise) and laboratory-confirmed influenza. General practitioners within the network provide weekly reports on case-patients with ILI as a proportion of total patients seen and send swabs from patients with ILI to the laboratory for testing. In 2010, a total of 87 practitioners participated in the program, which operated for 25 weeks, from May 3 (week 19) through October 24 (week 43). Practitioners were asked to collect nose and throat swabs from patients with an ILI (*20*) within 4 days after onset of the patient's symptoms. Samples were collected by using Copan dry swabs (Copan Italia, Brescia, Italy) and were placed in virus transport medium. Practitioners were also asked to provide data on the patient's age, sex, date of symptom onset, vaccination status, type of influenza vaccine (monovalent or trivalent/seasonal) received, and date of vaccination. Type of vaccine and date of vaccination were ascertained from medical records and patient report.

Laboratory Testing

RNA was extracted from clinical specimens by using a Corbett extraction robot (Corbett Robotics, Brisbane, Australia), followed by reverse transcription to cDNA by using random hexamers. PCR amplification and detection selective for the type A influenza virus matrix gene was performed by using primers and a Taqman probe on an ABI-7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Samples determined to be positive by this assay were confirmed as positive or negative for pandemic (H1N1) 2009 in a second real-time PCR that incorporated primers and probes specific for the hemagglutinin gene of the pandemic (H1N1) 2009 virus. Influenza B viruses were identified by a separate PCR. One practitioner chose to send samples to the state reference laboratory in South Australia for testing with equivalent diagnostic assays.

Ascertainment of Case-patients and Controls

Case-patients and controls were sampled prospectively throughout the study period. A case-patient was defined as a person with ILI for whom test results for pandemic (H1N1) 2009 were positive; a control was defined as a person with negative test results for influenza virus. Analysis of vaccine effectiveness against other influenza subtypes was not undertaken because of the almost exclusive circulation of pandemic (H1N1) 2009 virus during the season; therefore, patients with positive test results for other influenza viruses were excluded. A control could become a case-patient if another illness developed during the season, but a case-patient was no longer at risk and could not be included again.

Data Analysis and Calculation of Vaccine Effectiveness

All analyses were conducted by using Stata version 10.0 (StataCorp LP, College Station, TX, USA). The χ^2 test was used to compare proportions, and the Mann-Whitney U test was used to compare time from vaccination to time seen by practitioner; $p<0.05$ was considered significant. Patients were excluded from the vaccine effectiveness analysis if vaccination status was unknown, if the date of symptom onset was unknown, or if the interval between symptom onset and specimen collection was >4 days (because of decreased likelihood of a positive result after this time) (*21*,*22*). Patients were considered not vaccinated if time between date of vaccination and symptom onset was <14 days. If only the month of vaccination was reported, the date of vaccination was conservatively estimated to be the last day of the month. To avoid overestimation of vaccine effectiveness arising from recruitment of controls when influenza was not circulating in the population, analysis was restricted to case-patients and controls detected within the influenza season, defined as the period during which influenza-positive case-patients were detected (weeks 26–40).

Vaccine effectiveness was defined as (1–odds ratio) × 100%; the odds ratio is the odds of laboratory-confirmed pandemic (H1N1) 2009 case-patients having been vaccinated divided by the odds of controls having been vaccinated. In the test-negative case–control design,

the odds ratio estimates the incidence density (rate) ratio because controls are selected longitudinally throughout the course of the study (i.e., by density sampling) (*23*,*24*). The odds ratio in test-negative case–control studies has also been shown to approximate the risk ratio under conditions of varying attack rates and test sensitivity and specificity (*25*). Logistic regression was used to calculate odds ratios and 95% confidence intervals (CIs) for having laboratory-confirmed pandemic (H1N1) 2009, which were adjusted for the variables of age group and month of specimen collection against the following: seasonal vaccine, monovalent vaccine, both vaccines, and any (either or both the seasonal and monovalent) vaccine. Sensitivity analyses were conducted to determine the effects of the following on vaccine effectiveness: not censoring for specimens collected from ILI patients >4 days after symptom onset, including controls recruited outside the defined influenza season, and assuming that patients with unspecified type A influenza had pandemic (H1N1) 2009.

Ethical Considerations

Data in this study were collected, used and reported under the legislative authorization of the Victorian Public Health and Wellbeing Act 2008 and Public Health and Wellbeing Regulations 2009. Thus, the study did not require Human Research Ethics Committee approval.

Results

A total of 172,411 patients were seen by participating practitioners during the study period, of whom 678 (0.4%) had ILI. After a nadir ILI rate of 0.2% in week 21, the rate gradually increased to 0.4% in week 31 before increasing more sharply to a peak of 0.9% in week 36. Swabs were collected from 478 (71%) ILI patients, among whom 170 (36%) had positive influenza test results and the remainder were negative. Influenza-positive patients were detected during weeks 26–40, which was defined as the influenza season (Figure). A total of 142 patients were excluded from further analysis because vaccination status was unknown (n = 11), symptom onset date was unknown (n = 33), time between symptom onset and specimen collection was >4 days (n = 43), or the specimen was collected outside the influenza season (n = 82). A significantly higher proportion of influenza-negative patients (13%) than influenza-positive patients (4%) were excluded because >4 days had elapsed between symptom onset and specimen collection (p = 0.001). No significant difference was found by age group for whether study participants had a specimen collected within 4 days after symptom onset (p = 0.10).

Of the remaining 336 patients, 156 (46%) had positive influenza test results. Most (89%) influenza case-patients had pandemic (H1N1) 2009, 6% had unspecified type A influenza, 4% had influenza A (H3N2), and 1% had influenza type B (Figure). After exclusion of the other influenza patients, 139 pandemic (H1N1) 2009 case-patients and 180 controls were included in the study analysis. Most (57%) participants were 20–49 years of age, and case-patients were significantly younger than controls (p = 0.001); no case-patient was ≥65 years of age (Table 1). No statistically significant difference was found between male and female study participants by case or control status (p = 0.60) or by vaccination status (p = 0.09). The high proportion of case-patients detected in August resulted in a significant difference between case-patients and controls by month of swab collection (p<0.001).

Overall, 59 (18%) study participants were reported as vaccinated with any vaccine, but the proportion was higher among controls (26%) than among case-patients (9%; p<0.001). The proportion of controls, who were mostly older, who had received the trivalent seasonal vaccine was higher than the proportion of controls who had received the monovalent vaccine (Table 1). Similarly, controls who had received both vaccines were all ≥20 years of age. Only case-patients who were 5–19 and 20–49 years of age were

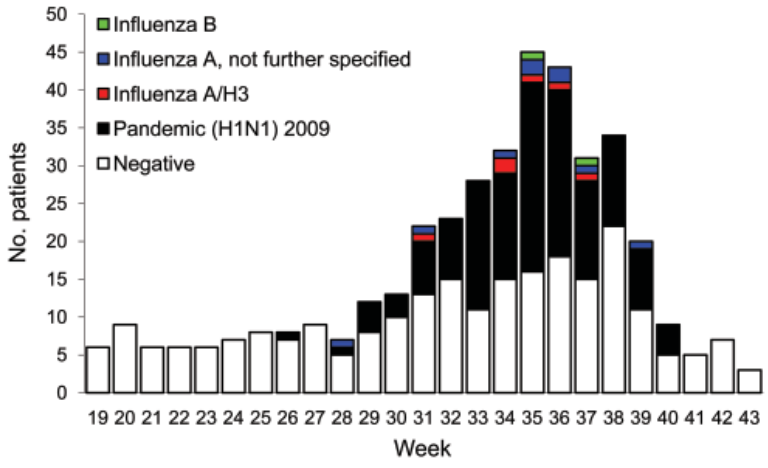

Figure. Influenza status of patients seen at sentinel general practices, Victoria, Australia, May 3 (week 19) through October 24 (week 43), 2010.

reported as vaccinated. Influenza vaccine type was not specified for 1 case-patient and 1 control, each of whom was reported as vaccinated.

Reflecting the availability of each vaccine, the median period between vaccination and visit to a general practitioner was significantly shorter for those who received seasonal vaccine (114 days) than for those who received monovalent vaccine (223 days; p<0.0001). No significant difference in the time from vaccination to practitioner visit was found between case-patients and controls for seasonal (p = 0.70) or monovalent vaccine (p = 0.95).

In general, point estimates of vaccine effectiveness adjusted for patient age and month of specimen collection differed little from crude estimates (Table 2). A significant protective effect was observed for seasonal vaccine only (adjusted vaccine effectiveness 79%; 95% CI 33%–93%) and seasonal and monovalent vaccines (adjusted vaccine effectiveness 81%; 95% CI 7%–96%). The adjusted vaccine effectiveness for receipt of any (either or both the seasonal and monovalent) vaccine was lower at 67% because of the 47% vaccine effectiveness for monovalent vaccine. The absence of vaccinated case-patients and controls meant vaccine effectiveness could not be estimated for several of the 5 age groups (Table 1); therefore, age was collapsed into 3 variables: children (0–19 years), working-age adults (20–64 years), and elderly persons (≥65 years). Estimates of vaccine effectiveness for working adults were 0%–14% higher than the overall adjusted estimates; estimates for children were either undefined because no controls were vaccinated or were without a significant protective effect. Vaccine effectiveness could not be calculated for elderly persons because there were no case-patients in this age group.

Sensitivity analyses to determine the effects of certain assumptions resulted in variations in the adjusted vaccine effectiveness point estimates of 0%–3% and no changes to their relative significance. The effects considered were as follows: assumption that those patients with unspecified influenza type A had pandemic (H1N1) 2009, no exclusion of patients if >4 days had elapsed between symptom onset and specimen collection, and no exclusion of patients if they were identified outside the defined influenza season.

Discussion

Our results indicate that the 2010 seasonal trivalent influenza vaccine is >80% effective against pandemic (H1N1) 2009 virus, regardless whether given by itself or in addition to monovalent vaccine. Groups in Europe and Canada have estimated the effectiveness of monovalent seasonal influenza vaccine against pandemic (H1N1) 2009 virus to be 72%–100% (*13–17*). However, the effectiveness of any vaccine (monovalent, seasonal, or both) against pandemic (H1N1) 2009 virus was lower (67%, 95% CI 33%–84%) because effectiveness for monovalent vaccine only was 47% (95% CI –62% to 82%). The lower effectiveness of monovalent influenza vaccine against pandemic (H1N1) 2009 virus compared with seasonal trivalent influenza vaccine is difficult to explain. Both vaccines contain the same quantities (15 μg) of hemagglutinin; and although the monovalent vaccine does not contain adjuvant and was available ≈6 months before the seasonal vaccine, it has been shown to be strongly immunogenic (*3*,*9*,*10*). Immunogenicity does not necessarily correlate directly with vaccine effectiveness, and we cannot exclude waning immunity as an explanation for the lower effectiveness of monovalent vaccine in our study. Waning immunity after receipt of monovalent vaccine has been suggested after an interim study from the United Kingdom for the 2010–11 influenza season (*26*). The finding could also be a product of the relatively small number of case-patients and controls who received only the monovalent vaccine, given that vaccine effectiveness estimates can change considerably by the inclusion or exclusion of 1–2 vaccinated study participants.

When stratified by age, estimates of vaccine effectiveness for working-age adults were higher and

Table 1. Participants in negative-test case–control study of efficacy of seasonal influenza vaccine for preventing pandemic (H1N1) 2009, Australia, 2010

Participants	Age group, y					Total, n = 319
	0–4, n = 19	5–19, n = 73	20–49, n = 181	50–64, n = 41	≥65, n = 5	
Controls						
Total*	13 (68)	27 (37)	107 (59)	28 (68)	5 (100)	180 (56)
Vaccinated with monovalent vaccine†	0	3 (11)	7 (7)	1 (4)	0	11 (6)
Vaccinated with seasonal vaccine†	0	0	9 (8)	10 (36)	2 (40)	21 (12)
Vaccinated with both vaccines†	0	0	7 (7)	4 (14)	2 (40)	13 (7)
Pandemic (H1N1) 2009 case-patients						
Total*	6 (32)	46 (63)	74 (41)	13 (32)	0	139 (44)
Vaccinated with monovalent vaccine†	0	3 (7)	3 (4)	0	0	6 (4)
Vaccinated with seasonal vaccine†	0	2 (4)	2 (3)	0	0	4 (3)
Vaccinated with both vaccines†	0	0	2 (3)	0	0	2 (1)

*No. (%) study participants.
†No. (%) controls/pandemic (H1N1) 2009 case-patients.

Table 2. Crude and adjusted vaccine effectiveness against pandemic (H1N1) 2009 virus, Australia, 2010

	Influenza vaccine effectiveness, % (95% confidence interval)			
Effectiveness	Seasonal	Monovalent	Both	Any
Crude	80 (39–93)	42 (–62 to 79)	84 (26 to 96)	70 (42 to 84)
Adjusted*				
0–19 y	Undefined†	44 (–231 to 91)	Undefined‡	–41 (–549 to 69)
20–64 y	89 (50 to 98)	56 (–88 to 90)	81 (7 to 96)	81 (52 to 92)
All ages	79 (33 to 93)	47 (–62 to 82)	81 (7 to 96)	67 (33 to 84)

*Adjusted for month of swab collection.
†No controls vaccinated.
‡No controls or case-patients vaccinated.

more precise than those for children. We previously demonstrated that the sentinel practitioner surveillance program in Victoria is well suited for estimating vaccine effectiveness among working-age adults, who account for most of the surveillance population (*18*), and the 2010 results were consistent with this observation. The relatively few participants in the young (childhood) age groups meant the study had insufficient power to produce defined or significant estimates of vaccine effectiveness. At the other end of the age spectrum, 2% of study participants (5 controls and 0 case-patients) in 2010 were ≥65 years of age compared with an average of 7% in this age group during 2003–07 (*18*). Although the absence of pandemic (H1N1) 2009 case-patients ≥65 years of age is not surprising, given that older adults have been shown to have relatively higher levels of cross-reactive antibodies to pandemic (H1N1) 2009 virus (*27*–*29*), the reason for the low proportion of controls in this age group remains unclear. Among the several explanations are a true lower rate of ILI in older persons during 2010, a lower rate of visits to practitioners for ILI by persons in this age group (or treatment at other health services such as hospitals), or preferential sampling of younger persons by practitioners (and perhaps awareness that pandemic [H1N1] 2009 was the predominant circulating influenza virus subtype).

In addition to having a sample size large enough to provide vaccine effectiveness estimates by age group and influenza type, several other considerations with regard to design of case–control studies of influenza vaccine effectiveness have been proposed: 1) whether the control group best represents the vaccination coverage of the source population and 2) whether collection and confounding variables have been adjusted for, particularly underlying chronic conditions for which vaccine is recommended and previous influenza vaccination history (*30*). A 2010 survey of pandemic vaccination suggests that monovalent vaccine coverage in the control group was generally consistent with that in the general population and that use of monovalent vaccine was ≈17% among those from Victoria, compared with 13% among controls (*31*). No equivalent survey of 2010 seasonal vaccine usage was available for comparison.

Data about concurrent conditions of study participants that would indicate need for influenza vaccination were not collected during the 2010 influenza season; thus, adjustment of the vaccine effectiveness estimates for this potentially confounding variable could not be conducted. Such confounding by indication (or negative confounding), in which persons at higher risk for influenza are more likely to be vaccinated, underestimates effectiveness of influenza vaccine but may be counteracted by healthy vaccinee bias (or positive confounding), which overestimates effectiveness (*30*,*32*). The extent to which these biases occur is likely to vary and may explain the positive and negative variation of crude influenza vaccine effectiveness estimates after adjustment for chronic conditions in several similar test-negative case–control studies (*33*–*35*). Speculation about the relative effects of these biases on how many received monovalent vaccine is also difficult; vaccination was funded for the entire population of Australia, but at the end of February 2010, only 18% had been vaccinated (*31*).

Similar methods using test-negative controls to assess seasonal and pandemic vaccine effectiveness against both seasonal and pandemic influenza viruses have been applied in North America and Europe (*13*,*16*,*17*,*33*–*39*). Observational studies provide a convenient and timely way to assess influenza vaccine effectiveness without the ethical, practical, and financial stringencies associated with clinical trials for vaccine efficacy, but they also have limitations. Modeling suggests that the test-negative case–control design generally underestimates true vaccine effectiveness under most conditions of test sensitivity, specificity, and the ratio of influenza to noninfluenza attack rates (*25*), although quantifying the extent of this effect in this study is difficult because the precise sensitivity and specificity of the test are not known. We attempted to limit ascertainment bias by censoring records that indicated specimen collection >4 days after symptom onset and restricting the analysis to case-patients and controls tested within the influenza season only, although sensitivity analyses indicated little effect if these restrictions were relaxed. Of note, these findings apply predominantly to working-age adults receiving medical care in the general practice setting; the study did not include those who did not seek medical care for ILI.

Thus, the study measured effectiveness of vaccine against illness severe enough to require a visit to a practitioner; the results cannot necessarily be generalized to other parts of the population, in particular young children and elderly persons. We were also unable to determine whether participants had previously been infected with pandemic (H1N1) 2009 virus, which may result in overestimation of vaccine effectiveness.

In conclusion, we applied a test-negative case–control study design to an established sentinel surveillance system to estimate effectiveness of a trivalent seasonal influenza vaccine, which included an A/California/7/2009 (H1N1)–like virus, the pandemic (H1N1) 2009 influenza virus strain. This strain is also a component of the trivalent influenza vaccine for the 2010–11 Northern Hemisphere influenza season (*40*). The trivalent vaccine provided significant protection against laboratory-confirmed pandemic (H1N1) 2009 virus infection.

Acknowledgments

We thank the general practitioners for participating in the surveillance system in 2010, the staff of the Viral Identification Laboratory at the Victorian Infectious Diseases Reference Laboratory for conducting the PCRs, and Alain Moren for suggestions regarding data analysis.

The General Practitioner Sentinel Surveillance system is partly funded by the Victorian Government Department of Health. Partial support was also provided by an Australian Government National Health and Medical Research Council grant (application ID 604925) for research on pandemic (H1N1) 2009 virus to inform public policy and by seed funding from the World Health Organization.

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Transmission of Influenza on International Flights, May 2009

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Understanding the dynamics of influenza transmission on international flights is necessary for prioritizing public health response to pandemic incursions. A retrospective cohort study to ascertain in-flight transmission of pandemic (H1N1) 2009 and influenza-like illness (ILI) was undertaken for 2 long-haul flights entering Australia during May 2009. Combined results, including survey responses from 319 (43%) of 738 passengers, showed that 13 (2%) had an ILI in flight and an ILI developed in 32 (5%) passengers during the first week post arrival. Passengers were at 3.6% increased risk of contracting pandemic (H1N1) 2009 if they sat in the same row as or within 2 rows of persons who were symptomatic preflight. A closer exposed zone (2 seats in front, 2 seats behind, and 2 seats either side) increased the risk for postflight disease to 7.7%. Efficiency of contact tracing without compromising the effectiveness of the public health intervention might be improved by limiting the exposed zone.

The emergence of pandemic influenza A (H1N1) 2009 in Mexico and the United States, with rapid spread to Europe, Asia, and the Pacific, is testament to the ease of spread of infectious disease across the globe (*1*). The World Health Organization activated level 5 pandemic alert on April 29, 2009, when sustained community transmission of the pandemic virus was demonstrated in Mexico and the United States. In her address to the United Nations on May 4, 2009, Margaret Chan, Director-General of the World Health Organization, called for heightened vigilance to limit international spread of the virus (*2*). Australia's response was rapid, with the introduction of a number of measures as outlined in the Australian Health Management Plan for Pandemic Influenza, 2008 (*3*). These measures included in-flight messages to incoming passengers, use of health declaration cards by all incoming travelers, and mandatory reporting by the pilot on the health status of crew and passengers before landing (*4*). The novel virus was also listed as a quarantinable disease under Australia's Quarantine Act 1908, which allows for the application of public health powers for intervention (*5*).

Reports documenting spread of disease during airline flight are limited (*6*–*9*). Specific policy stating that passengers sitting in the same row as and within 2 rows of a confirmed case-patient should be treated as suspected of having that disease relies on studies of air travel where the index case-patient was infected with *Mycobacterium tuberculosis* (*10*–*12*). The aim of this study was to investigate the spread of pandemic (H1N1) 2009 infection from persons with confirmed disease on flights to Australia during May 2009. The spread of other influenza-like illness (ILI) was also documented.

Methods

Study Population

A retrospective cohort study designed to determine exposure risk to known pandemic (H1N1) 2009 virus was undertaken for 2 long-haul flights that entered Australia the weekend of May 23–24, 2009. Flight 1 was chosen after identification of 6 passengers with confirmed pandemic (H1N1) 2009 infection within 24 hours after flight arrival from the United States. Flight 2 was chosen after identification of a confirmed case of pandemic (H1N1) 2009. This flight came from an area that lacked community

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DOI: 10.3201/eid1707.101135

transmission. Passenger details were obtained through collection of Health Declaration Cards and comparing the cards to flight manifests obtained from the airlines.

The definition of ILI was broad to capture as many persons as possible within the dataset. Passengers were asked to self-report development of any of the following signs or symptoms: fever, cough, sore throat, headache, runny nose, muscle aches, diarrhea, and lethargy. ILI was defined as ≥1 symptom (cough, runny nose, sore throat, or fever) within 7–14 days before the flight or during the flight or ≤7 days after arrival. The time periods were put in place to help determine when passengers were most likely to have contracted their ILI. Passengers were excluded who indicated bacterial infection (antibiotic prescription from medical personnel) or regular health issues, such as migraines. All but 4 passengers reporting symptoms had confirmation of ILI status from a qualified health professional.

Self-identification of other health conditions that were considered potential concurrent conditions for purpose of this study included obesity, diabetes mellitus, immunosuppression, asthma, chronic lung disease, and pregnancy. Seat location, concurrent condition status, and contraction of disease were compared. Ethics approval was given by the Australian Government Department of Health and Ageing Ethics Committee and the Australian National University Human Research Ethics Committee.

Data Collection

Surveys were distributed to passengers 3 months after flight arrival. The survey asked about influenza-like symptoms, symptom onset time, concurrent conditions, antiviral prophylaxis and treatment, isolation or quarantine dates, other potential exposure to ILI before and after the flight, contact with health professionals after the flight, and details of testing for pandemic (H1N1) 2009 virus. Two reminders were sent to improve the response rate of the study.

As a triangulation method, all passenger names, passenger sex, disease onset dates, and postal codes were cross-checked against those of passengers with known pandemic (H1N1) 2009 cases that were notified to national authorities for 1 month after flight arrival to verify information received from the survey responses and identify additional cases. Travel details were verified for pandemic (H1N1) 2009 case-patients identified through national notification. Contact tracing through public health authorities also identified ILI case-patients who had negative laboratory test results for pandemic (H1N1) 2009 virus.

Data Analysis

The increased risk of passengers contracting either laboratory-confirmed pandemic (H1N1) 2009 or an ILI (including pandemic [H1N1] 2009) was separately estimated by dividing the number of persons with the illness by the number of susceptible persons in the contact zones as described. For pandemic (H1N1) 2009, only passengers sitting in the economy class were considered exposed because of the location of persons displaying symptoms preflight or during the flight and the sectional layout of the aircraft.

Results

Of the 738 passengers on the 2 flights, 319 (43%) responded to a questionnaire; 143 (18%) of passengers could not be contacted. Cross-checking of mandatory notifications of pandemic (H1N1) 2009 against all passengers on both flights found 2 additional pandemic (H1N1) 2009 infection cases while also confirming symptom data on survey responses. Contact tracing by public health authorities found 5 additional passengers with ILI who had negative test results for pandemic (H1N1) 2009.

No passengers who had positive test results for pandemic (H1N1) 2009 had underlying health conditions considered to make them more susceptible to influenza; however, 5 of 32 passengers reporting ILI postflight had ≥1 potential concurrent conditions (1 each of obesity, diabetes mellitus, pregnancy, asthma, and chronic lung disease). Limited analysis demonstrated that the concurrent condition did not make these persons more susceptible than other passengers to contracting an ILI.

Flight 1

Flight 1, an Airbus A380, embarked from Los Angeles and arrived in Sydney on May 24, 2009, carrying 445 passengers. Of the 188 (42%) passengers who responded to a survey, 169 (90%) were Australian residents. Response rate varied with class of travel, with 11 (79%) of 14 first class passengers, 40 (56%) of 71 business class passengers, 19 (59%) of 32 premium economy class passengers, and 117 (36%) of 327 economy class passengers responding.

Combined results from the survey and disease notification data sources identified 8 passengers who had an ILI at the beginning of the 14-hour flight. For 4 of these passengers, pandemic (H1N1) 2009 infection was later laboratory confirmed; for 1, pandemic (H1N1) 2009 infection was confirmed as negative; 3 passengers were not tested. ILI symptoms developed in 2 other passengers during the flight, and pandemic (H1N1) 2009 was confirmed in both.

Twenty-four passengers were identified as developing ILI symptoms ≤7 days after arrival in Australia. Of these, 2 passengers had laboratory-confirmed pandemic (H1N1) 2009 infection, 15 had illness confirmed as negative for the pandemic virus, and 7 were not tested. Most passengers experienced onset of symptoms ≤3 days after flight arrival;

however, 6 passengers did not state exact date of disease onset (Figure 1).

Self-reporting of symptoms from passengers did not distinguish between different causes of ILI (Table). Fever was reported from 4 of 8 passengers with confirmed pandemic (H1N1) 2009, 7 of 16 passengers whose ILI was confirmed negative for pandemic (H1N1) 2009, and 2 of 10 passengers with ILI who were not tested. Two or fewer symptoms, not including fever, were reported for 3 of the 8 passengers with confirmed pandemic (H1N1) 2009, 7 for those testing negative for pandemic (H1N1) 2009, and 6 with ILI who were not tested.

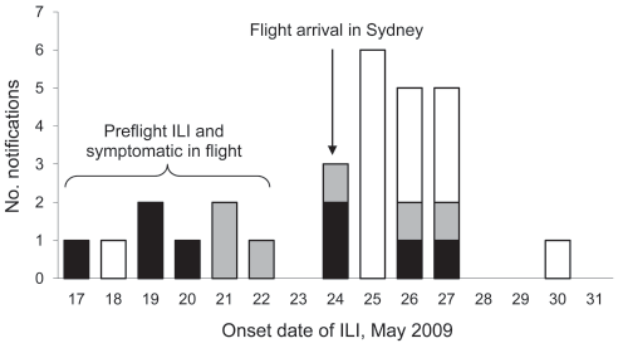

Figure 1. Onset date of influenza-like illness (ILI) in passengers traveling to Australia on flight 1, May 24, 2009. Six other passengers did not state exact ILI onset date. White bar indicates a negative test result for pandemic (H1N1) 2009 virus; black indicates a positive test result for pandemic (H1N1) 2009; gray bars indicate ILI with no test given.

Location and Transmission

Twenty (83%) of 24 passengers in whom an ILI developed postflight sat in aisle seats (Figure 2). This seat location increased the risk of contracting an ILI by 1.8×; however, it did not reach statistical significance. Survey respondents were 1.3× more likely to sit in an aisle seat.

Some clustering of cases was seen with the potential for spread of either ILI or pandemic (H1N1) 2009 from passengers who were infectious before and during the flight (Figure 2). The passengers who were symptomatic for pandemic (H1N1) 2009 before the flight departed were traveling as a family group; however, they were not connected to the persons who contracted the virus during the flight. Likewise, 2 other clusters of nonpandemic ILI with either symptomatic persons boarding the flight or showing symptoms postflight were in family groups and therefore would have had substantial contact before, during, and after the flight. Disease was deemed to have spread during the flight in passengers from seats 69H/69J/69K/70H to 68K and 71K, with all case-patients having positive test results for pandemic (H1N1) 2009; from 63C to 62D (neither passenger being tested); and from 84E/84F to 83G, with all case-patients testing negative for pandemic (H1N1) 2009. One cluster (seats 35B/36B/36D/37D) had no identifiable preflight index case-patient; however, 2 of the passengers were part of a family group.

In the cabin section of rows 66–77 was a cluster of passengers with symptoms before boarding who were later found to have positive test results for pandemic (H1N1) 2009. ILI symptoms developed in 7 passengers after the flight. In 2 of these passengers, pandemic (H1N1) 2009 was laboratory confirmed; for 4, ILI was confirmed as negative for pandemic (H1N1) 2009; and 1 person (seat 75G) was not tested.

Similarly, in the cabin section of rows 50–64, symptoms developed in 2 passengers during the flight or on the day of arrival (seats 52C and 58B); these passengers were found to be pandemic (H1N1) 2009 positive. Symptoms developed in 7 passengers after flight arrival; 5 of whom had negative test results for pandemic (H1N1) 2009, 2 (seats 54F and 62D) were not tested.

Risk of Contracting Disease and Contact Tracing

We examined the risk of contracting pandemic (H1N1) 2009 infection postflight to all susceptible passengers seated in the economy section of the aircraft. Health authorities contacted 145 passengers on flight 1 for quarantine and prophylactic treatment after potential exposure to pandemic (H1N1) 2009 (Figure 2, large black boxes). Of these, 52 (35%) passengers responded to the surveys, of whom 8 (15%) went into isolation 1 day after flight arrival, 17 (33%) by 2 days after flight arrival, and the others ≥3 days after flight arrival.

Initially, we looked at passengers exposed to this disease who sat in the same row as or within 2 rows either side of passengers who had symptoms develop before or during the flight. The increased risk of contracting pandemic (H1N1) 2009 by sitting in those seats was 1.4% (95% confidence interval [CI] –0.5% to 3.4%). If the contact

Table. Signs and symptoms reported by passengers on flight 1 with influenza-like illness, by pandemic (H1N1) 2009 testing status and results, Australia, May 2009*

Sign or symptom	Tested		Not tested, n = 10
	Positive, n = 8	Negative, n = 16	
Fever	4	7	2
Cough	6	8	3
Sore throat	1	9	6
Headache	4	8	3
Runny nose	5	10	5
Muscle aches	3	6	3
Diarrhea	1	2	1
Lethargy	5	7	2
Vomiting	0	1	0

*Signs and symptoms are not mutually exclusive.

Figure 2. Passenger configuration on flight 1 arriving in Sydney, New South Wales, Australia, on May 24, 2009. ILI, influenza-like illness.

zone was modified to sitting in the same row as or within 2 rows either side of passengers who had preflight symptoms (rows 67–72) and not those in whom symptoms developed during the flight, the risk of contracting pandemic (H1N1) 2009 postflight increased to 3.6% (95% CI –1.3% to 8.6%). No passengers were detected as acquiring pandemic (H1N1) 2009 from either of the passengers with symptoms that developed during the flight; however, 2 passengers in the same section of the aircraft who responded to the survey indicated having had ILI symptoms but not being tested for pandemic (H1N1) 2009.

The current zone for contact tracing is defined by passengers in the same row as and within 2 rows either side of the index case-patient. A closer zone forming a square delimited by 2 seats in front, 2 seats behind, and 2 seats on either side of the index case-patient could be prescribed (Figure 2, small black boxes [rows 67–72]). When the risk of contracting pandemic (H1N1) 2009 postflight was calculated for economy passengers sitting in the 2 × 2 square around the preflight symptomatic index case-patients in seats 69H–70H, the risk of becoming ill postflight increased to 7.7% (95% CI –2.6% to 17.9%).

Flight 2

On May 23, 2009, a Boeing 747-400 arrived in Sydney from Singapore carrying 293 passengers. Of the 131 (45%) passengers responding to a survey regarding the potential for contracting an ILI during their flight, 114 (87%) were Australian residents. Public health authorities were not alerted to the potential for passengers carrying pandemic (H1N1) 2009 virus on this flight until 6 days after arrival. They were alerted after mandatory notification of the virus infecting 1 passenger.

Survey data showed that 1 passenger was identified as having symptoms consistent with an ILI before the flight and that symptoms developed in 2 additional passengers on the flight. The passenger who was symptomatic before the flight was not tested for pandemic (H1N1) 2009; of the passengers whose symptoms developed during the flight, 1 was not tested and 1 (adult in seat 33D) was tested 6 days after flight arrival. At that time, the test returned a negative result.

Six passengers were identified from the survey as having ILI symptoms within 7 days after flight arrival (Figure 3). Of these, 1 passenger with symptom onset within 48 hours after flight arrival (a child in seat 33D) was laboratory confirmed to have pandemic (H1N1) 2009, 1 passenger (seat 24K) had negative test results for pandemic influenza A, and 4 passengers (seats 34A, 35B, 41G, and 63D) were not tested. The passenger in whom pandemic (H1N1) 2009 was confirmed was a child companion sharing a seat on the aircraft with the passenger whose symptoms developed during the flight but who tested negative for the virus.

Some clustering of cases was seen, with a potential spread of disease from passengers who were symptomatic during the flight (seats 62C–63D, 33D–34A, and 35B and the child sharing 33D). Transmission of disease between adult and child in 33D could have occurred before or during the flight. With lack of a confirmed index case-patient for pandemic (H1N1) 2009, the increased risk for disease was not calculated. No other passengers on this flight had positive test results for pandemic (H1N1) 2009 infection after triangulation methods with the notifiable diseases database.

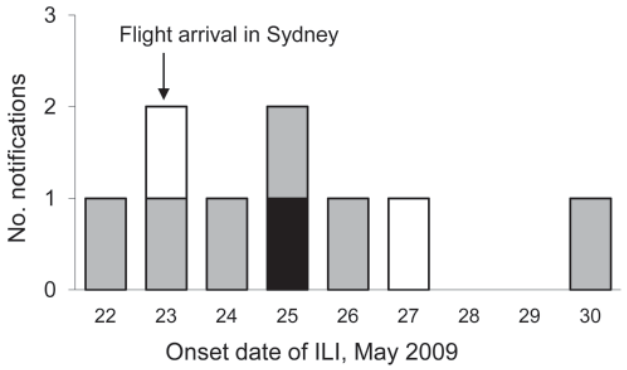

Figure 3. Onset date of influenza-like illness in passengers traveling to Australia on flight 2, May 23, 2009. White bar sections indicate a negative test result for pandemic (H1N1) 2009 virus; black bar section indicates a positive test result for pandemic (H1N1) 2009; gray bar sections indicate ILI with no test given. ILI, influenza-like illness.

Discussion

Of 2 long-haul flights entering Australia within the first month after declaration of a level 5 alert for pandemic (H1N1) 2009, a total of 45 (6%) of 738 passengers on 2 aircraft were identified as having the potential to spread an ILI into the local community. Follow-up confirmed 9 passengers with pandemic (H1N1) 2009 infection; 8 of these were from flight 1.

Flight 1, originating from a destination with documented widespread community transmission of pandemic (H1N1) 2009, had the greatest potential for introducing the pandemic virus into the Australian community, with 2% of its tested passengers being confirmed positive for pandemic (H1N1) 2009. Spread of the virus from a region known to have sustained community transmission was to be expected and formed part of the case definition in Australia during the early phases of the pandemic (*13*). However, flight 2 originated in Singapore, where the first recorded case of the disease was on May 26, 2009 (*14*), 3 days after the aircraft arrived in Australia, which suggests that a targeted approach to aircraft screening that relies on country-specific information is not completely reliable.

Transmission of ILIs on board these aircraft clustered closely with a passenger who was symptomatic during the flight or may have been in contact with an infectious passenger for >15 minutes during the flight. This finding is similar to transmission of pandemic (H1N1) 2009 noted on a long-haul flight to New Zealand in 2009 (*6*). Recent studies on the transmission of pandemic (H1N1) 2009 in ferrets demonstrated preference for aerosol and droplet transmission of the virus (*15*,*16*). A similar study investigating the disease in a tour group in China indicated droplet transmission from coughing or talking with the index case-patient as being the main mode of transmission (*17*). The cabin in the A380-800 (flight 1) allows for a 10% wider seat in economy class than does the 747-400 (flight 2) (*18*), and modern ventilation systems in aircraft circulate air around bands of seat rows rather than the through length of the aircraft (*19*). However, neither of these measures are enough to prevent droplet transmission from either talking (≈1 meter) or spread of smaller aerosol droplets (*7*,*8*).

Vigilance by health authorities and cooperation by the public assisted in detecting many ILIs that were not associated with pandemic (H1N1) 2009. These ILIs could be caused by different viruses, as seen by Follin et al. (*20*). Follin et al. reported that, although 5% of the 70 passengers examined in their study had pandemic (H1N1) 2009, rhinovirus, coronavirus, influenza B, and parainfluenza were also detected.

Contact tracing and implementation of public health intervention measures after in-flight exposure to disease is time and resource intensive (*21*). Southern Hemisphere estimates of the serial interval for pandemic (H1N1) 2009 varied from 1.5 days to 2.9 days (*22*,*23*), yet practicalities associated with disease diagnosis and contact tracing meant that quarantine dates began 1–5 days after flight arrival, thus minimizing opportunities to halt transmission by social isolation or chemoprophylaxis. Although compliance with the current practice of following up all passengers in the same row as and within 2 rows either side of the index passenger (*11*) was similar to a recent survey from Switzerland of air travelers in Europe (*24*), the increased risk of contracting disease as found in the current study would suggest that further limiting of the zone required for contacting exposed passengers could assist in efficient yet effective public health outcomes. Furthermore, use of risk assessment of different diseases would enable implementation of a public health response that would be proportionate to potential disease severity.

Pandemic (H1N1) 2009 and other ILIs can be spread to a community by passengers who were symptomatic before boarding the aircraft. Four of 9 of passengers in whom pandemic (H1N1) 2009 was diagnosed displayed symptoms preflight. This finding is similar to that in a recent study looking at the travel patterns of patients with pandemic (H1N1) 2009 reported from Singapore, where 25% of patients had symptoms before boarding their flights (*14*). Modeling predicting the global dynamics of disease spread and evidence obtained during the grounding of flights in the United States after September 11, 2001, demonstrated that travel restrictions can delay the intercity spread of influenza (*25*). Further modeling has shown that the intervention by preventing symptomatic passengers from boarding flights, particularly at airports considered major hubs, assisted in delaying influenza spread by up to 2 weeks (*26*). The control measure of exit screening, combined with the potential value of deterring passengers

from travel, also efficiently restricts the spread of other respiratory illnesses (*27*).

Potential limitations in this study include lack of knowledge of the health status of passengers who did not return the survey or inform health authorities of ILI symptoms after flight arrival, response bias resulting from contact with authorities after flight arrival, recall bias caused by the length of time between flight arrival and survey response; and potential for contracting an ILI postarrival from a source other than the flight. Cross-checking of data collected by local health authorities at the time of flight arrival showed no recall bias. Response levels from passengers contacted by health authorities were higher than those not contacted, thereby limiting response bias. The spectrum of signs and symptoms of passengers contracting ILI or pandemic (H1N1) 2009 varies; therefore, if a passenger did not return the survey or contact medical personnel or health authorities after the flight, some cases may have been missed (*28*). Media coverage of the arrival of flight 1 requesting passengers with ILI to contact health authorities was substantial. Although many passengers may be assumed to have then sought medical advice, the number of passengers who were not tested but reported ILI symptoms on their survey indicated this assumption was incorrect. Contracting pandemic (H1N1) 2009 postflight from a source in the community was unlikely. There was a small chance of contracting the disease preflight because of community transmission for flight 1; however, flight 2 originated from an area with no documented community transmission. At the time of investigation, community transmission of pandemic (H1N1) 2009 was not documented at the arrival port. The likelihood of community transmission is also low because all passengers with confirmed pandemic (H1N1) 2009 had symptom onset date within 48 hours after flight arrival.

Spread of pandemic (H1N1) 2009 and other ILIs occurred in limited zones of the aircraft during international flights into Australia during May 2009. The time required to contact passengers postflight resulted in the potential spread of disease into the community despite guidelines and policies in place to reduce the risk for disease importation. Nonetheless, application of these policies by Australian authorities may have assisted in delaying the importation of identified pandemic (H1N1) 2009 cases during the first month of the recent pandemic. The findings of this investigation suggest that efforts to prevent importation of respiratory diseases into a community and protection of individuals from in-flight exposure to ILI may require changes in international policies of both exit screening of symptomatic passengers preflight and contact tracing of those exposed to an ILI inflight. Further research on transmission of ILI in aircraft and into the effects of exit screening at international airport hubs to restrict travel of passengers with symptoms before flying would be of particular interest for respiratory disease of greater severity than pandemic (H1N1) 2009.

Acknowledgments

We thank the states and territories for provision of data from the NetEpi Database. We also thank Jeremy McAnulty, Paula Spokes, and Kate Ward for critical comment on the manuscript.

P.M.K. is funded by Australia's National Health and Medical Research Council.

Dr Foxwell is a scholar of the Master of Applied Epidemiology program, which is funded by the Australian Government Department of Health and Ageing. Her research interests include surveillance and response to all-hazard acute public health events, particularly developing risk assessment strategies for use in the acute phase of response to an event.

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Hantavirus Pulmonary Syndrome, United States, 1993–2009

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Release date: June 27, 2011; Expiration date: June 27, 2012

Learning Objectives

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

- Evaluate the epidemiology of hantavirus infection
- Distinguish the region in the United States with the highest prevalence of hantavirus pulmonary syndrome (HPS)
- Analyze the prognosis of HPS
- Identify factors associated with a higher risk for mortality in cases of HPS.

Editor

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

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Disclosures: ***Adam MacNeil, PhD, MPH; Thomas G. Ksiazek, DVM, PhD;*** *and* ***Pierre E. Rollin, MD,*** *have disclosed no relevant financial relationships.*

Hantavirus pulmonary syndrome (HPS) is a severe respiratory illness identified in 1993. Since its identification, the Centers for Disease Control and Prevention has obtained standardized information about and maintained a registry of all laboratory-confirmed HPS cases in the United States. During 1993–2009, a total of 510 HPS cases were identified. Case counts have varied from 11 to 48 per year (case-fatality rate 35%). However, there were no trends suggesting increasing or decreasing case counts or fatality rates. Although cases were reported in 30 states, most cases occurred in the western half of the country; annual case counts varied most in the southwestern United States. Increased hematocrits, leukocyte counts, and creatinine levels were more common in HPS case-patients who died. HPS is a severe disease with a high case-fatality rate, and cases continue to occur. The greatest potential for high annual HPS incidence exists in the southwestern United States.

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DOI: 10.3201/eid1707.101306

In May 1993, a series of cases of an acute illness associated with rapid development of respiratory failure were noted in the Four Corners region of the United States. Surveillance initiated in the area identified 24 cases of

compatible illness that had occurred in New Mexico, Arizona, Colorado, and Utah since December 1992; the case-fatality rate was 50%. Preliminary serologic data for case-patients suggested infection with an unknown virus in the family *Bunyaviridae* and genus *Hantavirus* (*1*). This observation was surprising, given that hantaviruses had not been associated with any human diseases in North or South America at that time, and the only known clinical syndrome associated with hantaviruses, hemorrhagic fever with renal syndrome, did not have a predominantly respiratory involvement. However, nucleic acid sequence from a novel hantavirus was rapidly identified in tissue samples of multiple patients, and similarly from deer mice (*Peromyscus maniculatus*) trapped near the residence of cases, implicating a novel hantavirus as the cause of the disease (*2*). Additional serologic and molecular data from case-patients and results of trapping studies in the Four Corners region supported these conclusions (*3*,*4*).

Since its identification in 1993, hantavirus cardiopulmonary syndrome (HPS) and numerous New World hantavirus species have been described across a wide geographic range of North, Central, and South America (*5*). In the United States, most HPS cases are likely caused by Sin Nombre virus (*6*), the virus responsible for the initially identified HPS cases. Other HPS-associated viruses include New York and Monongahela viruses (mice of the genus *Peromyscus* are reservoirs), associated with HPS in the eastern United States (*7*–*9*), Bayou virus, found in the southeastern United States (*Oligoryzomys palustris* rice rats are reservoirs) (*10*–*12*), and Black Creek Canal virus (*Sigmodon hispidus* cotton rats are reservoirs), which was associated with 1 case of HPS in Florida (*13*,*14*).

Hantaviruses are believed to be transmitted by inhalation of rodent secretions and excreta, or possibly through direct contact with an infected rodent. Although clusters of human cases have been identified in the United States, no evidence exists of human-to-human or nosocomial transmission of hantaviruses in North America (*15*,*16*) Infrequent but clear instances of human-to-human transmission of Andes virus in Argentina and Chile have been documented (*17*–*19*).

The incubation period of HPS is believed to range from 1 to 5 weeks (*20*). HPS typically begins with a prodromal syndrome, and common symptoms include fever, myalgias, headache, and nausea/vomiting (*21*,*22*). After the prodrome, the hallmark of HPS is rapid onset of a severe pulmonary illness, often involving hypoxia, pulmonary edema, and myocardial depression (*22*–*25*). Death typically occurs rapidly after hospitalization (*21*) and often as the result of cardiogenic shock (*25*). In this report, we evaluate the epidemiologic and clinical characteristics of all known laboratory-confirmed cases of HPS in the United States during 1993–2009.

Materials and Methods

After identification of HPS in 1993, the Viral Special Pathogens Branch at the Centers for Disease Control and Prevention (Atlanta, GA, USA) developed and maintained a registry of confirmed HPS cases in the United States. A clinically confirmed case of HPS is defined as 1) a febrile illness characterized by bilateral diffuse interstitial edema that may radiographically resemble acute respiratory distress syndrome (ARDS), with respiratory compromise requiring supplemental oxygen developing ≤72 hours after hospitalization, and occurring in a previously healthy person, or an unexplained respiratory illness resulting in death, with an autopsy examination demonstrating pulmonary noncardiogenic edema without an identifiable cause; and 2) laboratory evidence of hantavirus infection by detection of hantavirus-specific immunoglobulin M or increasing titers of hantavirus-specific immunoglobulin G, detection of hantavirus-specific RNA sequence by PCR in clinical specimens, or detection of hantavirus antigen by immunohistochemical analysis (*26*).

Information for the registry, including demographic, geographic, outcome, and (if possible) basic clinical data, is obtained by using a case-report form. Although many laboratory diagnoses are not made at the Centers for Disease Control and Prevention, case-report forms were reviewed to verify hantavirus laboratory diagnostics. Additionally, since 1995, HPS has been a nationally reportable disease in the United States; thus, parallel surveillance for HPS is conducted through the National Notifiable Diseases Surveillance System (*26*). To ensure completeness of the registry, we attempted to acquire case-report forms for all HPS cases reported to the National Notifiable Diseases Surveillance System. For all cases, we attempted to acquire qualitative data (yes or no) regarding certain clinical signs and symptoms, and laboratory values (fever, thrombocytopenia, increased hematocrit, increased creatinine levels, leukocyte counts, chest radiograph showing unexplained bilateral infiltrates or suggestive of ARDS, requirement of supplemental oxygen, and whether the patient was intubated). For a small number of patients for whom incomplete qualitative data were available, but for whom laboratory values were available, we assigned qualitative values on the basis of described clinical cutoff values (*24*).

Results

Number of HPS Cases and Demographics

During 1993–2009, we identified 510 laboratory-confirmed cases of HPS in the United States; 31 cases that occurred before 1993 had been retrospectively identified (*27*–*29*). HPS is primarily a disease of adults; most cases were in persons 20–50 years of age (mean age 38 years)

(Table 1); 7% of cases occurred in children ≤16 years of age. Among HPS cases, 64% occurred in male patients. Most cases occurred in white (78%) or American Indian/Native American (20%) persons, and 21% of case-patients reported their ethnicity as Hispanic.

Temporal and Geographic Characteristics of Cases

The largest annual number of HPS cases registered (n = 48) occurred in 1993 during the initial investigation. Since that time, annual case counts have varied considerably from year to year (Figure 1); counts have ranged from 11 to 45 cases/year (mean 30 cases/year). No significant trend was observed in increasing or decreasing case counts from after 1993 (p = 0.400, by general linear model). From a population perspective, HPS is rare in the United States; the annual incidence has ranged from 0.04 to 0.19 cases/million persons on the basis of a US Census Bureau estimate of the US population on July 1 of each respective year (www.census.gov). HPS displayed a strong seasonal distribution; the maximum number of cases occurred in May, June, and July, and the minimum occurred in December, January, and February (Figure 2).

We identified the probable geographic location of rodent exposure (at least to the state level) for 471 cases. Probable exposures occurred in 30 US states; most cases occurred in the western United States To examine trends in HPS occurrence, we grouped states into 4 regions on the basis of geography and hantavirus species

Table 1. Demographic characteristics of HPS case-patients, United States, 1993–2009*

Characteristic	No. (%) cases	Outcome	
		CFR, %	p value†
Age, y, n = 508			0.992
<10	5 (1)	20	
10–19	56 (11)	36	
20–29	116 (23)	36	
30–39	105 (21)	37	
40–49	101 (20)	33	
50–59	69 (14)	35	
60–69	48 (9)	33	
≥70	8 (2)	38	
Sex, n = 510			0.180
M	324 (64)	33	
F	186 (36)	39	
Race, n = 492			0.284
White	383 (78)	33	
American Indian or Native American	96 (20)	43	
Black	8 (2)	38	
Asian or Pacific Islander	5 (1)	60	
Ethnicity, n = 393			0.637
Non-Hispanic	312 (79)	37	
Hispanic	81 (21)	40	

*HPS, hantavirus pulmonary syndrome; CFR, case-fatality rate.
†χ^2 p value for testing the difference in proportion of HPS case-patients who died between categories for each demographic characteristic.

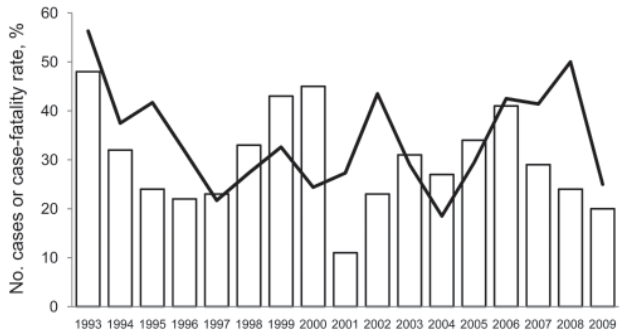

Figure 1. Annual number of cases (bars) of and case-fatality rate (line) for hantavirus pulmonary syndrome, United States, 1993–2009.

present: Southwest (Arizona, California, Colorado, New Mexico, Nevada, Utah); Northwest (Idaho, Montana, Oregon, Washington, and Wyoming); Midwest (Illinois, Indiana, Iowa, Kansas, Louisiana, Minnesota, North Dakota, Nebraska, Oklahoma, South Dakota, Texas, and Wisconsin); and East (Florida, Maryland, North Carolina, New York, Pennsylvania, Virginia, and West Virginia) (Table 2).

We assessed temporal trends in HPS for each of these regions (with the exception of the East because there were only 12 cases) (Figure 3, panel A). Overall case counts were relatively stable across the 17-year period in the Northwest (mean ± SD 5.9 ± 2.1 cases/year) and Midwest (mean ± SD 4.9 ± 2.8 cases/year). In contrast, case counts in the Southwest tended to fluctuate to a higher degree (mean ± SD 16.1 ± 9.8 cases/year). Overall variance in annual case counts was significantly higher in the Southwest than in the Northwest (F-statistic p<0.001) or the Midwest (F-statistic p<0.001). When combined with annual HPS case counts for the entire United States, peaks in HPS case counts in the Southwest corresponded directly with peaks for the entire country. This finding, in conjunction with stable case counts in the Northwest and Midwest, demonstrates that annual variability in HPS in the United States is primarily driven by fluctuations in number of HPS cases in the southwestern United States.

We assessed seasonality of HPS by geographic region (Figure 3, panel B). Similar to aggregate trends for the entire United States, HPS displayed a clear seasonal trend in the Midwest, Northwest, and Southwest. In contrast to the Midwest and Northwest, in which the highest proportion of cases occurred in May and decreased in the summer months, HPS cases peaked 2 months later (in July) in the Southwest.

Clinical Characteristics and Case-Fatality Rates

The overall case-fatality rate was 35%. Deaths varied noticeably from year to year (Figure 1); however,

Figure 2. Cumulative number of hantavirus pulmonary syndrome cases by month of onset, United States, 1993–2009.

no temporal trend in deaths was observed (p = 0.307, by Cochran-Armitage trend test). Additionally, case-fatality rates were similar across demographic characteristics; no differences in case-fatality rates were noted for age groups, or by sex, race, or ethnicity (Table 1). Similarly, from a geographic standpoint, case-fatality rates did not differ between geographic regions (p = 0.773, by χ^2 test) (Table 2). The mean time from onset of symptoms to death was 6.4 days (median 5 days).

As described (*21*,*22*), HPS case-patients had a severe respiratory illness (most persons had chest radiographs showing unexplained bilateral infiltrates or suggestive of ARDS, and required supplemental oxygen) and thrombocytopenia (Table 3). Other common findings included fever >101°F and increased hematocrits, creatinine levels, and leukocyte counts. Increased hematocrits, creatinine levels, and leukocyte counts; requirement for supplemental oxygen; and necessity for intubation were all associated with death of a patient. Additionally, although nearly all HPS case-patients had thrombocytopenia, platelet counts (lowest measured value during illness) were significantly lower among patients who died than among patients who survived (median platelet count in persons who died 33,500 cells/mL vs. median in persons who survived 51,500 cells/mL; p<0.001 by Wilcoxon rank-sum test), for 278 persons for whom data were available.

Discussion

In 1996, Khan et al. published a description of the first 100 cases of HPS identified in the United States (*21*). Some aspects of the epidemiology of HPS in the United States have since been discussed in the peer-reviewed literature. However, no studies have provided a comprehensive evaluation of the epidemiology of HPS in the United States. By maintaining a registry and obtaining information in standardized manner, we were able to evaluate the epidemiologic characteristics of HPS in >500 cases over 17 years of data collection. Although HPS is a nationally reportable disease in the United States, maintenance of our registry has enabled us to obtain more detailed and standardized information on HPS than otherwise possible through other national surveillance mechanisms.

HPS is often characterized as an emerging infectious disease. The discovery of a novel clinical syndrome and associated virus might constitute emergence from a public health perspective. However, several lines of evidence indicate the epidemiology of HPS constitutes that of an endemic or sporadic disease. First, although overall numbers clearly vary from year to year, our data demonstrate continual occurrence of HPS since 1993 without a trend toward an increasing or decreasing number of cases. Additionally, HPS cases before 1993 in the United States (*27*–*29*), possibly as far back as 1959 (*30*), and rodents infected with hantaviruses (*6*,*31*) have been identified retrospectively. Similarly, the evolutionary history of hantavirus species in the United States appears to have been closely linked with that of their primary rodent host species and to persist over time among these host species (*32*), consistent with the notion that hantaviruses are not newly emergent in the United States. Finally, HPS and wide distribution of associated hantavirus species across most of the New World do not support recent emergence of a novel pathogenic virus.

Table 2. HPS case-patients by geographic region, United States, 1993–2009*

Region	States†	Hantavirus species present in region	No. case-patients who died/ total no. case-patients (CFR, %)‡
Southwest	Arizona, California, Colorado, New Mexico, Nevada, Utah	Sin Nombre virus	92/273 (34)
Northwest	Idaho, Montana, Oregon, Washington, Wyoming	Sin Nombre virus	35/101 (35)
Midwest	Illinois, Indiana, Iowa, Kansas, Louisiana, Minnesota, North Dakota, Nebraska, Oklahoma, South Dakota, Texas, Wisconsin	Sin Nombre virus, Bayou virus	33/84 (39)
East	Florida, Maryland, North Carolina, New York, Pennsylvania, Virginia, West Virginia	New York virus, Monongahela virus, Black Creek Canal virus	5/12 (43)

*HPS, hantavirus pulmonary syndrome; CFR, case-fatality rate.
†Only states with ≥1 probable rodent exposure–related HPS case are shown.
‡There were no significant differences in case-fatality rates between geographic regions (p = 0.773 by χ^2 test).

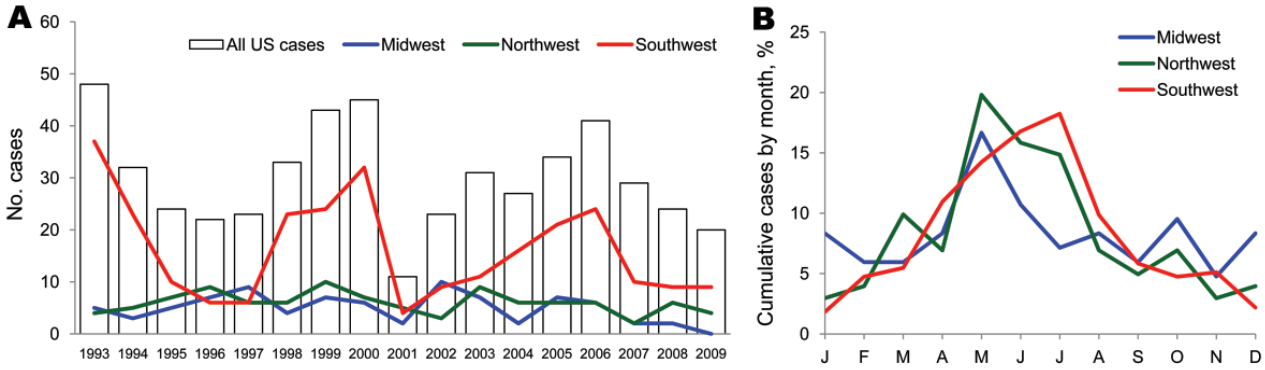

Figure 3. Annual number of cases of hantavirus pulmonary syndrome (HPS) (A) and percentage of cases by month of onset (B) by geographic region of probable HPS exposure, United States, 1993–2009.

The distribution of rodent reservoirs of pathogenic hantaviruses covers the entire mainland United States (*33*). During 1993–2009, HPS cases were associated with probable rodent exposures in 30 US states. However, in contrast to the wide distribution of rodent reservoirs, HPS is clearly more common in the western United States; only a small proportion (<3%) of cases are associated with exposures in the eastern United States. Although the virus species responsible for HPS is not typically assessed in diagnostic testing, it is likely that most cases of HPS in the United States are caused by Sin Nombre virus because of the western distribution of the reservoir host of this virus, the deer mouse, in comparison with reservoir hosts of other pathogenic hantavirus species, which are found primarily in the central and eastern United States.

Our examination of the epidemiology of HPS on the basis of geographic region has obvious limitations. For instance, state boundaries do not necessarily represent boundaries of ecosystems or distribution of reservoirs of different pathogenic hantavirus species. We noted major conclusions from this approach. First, we examined the hypothesis that hantavirus species may differ in their pathogenic potential. Although the overall number of HPS cases in the eastern United States was small (n = 12) and infections were potentially caused by multiple hantavirus species in the East and Midwest regions, our data do not suggest a difference in pathogenicity between hantavirus species endemic to the United States. Furthermore, systematic viral genotyping is needed to conclusively address the hypothesis that hantaviruses in the United States may differ in their pathogenic potential in humans. Second, annual numbers of HPS cases in the Northwest and Midwest were relatively consistent, whereas annual HPS case counts in the Southwest were significantly more variable, and peak years of HPS in the Southwest corresponded with high overall case-count years in the United States. These findings suggest greater potential for increases in HPS in the southwestern United States than in other regions of the country.

The HPS case-fatality rate in the United States was 35% during 1993–2009. No antiviral treatment is available for HPS, and we did not observe a trend in the case-fatality rate for HPS over time. No demographic factors were associated with deaths caused by HPS outcomes. The apparent rarity of HPS in younger persons is notable. However, similar case-fatality rates for HPS across age groups and results of case

Table 3. Clinical characteristics of HPS case-patients, United States, 1993–2009*

Characteristic	No. patients reporting characteristic/no. patients for whom data were available for that characteristic (%)			p value†
	All	Died	Survived	
Fever	356/397 (90)	116/132 (88)	239/264 (91)	0.414
Thrombocytopenia‡	405/418 (97)	131/134 (98)	273/283 (96)	0.477
Increased hematocrit‡	261/389 (67)	103/130 (79)	157/258 (61)	<0.001
Increased creatinine level‡	120/286 (42)	54/93 (58)	66/193 (34)	<0.001
Increased leukocyte count‡	169/234 (72)	72/83 (87)	96/150 (64)	<0.001
Chest radiograph showing unexplained bilateral infiltrates or suggestive of ARDS	390/406 (96)	135/141 (96)	255/265 (96)	0.812
Need for supplemental oxygen	390/405 (96)	134/135 (99)	255/269 (95)	0.025
Intubation of patient	265/430 (62)	129/139 (93)	135/290 (47)	<0.001

*HPS, hantavirus pulmonary syndrome; ARDS, acute respiratory distress syndrome.
†By χ^2 test for difference in the proportion of HPS case-patients with a clinical characteristic who died and those who survived.
‡Based on qualitative data (yes or no) regarding clinical characteristic from case investigation form. For a small number of case-patients for whom qualitati incomplete, but for whom laboratory values were available, qualitative values were assigned on the basis of described clinical cutoff values (*24*).

studies of HPS in children (*34*,*35*) indicate that severity of HPS is likely similar in adults and younger persons. We have limited data about the relationship between concurrent conditions and HPS outcome. However, similar case-fatality rates for HPS across age groups (particularly that the case-fatality rate remains similar in older persons) does not support the notion that underlying health conditions are the primary determinant of disease outcome. The actual level of virus exposure at the time of infection may also be a major determinant of disease severity. A recent study reported smoking as a significant risk factor for Puumala virus (genera *Hantavirus*) infection in Finland (*36*). We believe this finding warrants a study in the United States to determine whether smoking might increase the likelihood of development or the overall severity of HPS.

HPS is characterized by the rapid onset of a severe respiratory disease. Virtually all patients with laboratory-confirmed HPS in our registry for whom clinical data were available required supplemental oxygen (96%) and had a chest radiograph showing unexplained bilateral infiltrates or suggestive of ARDS (96%). Although thrombocytopenia is a common symptom of HPS, lowest platelet counts were lower in fatal HPS cases. Similarly, increased hematocrits, creatinine levels, and leukocyte counts occurred in a higher proportion in HPS case-patients who died. Although similar clinical findings have been reported for smaller case studies (*21*–*24*,*37*), our data demonstrate the role of these factors in predicting the outcome of HPS. We also noted similar associations between outcomes in patients who died and the requirement for supplementary oxygen and intubation. These 2 variables represent clinical procedures and thus would be expected to be more common in severe HPS cases. Although we do not have any data about the proportion of case-patients who received extracorpeal membrane oxygenation, some studies suggest that this procedure might improve the prognosis for severe HPS (*38*,*39*).

Because of the centralized and passive nature of data collection, our methods have some limitations. Data collection was limited to a short, standardized case investigation form; thus, we were unable to collect detailed clinical information and verify clinical information (such as radiographic findings). In addition, clinical aspects of our surveillance data were limited to a small number of specific criteria. Other investigators have reported signs, symptoms, radiographic characteristics, and pathologic features of HPS in greater detail (*21*–*24*,*40*). Studies have also demonstrated cardiopulmonary depression, and resulting cardiogenic shock, as a major pathologic aspect of HPS, particularly in patients who died (*25*). In addition, because of the passive nature of HPS surveillance, we may have missed some HPS cases in the United States. However, through continued outreach with state health departments and the ability to cross-check HPS cases with those reported through the National Notifiable Diseases Surveillance System, we have attempted to minimize the number of HPS cases that might go unregistered. However, frequently updated HPS case counts and geographic data are available (www.cdc.gov/hantavirus).

Despite its rarity, HPS continues to occur in the United States. With a case-fatality rate of 35%, HPS remains 1 of the most severe infectious diseases endemic to the United States.

Acknowledgments

We thank state health and local health department personnel for assisting in collection of data in the HPS registry; coordinators, laboratorians, epidemiologists, and Epidemic Intelligence Service Officers at the Viral Special Pathogens Branch, Centers for Disease Control and Prevention, for helping to maintain this registry since 1993; Arie Manangan for assisting in geographic classification of cases and development of map-based graphics; Timothy Fliestra for statistical assistance; James N. Mills for helpful discussions; and Stuart T. Nichol for support.

This study was supported by the Centers for Disease Control and Prevention.

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Hansen Disease among Micronesian and Marshallese Persons Living in the United States

Patricia Woodall, David Scollard, and Latha Rajan

An increasing proportion of Hansen disease cases in the United States occurs among migrants from the Micronesian region, where leprosy prevalence is high. We abstracted surveillance and clinical records of the National Hansen's Disease Program to determine geographic, demographic, and clinical patterns. Since 2004, 13% of US cases have occurred in this migrant population. Although Hawaii reported the most cases, reports have increased in the central and southern states. Multibacillary disease in men predominates on the US mainland. Of 49 patients for whom clinical data were available, 37 (75%) had leprosy reaction, neuropathy, or other complications; 17 (37%) of 46 completed treatment. Comparison of data from the US mainland with Hawaii and country of origin suggests under-detection of cases in pediatric and female patients and with paucibacillary disease in the United States. Increased case finding and management, and avoidance of leprosy-labeled stigma, is needed for this population.

At 11 cases per 10,000 population and 8 per 10,000, respectively, in 2007, the small Pacific Island nations of the Republic of the Marshall Islands and the Federated States of Micronesia have the highest prevalence of Hansen disease (HD), i.e., leprosy, in the world and have made little progress in the past decade toward the World Health Organization (WHO) leprosy elimination target of <1 per 10,000 (*1*,*2*). During the first quarter of 2010, 33 new cases were detected among the 54,000 residents of the Marshall Islands (*3*).

HD has been present in the United States for more than a century; the number of patients has remained relatively constant at 150–200 per year (*4*).The US National Hansen's Disease Program (NHDP) has noted an increasing number of cases among US-resident Marshall Islanders and Micronesians, including several persons with advanced disease. In 1996, the Hawaii HD program reported a cluster involving 16 (5%) of 321 persons screened from a Marshallese migrant community (*5*,*6*). In 2002, the US Army noted 3 cases in 1 month in soldiers from this region (*7*). The recent reporting of multiple cases among the Marshallese community in northwestern Arkansas (Centers for Disease Control and Prevention, unpub. data, 2006) has drawn attention in a region unaccustomed to leprosy, with its stigmatizing historical connotations (*8*,*9*).

Under the terms of the Compacts of Free Association (the legal documents governing the relationships between the United States, Federated States of Micronesia, and the Republic of the Marshall Islands), citizens of this former US Trust Territory of the Pacific Islands are not subject to usual immigration requirements but may freely enter, reside, and work in the United States for as long as they wish. They hold a unique legal status, are not classified as immigrants, and maintain their country-of-origin citizenship. Transportation data indicate net emigration of an average of 952 Marshallese and 1,443 Micronesians annually, with a total of 38,325 emigrants for 1991–2006; almost all of these persons are thought to have immigrated to the United States and its territories (*10*). The actual distribution of this population within the United States is unknown; a specific category included in the 2010 US Census should provide this information. As economic and climatologic pressure drive increasing emigration from this HD-endemic former US Trust Territory, the US HD case load is expected to continue to increase, worsening health disparities and requiring increased program and local resources, although this increased case load is unlikely to create a public health

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DOI: 10.3201/eid1707.102036

threat of transmission to the general population. Cultural and socioeconomic issues may affect case detection and long-term disease management in this population, including adherence to and completion of therapy.

The objective of this report is to describe, on the basis of secondary analysis of existing program data, the epidemiology of HD among Marshallese and Micronesian persons residing in the United States. The intent is to assist in providing resources to address a health disparity that disproportionately affects a group of a particular national origin, while at the same time avoiding worsening of ethnic and disease-related stigma.

Materials and Methods

Demographic and disease-related data were abstracted for January 1990–October 2009 NHDP surveillance and clinical records of cases that identified patients' place of birth as the former Trust Territory (Marshall Islands or Micronesia). To facilitate global comparison, US cases (reported by using the Ridley-Jopling classification system [*11*]) were reclassified into the WHO multibacillary/paucibacillary system (*12*). Country-of-origin HD data (case numbers or rates and age/sex/disease classification) were abstracted from WHO reports (*1*,*2*,*13*). Country-of-origin demographic data, US Census reports, and published reports relevant to migration patterns were abstracted to obtain denominator estimates where possible (*14*–*17*). Data were analyzed by using the SPSS version 16.0 statistical analysis program (SPSS Inc., Chicago, IL, USA). The study was approved by the Tulane University Institutional Review Board and exempted from review by the University of Arkansas for Health Sciences Institutional Review Board.

Results

The number of HD case-patients of Micronesian or Marshallese origin as a proportion of all US cases has risen over the past decade, with Micronesian and Marshallese patients constituting 90 (13%) of all 686 cases in the United States during 2004–2008 (Figure 1); the total source population of these 2 countries is only ≈170,000. Data are summarized in Table 1.

During January 2000–October 2009, 72 (55%) of US HD cases in Micronesian or Marshallese patients were reported in Hawaii. Of 29 Micronesian patients, 22 (76%) had multibacillary HD, 5 were female, and 3 were children. Of 43 Marshallese patients, 31 (72%) had multibacillary HD, 17 (17%) were female, and 1 was a child. Cases in Hawaii were not consistently reported to NHDP until the late 1990s; however, Bomgaars et al. reported that HD cases in 15 Micronesian and 23 Marshallese patients were noted there from 1990 through mid-1998, including the Marshallese cluster summarized in Table 2 (*6*).

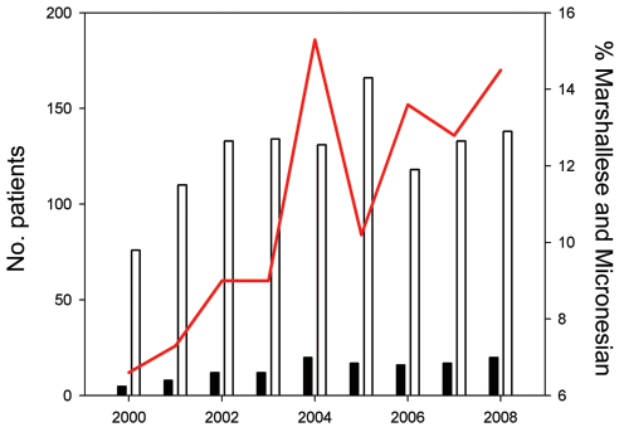

Figure 1. New patients of Marshallese or Micronesian origin with Hansen disease, compared with total new US patients with Hansen disease, 2000–2008. White bars, total US patients; black bars, total patients of Marshallese or Micronesian origin; red line, patients of Marshallese or Micronesian origin as percentage of total US patients.

Since 1990, 88 cases have been reported in 26 states of the US mainland (Figures 2, 3), 66 of these since 2000. Cases are increasingly being reported in inland locations. In all groups, the majority of patients were young men with multibacillary disease.

Among Micronesians, 55 cases occurred in 22 states of the US mainland (Table 1), 41 of these since 2000. All but 2 patients were <40 years of age, 42 (76%) had multibacillary disease, 10 (18%) were female, and 6 (10%) were children. Six patients reported having a previous HD diagnosis in the Federated States of Micronesia; 4 of these patients reported having completed the WHO-recommended 1 year of multidrug therapy (MDT) there. Of these patients, 14 (25%) originated in Chuuk State and 13 (24%) in Pohnpei State; for 28 (51%), the specific Federated State was not documented. During the 1990s, 11 of 14 US-mainland HD cases were reported in the western coastal states of California, Washington, and Oregon. Since 2000, a total of 28 of 41 cases were reported in midwestern and southern states (e.g., 6 in Iowa, 5 each in Texas and Florida, 3 in Missouri). Before moving to the reporting state, 21 patients had resided in other mainland states (often in multiple locations), 5 in Hawaii, and 8 in Guam. Of the 33 patients for whom clinical data were available, 25 (76%) had ≥1 complication, including 15 patients with erythema nodosum leprosum (ENL) and 3 with severe facial involvement (lagophthalmos, blindness, nasopalatal destruction). One patient was pregnant when her HD was diagnosed. Of the 24 patients for whom pharmacy data were available (excluding 8 who had recently begun treatment), the complete US-recommended MDT course had been dispensed to 10 (42%) (Table 1).

Table 1. Hansen disease in residents of Micronesian and Marshallese origin, United States*

Population group	No. (%) patients				Median time from US entry to report, y (range)	MDT,† no. (%)	Complications, no. cases					
	Total	MB	F	Age <15 y			ENL	RR	Hand/foot neuropathy	Eye	Other	Any (%)‡
Hawaii-residing Micronesians, 2000–2009§	29	22 (76)	5 (17)	3 (10)	1.5 (0–13)	NA	NA	NA	NA	NA	NA	NA
Hawaii-residing Marshallese, 2000–2009§	43	31 (72)	17 (39)	1 (2)	1 (0–14)	NA	NA	NA	NA	NA	NA	NA
US Mainland Micronesians, 1990–2009	55	42 (76)	10 (18)	6 (10)	3 (0–10)	10 (42)	15	3	8	6	3¶	25 (77)
US Mainland Marshallese, 1990–2009	33	25 (76)	11 (33)	3 (10)	2 (0–12)	7 (32)	6	3	5	1	1#	12 (75)
Arkansas-residing Marshallese, 1990–2009	17	15 (88)	5 (30)	0 (0)	2 (0–5)	3 (20)	4	2	2	0	0	12 (70)

*Data from National Hansen's Disease Program surveillance and clinical records, January 1990–October 2009. MB, multibacillary; MDT, multidrug therapy; ENL, erythema nodosum leprosum; RR, reversal reaction; NA, data not available.
†MDT completed, excluding patients currently under active treatment.
‡Patients for whom clinical data were available.
§Pre-2000 data were not reported to National Hansen's Disease Program.
¶1 renal, 1 testicular, 1 palatal.
#1 renal.

Of the 33 US mainland Marshallese patients since 1990 (25 of these since 2000), single cases were reported in 9 states, 2 in California, 5 in Washington, and 17 in Arkansas. All but 1 patient were <40 years of age, 25 (76%) had multibacillary disease, 11 (33%) were female, and 3 (10%) were children. Three patients reported that their HD had been diagnosed in the Marshall Islands; none had completed MDT there. Nine patients had resided in other mainland states before moving to the reporting state; 5 had first lived in Hawaii. Of the 16 patients for whom clinical data were available, 12 had ≥1 complication, including 6 patients with ENL. Two patients were pregnant at the time of diagnosis. Of the 22 patients for whom pharmacy data were available (excluding 3 currently being treated), the complete US-recommended MDT course had been dispensed to 7 (32%) (Table 1).

Beginning in 1996, 17 HD cases have been reported among the ≈8,000–10,000 Marshallese living in northwestern Arkansas, 10 of these since 2004. All patients were <40 years of age, 15 (88%) had multibacillary disease, 5 (30%) were female, and none were children. (Two 25-year-old men with multibacillary disease, reported in November 2009 after completion of data collection for this study, are not included in this analysis.) Four patients had previously lived in other states, none in Hawaii. Specific island origin within the Marshall Islands was not reported; however, the general Arkansas Marshallese population is known to come from several different atolls. Of the 10 patients for whom clinical data were available, 7 had ≥1 complication, including 4 patients with ENL. Excluding the 2 patients currently being treated, the US-recommended MDT course had been dispensed to 3 (20%) (Table 1).

HD prevalence reported in the countries of origin is summarized in Figures 4 and 5. Because of the epidemiologic characteristics of HD (e.g., lack of biomarker, prolonged latency), surveillance is generally based on program records rather than population surveys; prevalence and case-detection rate (an approximation of incidence) are highly dependent on operational factors (Figure 5). Active case-detection programs were done in the Federated States of Micronesia in 1996 and 2005 and in the Marshall Islands in 1996 and 1998–1999 (*18–20*). Patterns of age/sex/classification distribution differed markedly during these years, with more children and paucibacillary disease

Table 2. Hansen disease patient data for multibacillary disease status, female sex, and age <15 y, as reported by sources other than the US National Hansen's Disease Program*

Data source	Multibacillary disease, %	Female sex, %	Age <15 y
Federated States of Micronesia national data, 2000–2007†	44	35	34
Federated States of Micronesia Leprosy Elimination Campaign, 2005‡	30	36	32
Republic of the Marshall Islands national data, 2000–2007†	55	42	23
Republic of the Marshall Islands Leprosy Elimination Campaign, 1999‡	31	NA	35
Hawaii Marshallese cluster, 1998‡	28	NA	39

*NA, data not available.
†World Health Organization annual reports (national surveillance).
‡Published reports from active case-finding campaigns (*5*,*13*,*18–20*).

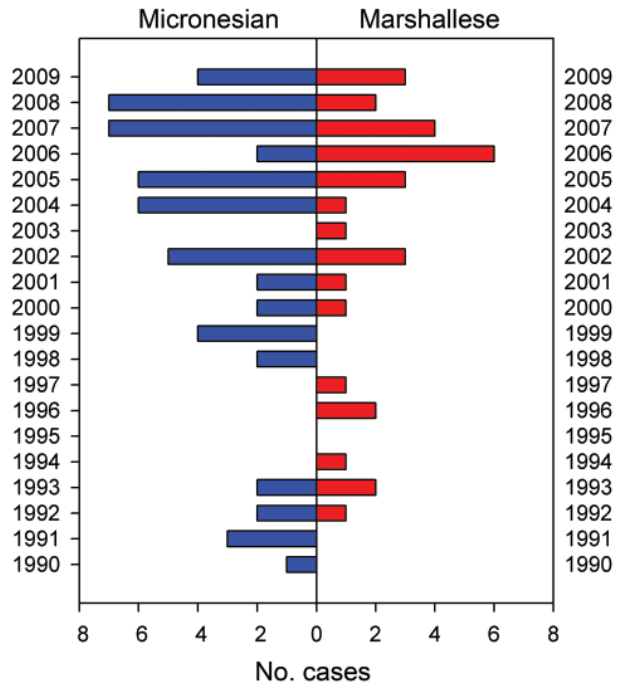

Figure 2. Hansen disease in patients of Marshallese or Micronesian origin, US mainland, by year reported, January 1990–October 2009.

being identified than during the usual passive surveillance years (Table 2); this pattern was also noted in the 1998 Marshallese cluster in Hawaii.

Discussion

This study was descriptive; thus, comparisons among the various population subgroups are not statistically valid because of small numbers, varied data sources, and unknown age/sex distribution and denominators for the overall migrant populations. However, because age and sex distributions for the Arkansas, Hawaii, and Republic of the Marshall Islands Marshallese populations are similar (*17*), some crude comparisons can be made for these groups. Unless an unidentified confounder is present, rates and distribution of cases would be expected to be similar in the 3 groups.

The Arkansas and Hawaii Marshallese populations were approximately the same size (3,000 each) and age/sex distribution (median age 20 years, M:F = 1) in 1998–2001; rough estimates place the current Arkansas population at 8,000–10,000 and Hawaii at 6,000–8,000. However, Hawaii has identified 2.6× more cases since 2000, more female patients, and more paucibacillary disease and has found disease in children. Estimated case detection rates for Arkansas Marshallese persons are approximately half those for the Marshall Islands (Figure 6) and more skewed toward multibacillary disease, male, and adult patients (Table 1). This Arkansas and general US mainland pattern of predominantly multibacillary disease in men does not reflect disease distribution patterns in the countries of origin or in Hawaii, suggesting under-detection of child, female, and paucibacillary disease patients rather than a biologic disease pattern (Tables 1, 2).

Of 49 mainland patients from both the Federated States of Micronesia and the Marshall Islands for whom

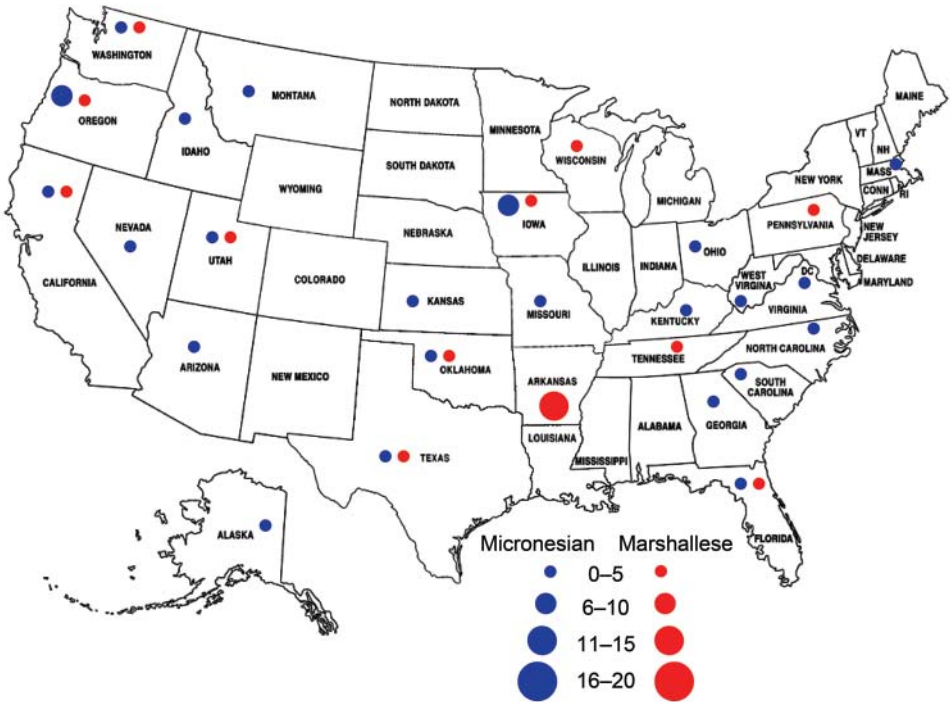

Figure 3. States reporting Hansen disease in patients of Marshallese or Micronesian origin, US mainland, January 1990–October 2009. The number of cases reported from each state is indicated.

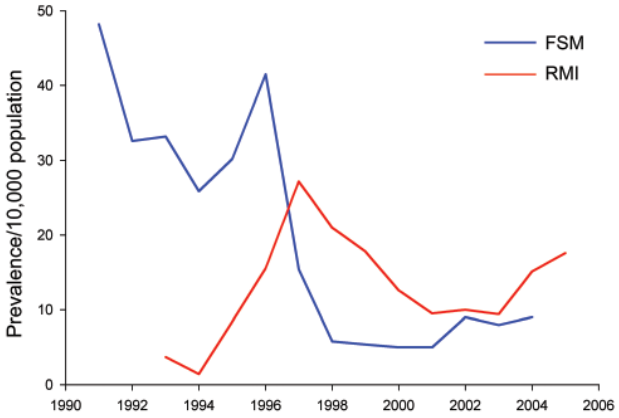

Figure 4. Hansen disease prevalence as cases per 10,000 population, Federated States of Micronesia (FSM) and Republic of the Marshall Islands (RMI) 1991–2005 (*1*,*2*,*13*).

clinical data were available, 37 (75%) had ≥1 complication, such as leprosy reaction, neuropathy, or other tissue involvement (e.g., palatal, renal, testicular). Although this finding could certainly reflect reporting bias, with the most severely affected being more likely to seek care and to be reported, it does indicate the magnitude of the need for treatment resources for this population with limited health care access. The most severe cases of blindness and disfigurement occurred in Micronesians living alone or in very small ethnic communities, limiting the potential impact of ethnic-targeted services. The high number of ENL cases is of note because these patients may require corticosteroid treatment for many years. The low rates of MDT completion may contribute to poor clinical outcomes and possibly to drug resistance and ongoing transmission.

This study has several limitations. Hawaii did not report to the NHDP system until the late 1990s. Cases in Guam and Northern Marianas and among US military personnel are not reported to the NHDP. Reporting is not mandatory in all states but is likely to be fairly complete because of US physicians' need for consultation on management of this unfamiliar condition. Since the 2004 US Food and Drug Administration designation of clofazimine as an investigational drug, any patients with multibacillary disease are likely to have been reported so they would be able to receive the recommended MDT, although paucibacillary disease may be underreported. The increase in case reports since 2004 could reflect this requirement.

Measures of adherence and completion of treatment, complications, and other clinical data were available only for mainland patients receiving direct or pharmacy care from the Baton Rouge NHDP facility. MDT is dispensed directly to active patients or provided to local physicians or health departments for supervision of treatment. Although adherence to and completion of treatment are monitored at national program level for HD patients attending clinics funded through contracts with the NHDP, for whom compliance exceeds 90% (D. Scollard, unpub. data), this level of follow-up has not been possible with private physicians, who are the providers for all of the Marshallese and Micronesian patients. In this situation, dispensing of MDT by the NHDP pharmacy was the only treatment measure available; actual adherence to treatment is unknown but is likely to be much lower and could be a subject for future study. A variety of cultural factors are likely to contribute to the low rate of treatment completion in this group, but evaluation of these cultural issues is beyond the scope of this study

Since achievement of the global leprosy elimination target of <1 case per 10,000 persons, attention has waned in some regions in which this neglected tropical disease persists. Although prevalence in the Republic of the Marshall Islands and the Federated States of Micronesia fell during the 1990s, rates have remained stable at high levels for the past decade. In these nonindustrialized nations, leprosy receives low priority in relation to more urgent public health concerns (rising rates of multidrug-resistant tuberculosis, 30% adult prevalence of type 2 diabetes, high infant mortality, and many others). Under the Compacts of Free Association, since the 1986 independence of the Federated States of Micronesia and the Republic of the Marshall Islands most health funding to these former territories flows from the United States. Assistance with leprosy control in the countries of origin would, in addition to improving health conditions there, be the first step toward preventing importation of cases to the United States. Because of the unique status of migrants from these nations to the United States, Micronesian and Marshallese

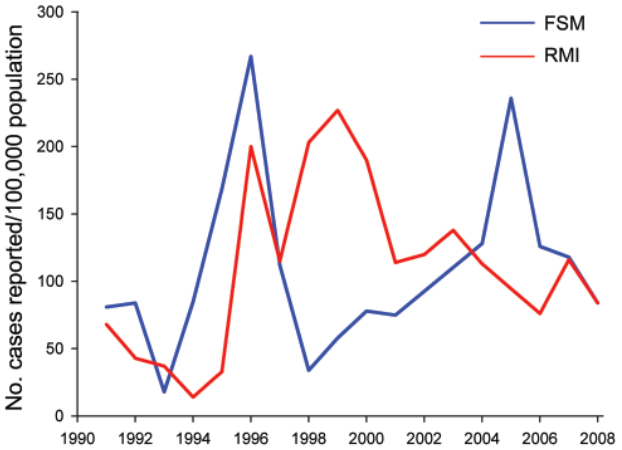

Figure 5. Hansen disease cases per 100,000 population, Federated States of Micronesia (FSM) and Republic of the Marshall Islands (RMI) (*1*,*2*,*13*), 1990–2008.

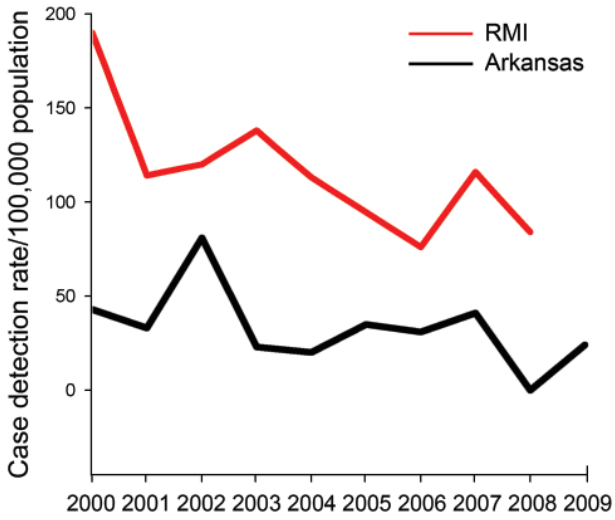

Figure 6. Estimated Hansen disease cases per 100,000 population, Arkansas Marshallese and Republic of the Marshall Islands (RMI), 2000–2009. RMI rates from World Health Organization reports (*1*,*2*,*13*); Arkansas cases from National Hansen's Disease Program records. Arkansas Marshallese population (denominator) derived from US Census estimates (*15*,*17*).

migrants receive neither the health screening required for immigrants (which is not effective at preventing importation of HD because of its decades-long incubation period) nor targeted health services often available to large immigrant or refugee populations. Although legally residing in the United States, they remain citizens of their home nations and are thus ineligible for US health programs such as Medicare and Medicaid, although NHDP services such as biopsy interpretation, MDT, and direct outpatient care at the Baton Rouge facility are available. Many remain uninsured (>50% of the Arkansas Marshallese population) (*17*). Mainland state and local health departments are not typically resourced to serve either this population or this otherwise-rare condition. Hawaii receives more than $15 million annually in Compact impact funds to partially address the multiple health issues of the Marshallese and Micronesian migrant populations, but these additional US funds have not been available to the mainland states. (*21*).

With the goal of decreasing health disparity and preventing disability, case-finding and case-management interventions are needed in US-resident Marshallese and Micronesian communities that are integrated into general health services and avoid the stigma of leprosy-labeled activities. Special efforts may be necessary to increase case detection among women and children. Large populations, such as the Arkansas Marshallese community, may be easier to reach with targeted but nonstigmatizing efforts, as has occurred in Hawaii. The small, widely distributed Micronesian communities, where the most severe, disabling cases have been identified, may be more difficult to reach.

Acknowledgment

We thank the staff of the NHDP for assistance with data collection. We extend special thanks to Jerry Simmons and Kathleen Leonard for assistance in preparation of the figures.

Dr Woodall worked as a US Public Health Service medical officer in the Marshall Islands for several years and completed this work as a Master in Public Health and Tropical Medicine candidate at Tulane University. Her current interest is control of the neglected tropical diseases.

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Epidemiology and Control of Legionellosis, Singapore

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To determine trends and clinical and epidemiologic features of legionellosis in Singapore, we studied cases reported during 2000–2009. During this period, 238 indigenous and 33 imported cases of legionellosis were reported. Cases were reported individually and sporadically throughout each year. Although the annual incidence of indigenous cases had decreased from 0.46 cases per 100,000 population in 2003 to 0.16 cases per 100,000 in 2009, the proportion of imported cases increased correspondingly from 6.2% during 2000–2004 to 27.3% during 2005–2009 ($p<0.0005$). The prevalence of *Legionella* bacteria in cooling towers and water fountains was stable (range 12.1%–15.3%) during 2004–August 2008.

Legionellosis (Legionnaires' disease and Pontiac fever) is an environment-related, acute respiratory infection caused by gram-negative, rod-shaped bacteria of the genus *Legionella*. Pontiac fever is a self-limiting influenza-like syndrome; Legionnaires' disease is more severe, has pneumonia as the predominant clinical finding, and is a potentially fatal illness. Currently, there are 52 *Legionella* species (*1*) and 70 serogroups (*2*). Of these species, 25 species are known to cause human disease (*1*). Most human infections are caused by *Legionella pneumophila* (*3*), and the predominant serogroup is serogroup 1 (*4*). Other species, which together with *L. pneumophila*, account for most human infections include *L. longbeachae* and *L. micdadei*. The mode of transmission of legionellosis is believed to be by inhalation of aerosols (*5*). Other possible modes of transmission such as aspiration of contaminated potable water have also been widely discussed (*6*–*8*).

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DOI: 10.3201/eid1707.101509

Legionellosis was first identified in 1976 during an outbreak of severe pneumonia among delegates to the 1976 American Legion Convention in Philadelphia (*9*). Since then, several outbreaks linked to a variety of aerosol-producing devices, such as cooling towers (*10*–*13*), whirpool spas (*14*), decorative fountains (*15*), mist machines (*16*,*17*) and industrial air scrubbers (*18*), have been reported. The largest outbreak of the disease (449 confirmed cases) was attributed to cooling towers in a city hospital in Murcia, Spain, in July 2001 (*10*).

Risk factors for legionellosis include cigarette smoking, chronic lung disease, and immunosuppression (especially that caused by corticosteroid therapy and organ transplantation) (*8*). Environmental factors such as high humidity and increased rainfall also increase the risk for legionellosis (*19*).

In Singapore, a densely populated, tropical city-state with many high-rise commercial, office, and residential air-conditioned buildings, Legionnaires' disease was recognized as a potential public health threat because environmental surveys showed that cooling towers were heavily colonized by *Legionella* spp. bacteria. A study conducted in 1987 showed that *Legionella* spp. were present in 7 (46.7%) of 15 cooling towers sampled (*20*). Because air-conditioning systems are operated most of the year, a large heat load is imposed on water cooling systems, thus facilitating an increased rate of colonization and multiplication of *Legionella* spp. (*21*). Furthermore, the viability of *Legionella* spp. in contaminated aerosols is increased by high relative humidity (*21*).

Legionella spp. pneumonia accounts for 2%–7% of community-acquired pneumonia among hospitalized patients in Singapore (*22*). Legionnaires' disease was made a notifiable infectious disease in Singapore in 1985 and legally notifiable in 2000. A code of practice for prevention

and control of *Legionella* spp. bacteria in cooling towers for building owners and water treatment contractors was published in 1992 and revised in 1994 and 1998. Subsequently, Environmental Public Health (Cooling Towers and Water Fountains) Regulations were enacted and implemented nationwide in 2001 (*23*).

We studied trends and clinical and epidemiologic features of legionellosis in Singapore during 2000–2009. We also reviewed the prevalence of *Legionella* spp. bacteria in cooling towers and water fountains during the same period to determine whether any relationship existed between disease incidence and prevalence of *Legionella* spp. bacteria in these artificial water systems during the past decade.

Materials and Methods

Case Surveillance

The Ministry of Health (MOH) is responsible for surveillance of legionellosis in Singapore. The Infectious Diseases Act requires all registered medical practitioners and directors of clinical laboratories to notify the MOH of all cases of legionellosis by fax or electronically through a dedicated website by using a standard notification form within 24 hours after diagnosis. Clinical and laboratory criteria for notification are based on the guidelines published by the MOH (*24*). Pontiac fever is defined as a self-limiting influenza-like illness with malaise, myalgia, fever, chills, and headache without pneumonia, and Legionnaires' disease is defined as a clinical syndrome and symptoms with fever, myalgia, headache, cough, chest pain, anorexia, nonbloody diarrhea, encephalopathy, or change in sensorium, with pneumonia as the predominant clinical finding. A confirmed case of Legionnaires' disease or Pontiac fever is defined as a clinically compatible disease with a 4-fold increase in *Legionella* spp. antibody titer in paired serum samples, or presence of *Legionella* spp. antigen in urine (BinaxNOW *Legionella* immunochromatographic test; Binax Inc., Portland, OR, USA) or positive immunofluorescence or isolation of *Legionella* spp. from respiratory specimens. If clinical diagnosis was based on a positive *Legionella* spp. antibody titer ≥1,024 in a serum sample, the case was classified as presumptive.

Each reported case was investigated thoroughly by a trained public health officer by using a standard questionnaire. Investigations included interviews with the case-patient or family members and a review of clinical and laboratory records. Relevant epidemiologic and clinical data obtained included age, sex, ethnic group, occupation, residential status, residential and work place addresses, clinical features, onset of illness, recent history of travel and hospitalization before onset of illness, concurrent conditions, and clinical outcomes. Investigators paid special attention to identify any clustering of cases by person, place, and time. A cluster was defined as a locality where ≥2 cases occurred within 6 months of each other and with the same residential or workplace addresses within 500 m of each other. If the case-patient had a recent travel history outside Singapore 10 days before the onset of illness, the case was considered imported. We analyzed clinical and epidemiologic data maintained by the Communicable Diseases Division, MOH, during 2000–2009.

Environmental Surveillance

The National Environment Agency, Ministry of Environment and Water Resources, is responsible for environmental surveillance and control of *Legionella* spp. bacteria in cooling towers, water fountains, and other artificial water systems in Singapore. It works closely with the MOH to prevent outbreaks of legionellosis.

Under legislation enacted in Singapore, water samples obtained from cooling towers and water fountains are submitted to any of the 7 Singapore Accreditation Council-Singapore Laboratory Accreditation Scheme laboratories, which have been accredited to perform testing for *Legionella* spp. in Singapore. Water samples of ≈500 mL were obtained from the pond of the cooling tower or fountain by using a pump that had been disinfected with sodium hydrochlorite and neutralized with sodium thiosulfate. Testing of *Legionella* spp. is based on international laboratory standards such as the BS 6068–4.12:1998, AS/NZS 3896:1998, and ISO 11731:1998 (*25*). The limit of detection for the most commonly used standard (BS 6068–4.12:1998) in the Food and Water Microbiology Laboratory at the Singapore General Hospital (SGH) was 100 CFU/L.

Samples were first concentrated 100-fold by using membrane filtration, heated at 50°C for 30 min, and treated with acid by using HCl–KCl buffer, pH 2.2, for 5 min. Concentrated samples were then plated on charcoal yeast extract agar supplemented with cysteine, ferric ions, and antimicrobial drugs (Oxoid Ltd., Basingstoke, UK). These culture plates were incubated at 36°C ± 1°C and examined at regular intervals for a maximum of 10 days. Colonies suspected of being *Legionella* spp. were then investigated by using biochemical and serologic tests. Speciation or serogrouping of *Legionella* spp. isolates was done by using a direct fluorescent antibody test and latex agglutination or slide agglutination. For every sample, the culture plate showing the maximum number of confirmed *Legionella* spp. colonies was used to estimate the number of CFUs of *Legionella* spp. in the original water sample.

The Food and Water Microbiology Laboratory at SGH was the reference laboratory for the testing of *Legionella* spp. bacteria in Singapore. However, as of September 2008, it ceased testing of *Legionella* spp. bacteria in water

samples. We analyzed data maintained by the Food and Water Microbiology Laboratory, SGH, because it was the only public sector laboratory with reliable records.

Data Analysis

The estimated mid-year population of the corresponding years obtained from the Department of Statistics, Singapore, was used to calculate incidence rates. Statistical analyses were performed by using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Linear patterns in age distribution of legionellosis case-patients over time were assessed by using the χ^2 test for trend. Association between medical conditions and whether the case-patient survived or died was examined by using the Fisher exact test. In all data analyses, a p value <0.05 was considered significant.

Results

During 2000–2009, a total of 238 indigenous and 33 imported cases of legionellosis were reported in Singapore. An additional 4 cases reported among tourists and 48 among foreigners who sought medical treatment in Singapore were excluded. Of the 271 reported cases included in the study, 195 were classified as Legionnaires' disease (72 confirmed and 123 presumptive) and 76 as Pontiac fever (9 confirmed and 67 presumptive) (Table 1). Although none of the cases were diagnosed by immunofluorescent assay or culture of respiratory specimens, the percentage of cases diagnosed by using the *Legionella* urinary antigen test increased from 10.8% in 2000 to 19.0% in 2005 and 45.5% in 2009.

The highest mean annual age-specific incidence per 100,000 population was in persons 75–84 years of age; the male:female ratio was 1.4:1 (Table 2). The proportion of case-patients ≥55 years of age increased significantly from 44.4% in 2004 to 76.1% in 2009 (p<0.0005). Among the 3 major ethnic groups, Indians had the highest mean annual incidence rates (0.83 cases/100,000 population), followed by Chinese (0.70 cases/100,000 population) and Malays (0.55 cases/100,000 population). Retirees (32.8%) constituted most of the reported case-patients, followed by professionals, self-employed persons, and managers (17.7%); and housewives (17.2%). Cases occurred singly and sporadically throughout the year, and no cluster was detected. None of the case-patients had a recent history of hospitalization within 2 weeks before onset of illness.

The annual incidence rate of indigenous cases (confirmed and presumptive) decreased markedly from 1.37/100,000 population in 2000 to 0.28/100,000 population in 2009 (p = 0.001) (Figure) and correspondingly increased in imported cases from 6.2% during 2000–2004 to 27.3% during 2005–2009 (p<0.0005) (Figure). The countries of origin of these imported cases were mainly in Asia. The annual incidence rate for confirmed indigenous cases also decreased from 0.46 per 100,000 population in 2003 to 0.16 per 100,000 population in 2009. The overall case-fatality rate (CFR) was 2.2% for confirmed and presumptive cases and 3.7% for confirmed cases.

The main clinical features of reported cases were cough (77.9%), fever (72.7%), shortness of breath (32.5%), chest pain or discomfort (13.3%) and nausea or vomiting (11.8%) (Table 3). Most (52.8%) case-patients had concurrent conditions such as hypertension (31.5%), diabetes mellitus (23.8%), ischemic heart disease (16.1%), renal failure (11.9%), and asthma (11.2%). Six deaths were reported; 3 in 2000, 1 in 2001, 1 in 2002, and 1 in 2009. All but 1 death occured in persons ≥55 years of age, and all persons who died had concurrent conditions of the cardiovascular system. The CFR for persons with concurrent conditions (6/143, 4.2%) was significantly higher than that for persons without these conditions (0/128, 0%) (p = 0.031).

Surveillance of *Legionella* Bacteria in the Environment

On the basis of available data, 18,164 water samples from cooling towers and 1,277 water samples from water fountains were tested at the Food and Water Microbiology Laboratory, SGH, during 2000–2008. All samples were random samples routinely collected by water treatment contractors, building managers, and environmental health officers from the former Quarantine and Epidemiology Department, Ministry of the Environment and Water Resources. Data for 2000–2002 was obtained from annual reports of the Quarantine and Epidemiology Department and was a subset of the total number of samples tested in this laboratory. Data for 2003 were unavailable because this department kept its records for only 5 years before discarding them. The mean positivity rates for *Legionella*

Table 1. Classification of reported indigenous and imported legionellosis cases, by year, Singapore, 2000–2009*

Classification	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total
Confirmed											
Pontiac fever	0	4	1	3	1	0	0	0	0	0	9
Legionnaires' disease	3	15	12	16	4	5	1	0	5	11	72
Presumptive											
Pontiac fever	7	12	9	16	4	0	0	4	8	7	67
Legionnaires' disease	47	15	15	6	4	13	12	8	2	1	123
Total	57	46	37	41	13	18	13	12	15	19	271

*Four tourists and 48 foreigners seeking medical treatment in Singapore were excluded.

Table 2. Age and sex distribution, age-specific incidence, number of deaths, and CFRs among 271 case-patients with indigenous or imported legionellosis, Singapore, 2000–2009*

Age group, y	M	F	Total	Mean incidence†	No. deaths	CFR, %
0–4	1	0	1	0.05	0	0
5–14	1	0	1	0.02	0	0
15–24	7	1	8	0.13	0	0
25–34	11	6	17	0.20	0	0
35–44	19	8	27	0.36	1	3.7
45–54	24	12	36	0.61	0	0
55–64	27	18	45	1.38	2	4.4
65–74	37	33	70	3.73	1	1.4
75–84	28	25	53	6.66	2	3.8
≥85	3	10	13	5.83	0	0
Total	158	113	271	0.65	6	2.2

*Four tourists and 48 foreigners seeking medical treatment in Singapore were excluded. CFR, case-fatality rate.
†Per 100,000 population. Based on estimated mid-year population of 2004.

spp. bacteria were 15.6% for cooling tower samples and 12.4% for water fountain samples. Isolation rates for *Legionella* spp. decreased significantly in cooling towers and water fountains from an average of 58.4% during 2000–2002 to an average of 13.7% during 2004–2008 ($p<0.0005$) (Table 4).

Discussion

The incidence of legionellosis in Singapore has decreased during the past decade. Its incidence for indigenous cases in 2009 was 0.28 cases per 100,000 population (0.16/100,000 population for confirmed cases only), which was much lower than that in Europe (1.18/100,000 in 2008) (*26*) and Hong Kong (0.53/100,000 in 2009) (*27*). The percentage of imported cases of legionellosis has also increased over the past 5 years from 6.2% during 2000–2004 to 27.3% during 2005–2009 ($p<0.0005$). Imported legionellosis is often related to overnight stays in public accommodations (*28*). The number of imported cases is expected to increase further because of improved reporting and surveillance and increasing regional and international travel. A surveillance information exchange system similar to the European Surveillance Scheme for Travel Associated Legionnaires' Disease (*28*) should be established in the Asia–Pacific region so that member countries could detect possible clusters of imported legionellosis that would have been undetected by national surveillance systems in individual countries. Regional and international cooperation in the sharing of best practices would also be useful.

The epidemiologic and clinical features of legionellosis in Singapore are comparable to those in temperate countries (*2*,*27*,*29*). The incidence rate was highest for persons 75–84 years of age; there was a slight predominance in male patients and in patients with concurrent conditions. Retirees were more susceptible to legionellosis than were persons with other occupations, which also reflected higher disease incidence for persons ≥65 years of age. During 2000–2009, the overall CFR was 2.2% for confirmed and presumptive cases and 3.7% for confirmed cases, which was lower than that in Europe (6.5% in 2008) (*26*). The CFR was higher for persons with concurrent conditions. The CFR was recently reduced in comparison with the rate (14.7%) during 1986–1996 (*30*). This reduction could be attributed to wider use of *Legionella* urinary antigen tests in the diagnosis of Legionnaires' disease, which led to faster diagnosis than with traditional serologic tests. The number of cases diagnosed by using the *Legionella* antigen test increased from 10.8% in 2000 to 19.0% in 2005 and 45.5% in 2009. A delay in appropriate antimicrobial drug therapy has been associated with an increased mortality rate (*31*).

Clinical practice guidelines of the Singapore MOH for use of antimicrobial drugs in adults, which have been in use since 2000, had recommended antimicrobial drugs active against *Legionella* spp., specifically macrolides (erythromycin, clarithromycin, and azithromycin) or fluoquinolones (levofloxacin) (*32*,*33*) as first-line empirical treatment for community-acquired pneumonia in persons because of their risk category. However, laboratory testing for Legionnaires' disease is needed to ensure that patients are adequately treated for this disease. Thus, results of *Legionella* urinary antigen tests would assist clinicians in formulating antimicrobial drug therapy and result in a lower CFR.

During the same period, prevalence of *Legionella* spp. bacteria decreased in cooling towers and water fountains in Singapore. This finding could have been caused by the Environmental Public Health (Cooling Towers and Water Fountains) Regulations, which were enacted and implemented nationwide in 2001. This legislation specifies the frequency of inspection, maintenance, and testing of water for *Legionella* spp. bacteria. Cooling towers or water fountain should be thoroughly cleaned and disinfected at least once every 6 months and inspected at least once a

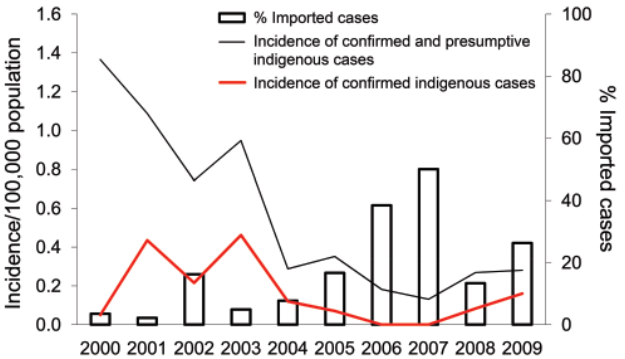

Figure. Incidence rate (per 100,000 population) of indigenous legionellosis cases and proportion (%) of imported cases, Singapore, 2000–2009.

Table 3. Signs and symptoms among 271 case-patients with indigenous or imported legionellosis, Singapore, 2000–2009*

Sign or symptom†	No. (%) case-patients
Fever (with/without chills and rigors)	197 (72.7)
Respiratory	
Cough (productive or nonproductive)	211 (77.9)
Shortness of breath	88 (32.5)
Chest pain and discomfort	36 (13.3)
Runny nose	4 (1.5)
Rhinorrhea	1 (0.4)
Bronchitis	2 (0.7)
Sore throat	7 (2.6)
Gastrointestinal	
Nausea/vomiting	32 (11.8)
Abdominal/epigastric pain	13 (4.8)
Diarrhea	8 (3.0)
Neurologic	
Drowiness/giddiness	13 (4.8)
Headache	9 (3.3)
Other signs or symptoms	
Chills	31 (11.4)
Generalized weakness	10 (3.7)
Myalgia	5 (1.9)
Lethargy	8 (3.0)
Loss of appetite	15 (5.5)
Others	22 (8.2)

*Four tourists and 48 foreigners seeking medical treatment in Singapore were excluded.
†Case-patients may have ≥1 sign or symptom.

week for any physical defect, general cleanliness, organic fouling, and physical debris. Furthermore, water samples should be sampled and tested by a government laboratory or any Singapore Accreditation Council–Singapore Laboratory Accreditation Scheme–accredited laboratories at least once a month to determine standard plate counts and at least once every 3 months to detect *Legionella* spp.

The owner or occupier of these water facilities is required to keep records of any remedial or maintenance work, inspection, or test conducted and to make available such records for inspection by any public health officer from the National Environment Agency. If cooling towers or water fountains were found by the Director-General of Public Health to endanger health of any person, the health authority could require the owner or occupier to stop using the cooling tower or water fountain and to cordon off the immediate vicinity. For first offense, the penalty is a fine not exceeding 5,000 Singapore dollars. For a second offense and subsequent offenses, the penalty is a fine not exceeding 10,000 Singapore dollars (*23*).

It is tempting to attribute the decreasing disease trend to introduction of legislation to prevent and control *Legionella* spp. bacteria in cooling towers and water fountains. However, there are several limitations that should be considered before any conclusion could be made for this relationship. In addition, other factors could have also contributed to the decrease of indigenous cases of legionellosis in Singapore.

First, no epidemiologic evidence exists to link reported cases of legionellosis to environmental isolates of *Legionella* spp. because these bacteria has never been isolated from infected patients in Singapore since the disease became notifiable in 1985. Although isolation of *Legionella* spp. from respiratory specimens is the standard in the diagnosis of legionellosis, this laboratory method is not the method of choice for many clinicians because there are other more convenient methods for testing.

Second, *Legionella* spp. bacteria are ubiquitous in the environment. Their prevalence in other artificial water systems was 31.8% in spa establishments (*34*). In another study conducted during 1998–2002, *Legionella* spp. bacteria were found in 16.2% of mist fans and 23.7% of water taps and shower heads (*35*). However, these bacteria were not detected in the municipal potable water system because the surveillance program of the Public Utilities Board (National Water Agency) started in 2003.

A major contributing factor that has possibly resulted in the decrease of *Legionella* spp. colonization in the potable water system was replacing chlorination with chloramination in disinfecting the potable water system since 2005. In 2008–2009, the Public Utilities Board (National Water Agency) further intensified its monitoring program by obtaining >130 samples quarterly from hospitals, medical centers, dead ends of the distribution system, and water taps but no *Legionella* spp. bacteria were detected. The results were again negative in a special study to detect *Legionella* spp. bacteria in the biofilm of the municipal potable water distribution system. The findings were reviewed by an external audit panel comprising local and overseas experts. Monochloramine disinfection of municipal water supplies is associated with decreased risk for Legionnaires' disease (*36*,*37*). Thus, the use of chloramine since 2005 could have also led to the decreasing

Table 4. Isolation rate for *Legionella* bacteria in environmental samples obtained from cooling towers and fountains, Singapore, 2000–August 2008*

	No. positive/no. tested (%)							
Characteristic	2000†	2001†	2002†	2004	2005	2006	2007	2008‡
Isolation rate	114/193 (59.1)	220/323 (68.1)	140/291 (48.1)	883/7,284 (12.1)	635/4,160 (15.3)	448/3,073 (14.6)	385/2,711 (14.2)	172/1,406 (12.2)

*Data were unavailable for 2003.
†Data were incomplete.
‡January–August only.

incidence of indigenous cases of legionellosis (confirmed and presumptive).

Third, the disease surveillance system favors identification of the more severe form of legionellosis, which represents a fraction of the actual extent of infections in the community. A seroepidemiologic survey of the general population in Singapore showed a prevalence of 10.3% for antibodies against *Legionella* spp. in persons <20 years of age and 21.9% in persons ≥20 years of age (*30*).

Fourth, the rapid immunochromatographic assay is more widely used for detection of *L. pneumophila* serogroup 1 antigen in urine specimens (*38*). This finding suggests that legionellosis caused by other species or serogroups would have been missed.

Fifth, most (70.1%) reported legionellosis cases were classified as presumptive, which would not constitute a definite case of Legionnaires' disease in the United States and some other countries. A decreasing trend was also observed when we considered only confirmed indigenous cases. Although, the annual incidence rate for confirmed indigenous cases decreased from 0.46 per 100,000 population in 2003 to 0.16 per 100,000 population in 2009, this difference was not significant. This finding could have been caused by the smaller sample size (n = 67) of confirmed indigenous cases of legionellosis during 2000–2009.

Sixth, changes in clinical testing patterns may have resulted in the observed decrease in incidence rate. Unfortunately, except for the clinical laboratories at SGH, we did not have data from other hospitals and clinical laboratories where *Legionella* spp. testing was performed.

Seventh, the results for water samples obtained from cooling towers and water fountains and submitted to the Food and Water Microbiology Laboratory at SGH may not be representative of the situation in Singapore. Thus, results and conclusions derived from this study should be interpreted with caution. More research should be planned and conducted to evaluate the effectiveness of legislation in the control of legionellosis.

The absence of outbreaks of legionellosis, and the decrease in the incidence rate, CFR, and prevalence rate of *Legionella* spp. bacteria in the cooling towers and water fountains is reassuring. However, a high level of vigilance should continue in the maintenance and inspection of cooling towers and water fountains. Studies should be conducted to determine epidemiologic and molecular relationships between legionellosis cases and environmental *Legionella* isolates. At the same time, the prevalence of *Legionella* spp. bacteria in other aerosol-producing artificial water systems should be periodically assessed and appropriate preventive and control measures taken.

Acknowledgment

We thank Chan Yoon Kum for providing data on the surveillance program for *Legionella* spp. in the municipal potable water system of Singapore.

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Extended-Spectrum β-Lactamase Genes of *Escherichia coli* in Chicken Meat and Humans, the Netherlands

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We determined the prevalence and characteristics of extended-spectrum β-lactamase (ESBL) genes of *Enterobacteriaceae* in retail chicken meat and humans in the Netherlands. Raw meat samples were obtained, and simultaneous cross-sectional surveys of fecal carriage were performed in 4 hospitals in the same area. Human blood cultures from these hospitals that contained ESBL genes were included. A high prevalence of ESBL genes was found in chicken meat (79.8%). Genetic analysis showed that the predominant ESBL genes in chicken meat and human rectal swab specimens were identical. These genes were also frequently found in human blood culture isolates. Typing results of *Escherichia coli* strains showed a high degree of similarity with strains from meat and humans. These findings suggest that the abundant presence of ESBL genes in the food chain may have a profound effect on future treatment options for a wide range of infections caused by gram-negative bacteria.

Infections with drug-resistant bacteria are associated with higher rates of illnesses and deaths, which have a serious effect on costs of health care (*1*,*2*). During the past decade, drug resistance in *Enterobacteriaceae* has increased dramatically worldwide. This increase has been caused mainly by an increased prevalence of extended-spectrum β-lactamase (ESBL)–producing *Enterobacteriaceae* (*3*,*4*) and has increased the use of last-resort antimicrobial drugs (i.e., carbapenems).

ESBL genes are located on plasmids that can be easily transferred between and within bacterial species. Some ESBL genes are mutant derivatives of established plasmid-mediated β-lactamases (e.g., $bla_{\text{TEM/SHV}}$), and others are mobilized from environmental bacteria (e.g., $bla_{\text{CTX-M}}$). During the 1990s, most reports on ESBL genes concerned $bla_{\text{TEM/SHV}}$ types, which were related to cross-infections in hospitals. However, the recent global increase has been caused mainly by $bla_{\text{CTX-M}}$–type genes. The epidemiology of ESBL genes is changing rapidly and shows marked geographic differences in distribution of genotypes of $bla_{\text{CTX-M}}$ β-lactamases (*5*). In the United States, the most prevalent drug resistance gene in humans is currently $bla_{\text{CTX-M-15}}$, which is often associated with a widely distributed variant of *Escherichia coli* O:25b, sequence type 131 (ST131). Bacteria containing ESBL genes are currently a common cause of infections originating in community-dwelling persons without a history of hospitalization, and these organisms can then be introduced into hospitals (*6*–*9*).

Fecal carriage of ESBL genes has been identified as the major reservoir in the environment, but the original source of this colonization has not been clearly identified. Because bacterial species that carry ESBL genes are normal inhabitants of the gastrointestinal tract, food is a potential source of them. The presence of ESBL genes has been clearly documented in food-production animals, especially chickens (*10*,*11*). Drug resistance in animals is caused mainly by the large amount of antimicrobial drugs used in food production. In addition to their presence in

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DOI: 10.3201/eid1707.110209

farm animals, ESBL genes have been found in retail meat (*12*,*13*). A recent survey of broiler chickens in Great Britain found that $bla_{CTX\text{-}M\text{-}1}$ was the most prevalent ESBL gene (*14*). Although ESBL genes in food-production animals pose a potential threat to humans, Randall et al. concluded that drug resistance genes in chickens ($bla_{CTX\text{-}M\text{-}1}$) differed from the drug resistance genes most frequently found in humans ($bla_{CTX\text{-}M\text{-}15}$) (*14*).

In the Netherlands, use of antimicrobial drugs and associated drug resistance in humans is among the lowest in Europe (*15*). Paradoxally, use of antimicrobial drugs in food-production animals in this country is among the highest in Europe (*16*). Therefore, the Netherlands provides a good setting to monitor spread of drug resistance from an animal reservoir into the human population. This spread was recently exemplified by emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* in pigs and veal calves. This was first reported in the Netherlands in 2004 and has now been reported worldwide (*17*). The aim of our study was to determine the prevalence of ESBL genes in retail meat and hospitalized patients in the Netherlands and to compare ESBL genes and bacterial strains involved.

Methods

Meat Survey

During August 17–October 30, 2009, a prospective observational study was conducted in 4 hospitals in the southern part of the Netherlands (Sint Elisabeth Hospital in Tilburg, Twee Steden Hospital in Tilburg, Amphia Hospital in Breda, and Lievensberg Hospital in Bergen op Zoom). Randomly chosen packages of meat from major grocery stores in the region of the 4 participating hospitals were included. Each sample was derived from a different package containing raw and unspiced meat. All samples were incubated for 16–18 h at 37°C in 15 mL of tryptic soy broth (TSB). Subsequently, 100 µL of the initial broth sample was transferred into a second sample of TSB broth containing 8 mg/L vancomycin and 0.25 mg/L cefotaxime. After overnight incubation, 10 µL of the broth was placed on a chromogenic agar plate selective for ESBL (bioMérieux, Marcy l'Etoile, France), and the plates were incubated overnight. Colonies with distinct morphologic appearance were further characterized.

Species and resistance patterns of oxidase-negative, gram-negative rods were determined by using the Vitek2 System (bioMérieux). Phenotypic confirmation of ESBL was performed by using Etest (bioMérieux) for all isolates. A combination of ceftazidime, cefotaxime, and cefepime with and without clavulanic acid was used. If the MIC of ≥1 of the cephalosporins showed an 8-fold reduction in combination with clavulanic acid, the isolate was considered to be an ESBL producer. If the Etest result was inconclusive, a combination disk diffusion test (Rosco, Taastrup, Denmark) was performed. All tests were performed and interpreted according to the National Guideline for the Laboratory Detection of ESBL (*18*).

Fecal Carriage Survey

In the 4 hospitals in the same area were the meat had been obtained, 2 consecutive prevalence surveys were performed 3 weeks apart (November 1–December 20, 2009) as part of each hospital's infection control program. Patients who had positive results for ESBL at the first sampling were excluded from subsequent sampling. These hospitals provide care to ≈1 million persons. Rectal swabs specimens were obtained from all patients admitted to the internal medicine, surgery, urology, pulmonology, and intensive care unit wards. Patients <18 years of age or those who had a colostomy were excluded. Rectal swab specimens were incubated in TSB broth cultures by using broth enrichment as described for the meat samples. Duration of hospitalization on the day of the survey was recorded for all participating patients.

Blood Cultures

All *E. coli* and *Klebsiella* spp. resistant to cefotaxime, including all strains presumably producing ESBL on the basis of microbiologic results, and isolated from clinical blood cultures, were obtained during July 2008–December 2009 from the 4 study hospitals. Confirmation of ESBL genes was performed as described for meat samples. Blood culture isolates were obtained from individual patients.

Genetic Characterization of Drug Resistance Genes

Characterization of drug resistance genes in all strains that were phenotypically ESBL producers was conducted 2 ways. First, we tested all isolates for $bla_{CTX\text{-}M}$ by using denaturing high-performance liquid chromatography as described (*19*). Second, we screened for ESBL genes by using a micro-array (Check-Points, Wageningen, the Netherlands) that was designed to detect single nucleotide polymorphisms in essential bla_{TEM} and bla_{SHV} genes, variant genes, and $bla_{CTX\text{-}M}$ group genes ($bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, and $bla_{CTX\text{-}M\text{-}8/25}$). The procedure has been reported (*20*). Subsequently, sequencing was performed to further specify ESBL genotypes. On the basis of micro-array results, bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ genes were amplified by PCR and specific primers. PCR amplicons were selected and sequenced after purification (Agencourt Ampure; Beckman Coulter, Leiden, the Netherlands). Sequence analysis and alignments were performed by using Bionumerics 6.01 software (Applied Maths, Sint-Martens-Latem, Belgium), the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and information from the Lahey Clinic (Burlington, MA, USA) (www.lahey.org/

studies). If results of denaturing high-perfomance liquid chromatography and DNA sequence were discordant, the DNA sequence was used as the correct result.

Multilocus Sequence Typing of *E. coli* Strains

All *E. coli* isolates from meat, rectal swab specimens, and blood cultures were typed by using multilocus sequence typing (MLST) as described by Wirth et al. (*21*). If patient or meat samples contained >1 morphologically distinct ESBL-producing *E. coli* strain, all strains were included in the MLST analysis.

Statistical Analysis

Data were analyzed by using SPSS version 18 software (SPSS, IBM, Somers, NY, USA). Univariate analysis was performed for calculation of difference in prevalences by using the χ^2 test. One sample could contain >1 strain because morphologically distinct colonies with different drug resistance genes or a different MLST result were all included in the final analysis.

Results

Meat Survey

A total of 262 fresh meat samples were included in this study (mean weight 11.9 g). The type of meat was chicken (n = 89, 34.0%), beef (n = 85, 32.4%), pork (n = 57, 21.8%), mixed or ground meat (n = 22, 8.4%), and other types of meat (n = 9, 3.4%). Phenotypic screening initially identified 112 samples containing possible ESBL producers. Genotypic confirmation identified 79 (30.2%) ESBL-producing samples. Some samples contained >1 type of ESBL gene. Prevalence of ESBL genes differed among the 4 meat groups: 71 (79.8%) in chicken, 4 (4.7%) in beef, 1 (1.8%) in pork, 2 (9.1%) in mixed or ground meat, and 1 (11.1%) in other types of meat. ESBL gene prevalence was significantly higher in chicken ($p<0.001$ for all comparisons with other meat types).

Fecal Carriage Survey

A total of 927 rectal swab specimens were obtained from 876 patients (461 male patients and 415 female patients, mean ± SD age 65.7 ± 16.8 years). Phenotypic screening identified 59 patients as possibly being infected with ESBL-producing *E. coli*. Confirmatory test results for ESBL genes were inconclusive for 2 samples and excluded (no inconclusive results were obtained with meat or blood cultures). A total of 45 (4.9%) samples contained confirmed ESBL genes.

Blood Cultures

Thirty-one clinical blood cultures suspected of containing ESBL genes on the basis of phenotypic screening were available for further analysis. Genetic characterization confirmed that 23 (74.2%) samples contained ESBL genes.

ESBL-producing Bacterial Species

Sixty-eight (76.8%) chicken meat samples contained ESBL-producing *E. coli*, 6 (7.7%) contained ESBL-producing *Klebsiella* spp., and 4 (5.1%) contained other ESBL-producing species. Of the 8 types found in other meat, all were ESBL-producing *E. coli.* Rectal swab specimens of hospitalized patients showed that 39 (69.6%) patients had *E. coli*, 11 (19.6%) had *Klebsiella* spp., and 8 (10.7%) had other bacterial species. Blood cultures showed that 16 (64.0%) patients had *E. coli* and 9 (36.0%) had *Klebsiella* spp.

Drug Resistance Genes

The distribution of drug resistance genes from various sources is shown in Figure 1. The predominant ESBL genotype in chicken meat was $bla_{\text{CTX-M-1}}$ (n = 50, 58.1%). This genotype was also the most frequent ESBL genotype in rectal swab specimens (n = 22, 45.8%) and the second most common in blood cultures (n = 5, 20.8%). In chicken meat, $bla_{\text{CTX-M-1}}$ (n = 50, 58.1%) was the most common genotype, followed by $bla_{\text{TEM-52}}$ (n = 12, 14.0%) and $bla_{\text{SHV-12}}$ (n = 12, 14.0%). In other types of meat, 5 (62.5%) of 8 ESBL genotypes were $bla_{\text{CTX-M-1}}$. In rectal swab specimens of hospitalized patients, $bla_{\text{CTX-M-1}}$ (n = 22, 45.8%) was the most common genotype, followed by $bla_{\text{CTX-M-15}}$ (n = 8, 16.7%) and $bla_{\text{TEM-52}}$ (n = 6, 12.5%). In blood cultures, $bla_{\text{CTX-M-14}}$ was the predominant genotype (n = 8, 33.3%).

Epidemiology of Patients and Drug Resistance Genes

When we compared the most prevalent drug resistance genes in 346 patients who had been hospitalized ≤48 h at the time of screening with 581 patients who had been hospitalized >48 h, prevalence was similar for $bla_{\text{CTX-M-1}}$ (2.3% and 2.4%, respectively) and for TEM-52 (0.6% and 0.7%, respectively) in the 2 groups. Prevalence of $bla_{\text{CTX-M-15}}$ was 4× higher in the group that was hospitalized longer (0.3% and 1.2%, respectively; p = 0.27). There was only 1 possible cluster of cases (same resistance gene at the same time at the same ward), which involved 3 patients with $bla_{\text{CTX-M-15}}$. One patient was infected with *E. coli* and 2 were infected with *K. pneumoniae*.

MLST of *E. coli*

MLST results of 158 ESBL-positive *E. coli* strains isolated from chicken meat, other meat types, rectal swab specimens, and blood cultures are shown in Figure 2. *E. coli* containing ESBL genes showed a heterogeneous population that contained several clusters. Most clusters contained strains isolated from meat and humans. All but 1 of the ESBL-producing strains from other meat types

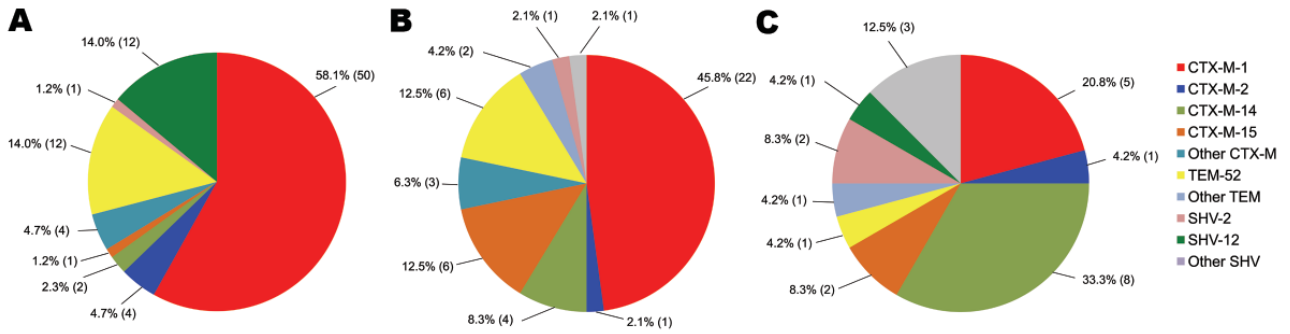

Figure 1. Distribution of extended-spectrum β-lactamase genes in chicken meat (A), human rectal swabs (B), and human blood cultures (C), the Netherlands. Values in parentheses are no. positive.

clustered with strains from chicken meat. Twenty-five (56.8%) of 44 strains from rectal swab specimens and 9 (56.3%) of 16 strains from blood cultures clustered with strains from chicken meat.

MLST results for strains with $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{TEM-52}}$ are shown in Figure 2. Genotypes $bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-52}}$ showed a heterogeneous population and clusters containing strains from humans and meat. *E. coli* harboring $bla_{\text{CTX-M-15}}$ was found less frequently, and no clusters with human-derived and meat-derived strains were observed. The widely disseminated ST131 clone was found in human samples only: 4 times in combination with $bla_{\text{CTX-M-15}}$, twice with $bla_{\text{CTX-M-14}}$, and once with $bla_{\text{SHV-12}}$.

Discussion

ESBL genes were found in a high (79.8%) proportion of retail chicken meat samples in the Netherlands. A comparison of ESBL-producing *Enterobacteriaceae* derived from meat and hospitalized patients showed a high degree of similarity of resistance genes and MLST patterns. Genotype $bla_{\text{CTX-M-1}}$ was the most frequent drug resistance gene in chicken meat and humans and the second most frequent in blood cultures. Other meat types contained similar drug resistance genes, but the prevalence of ESBL genes was much lower. It is unclear whether ESBL genes in other meat types are related to a reservoir in food-production animals or contamination at meat-processing facilities. An extensive reservoir of ESBL genes on farms was repeatedly shown in poultry (*10*,*11*). Our findings suggest a relationship between contamination of chicken meat with drug-resistant bacteria and appearance of ESBL genes in humans in the Netherlands. This relationship was further supported by genomic comparison of strains detected in chicken meat with those detected in human fecal specimens. MLST showed that most *E. coli* strains harboring $bla_{\text{CTX-M-1}}$ or $bla_{\text{TEM-52}}$ from humans and meat belong to clusters containing strains from both sources. These findings suggest a relationship between contamination of chicken meat and appearance of ESBL genes in humans in the Netherlands.

The high prevalence of ESBL genes in chicken meat is consistent with findings of other investigators. Doi et al. reported that 67% of retail meat samples in Seville, Spain, contained ESBL or ESBL-like resistance genes (*12*). A survey of imported raw chicken in the United Kingdom reported ESBL genes in 10 of 27 samples (*13*). The authors concluded that ESBL genes in meat pose a potential threat to humans, but that the most prevalent ESBL genotype in humans in the United Kingdom ($bla_{\text{CTX-M-15}}$) was not found in imported meat.

In our study, we found a high degree of similarity between drug resistance genes in humans and retail meat. A possible explanation for this finding is that in the Netherlands, where drug resistance in bacterial isolates in humans is less frequent (*15*) and cross-transmission in hospitals is controlled effectively (*22*), the role of acquiring drug-resistant strains from food is more easily detected. In addition, the Netherlands is one of the highest users of antimicrobial agents in food-production animals (*16*), which results in high rates of drug resistance among these animals. A report by the Veterinary Antibiotic Usage and Resistance Surveillance Working Group showed that cefotaxime-resistant *E. coli* in poultry meat in the Netherlands has emerged since 2005 (*23*). This finding coincides with the increase in third-generation cephalosporin-resistance in *E. coli* and *K. pneumonia* bacteria in invasive infections in humans reported by the European Antimicrobial Resistance Surveillance Network (*24*).

We performed a prevalence survey among hospitalized patients to determine the size and nature of the reservoir of ESBL genes. Nearly 5% of all hospitalized patients were carriers of ESBL genes. It is difficult to put this rate into context because no screening studies have been conducted in the Netherlands. A large study in Chicago, Illinois, USA, found that during 2000–2005, the rate of ESBL gene carriage among high-risk, hospitalized patients

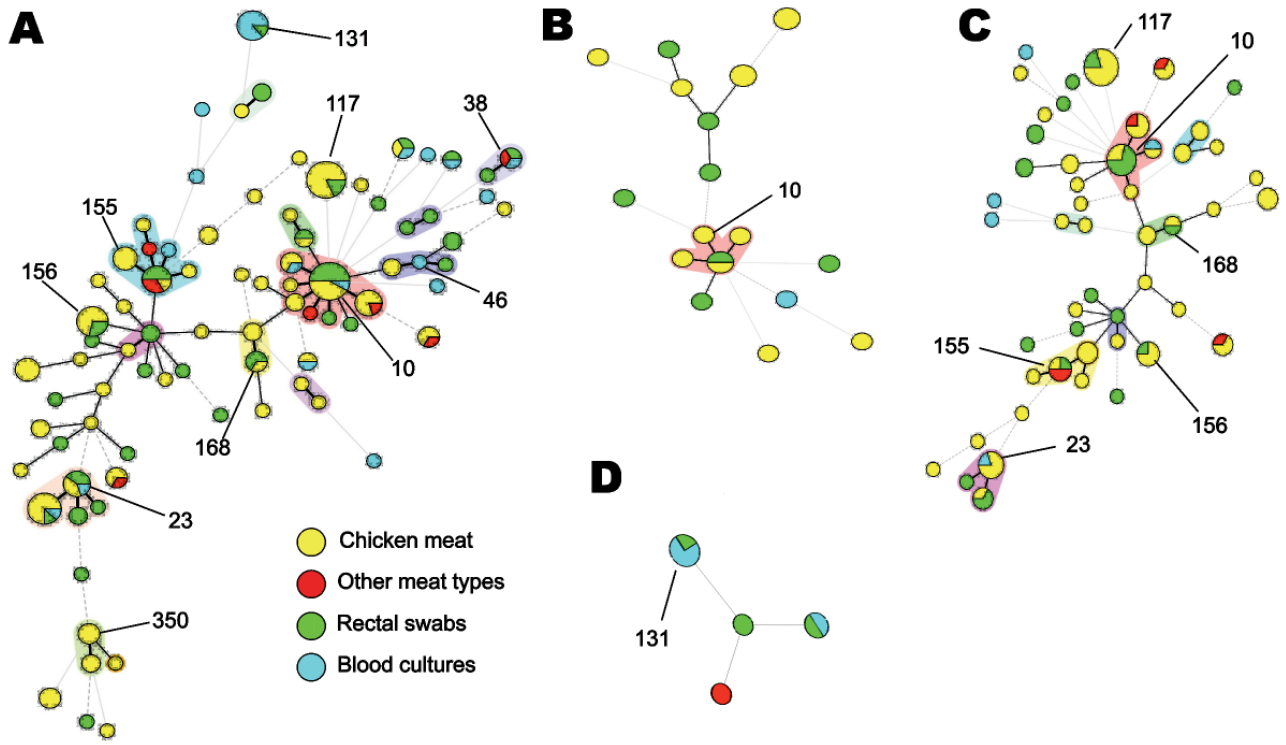

Figure 2. Multilocus sequence typing patterns of *Escherichia coli* from chicken meat, other meat types, human rectal swabs, and human blood cultures, the Netherlands. A) All *E. coli* containing extended-spectrum β-lactamase genes; B) *E. coli* containing bla_{TEM-52}; C) *E. coli* containing $bla_{CTX-M-1}$; D) *E. coli* containing $bla_{CTX-M-15}$. Major sequence types are shown as numbers. Black connecting lines indicate single-locus variants; gray connecting lines indicate double-locus variants; dashed connecting lines indicate strains with ≥3 loci that are different; and shadowing indicates that >2 sequence types belong to 1 complex.

increased from 1.3% to 3.2%, and bacteremia developed in 8.5% of all previously identified ESBL gene carriers during hospitalization (*25*). In Spain, an increase was also observed in fecal carriage in hospitalized patients from 0.3% in 1991 to 11.8% in 2003 (*7*).

The rate we observed in 2009 in the Netherlands was lower than rates in Spain in 2003, which would be expected because of low antimicrobial drug resistance rates in the Netherlands (*24*). Conversely, a prevalence of 5% extrapolated to the Dutch population in 2011 (16,700,000 inhabitants) would indicate that currently >800,000 persons in the Netherlands are colonized with ESBL-producing bacteria. Because use of antimicrobial drugs in the health care setting in the Netherlands is low and has not changed during the past decade, alternative factors for increased drug resistance are not known. An indication for the role of a community reservoir is the prevalence of drug resistance genes in patients who had been hospitalized ≤48 h and those hospitalized >48 h. Drug resistance genes that are associated with a proposed food reservoir (*26*) ($bla_{CTX-M-1}$ and bla_{TEM-52}) were already present at hospitalization. However, $bla_{CTX-M-15}$, which is reported to be associated with health care settings (*26*), had a higher, albeit not significant, prevalence in the group who had been in the hospital >48 h.

Considering what is known about the epidemiology of *E. coli*, the abundance of ESBL genes in chicken meat is a likely explanation for current findings in humans. Although there are extensive campaigns promoting safe handling of chicken meat during processing, enteric pathogens are frequently transferred to humans and pose a continuous public health threat (*27*). Johnson et al. studied geographically and temporally matched *E. coli* isolates from humans and poultry (*28*). Drug-susceptible *E. coli* isolates from humans differed from drug-resistant isolates from humans and from isolates in poultry irrespective of their drug resistance pattern. Drug-susceptible isolates from poultry were similar to drug-resistant isolates of poultry and humans. Their conclusion was that drug-resistant human fecal *E. coli* isolates likely originate from poultry, whereas drug-resistant *E. coli* isolates from poultry likely originate from susceptible precursors in poultry. In vitro experimental support for our hypothesis comes from a recent study showing transfer of a bla_{TEM-52}–carrying plasmid from an avian *E. coli* strain to 2 human *E. coli* strains in a continuous flow culture model (*29*).

Emergence of ESBL genes in poultry has been associated with use of third-generation cephalosporins (particularly ceftiofur) in chickens. In Canada, a strong correlation was found between incidence of ceftiofur-resistant *Samonella enterica* serovar Heidelberg in humans and retail chickens (*30*). Use of ceftiofur in animals was stopped voluntarily, and ceftiofur resistance rates subsequently decreased in retail chicken meat and humans. After partial reintroduction of this drug, resistance rates in poultry and humans increased, providing further evidence for a zoonotic source of ESBL genes.

Overrepresentation of ST131 in blood cultures confirms the virulent properties attributed to this clone. Typically, ST131 is found in association with $bla_{\text{CTX-M-15}}$. In our survey, ST131 strains were associated with 3 drug resistance genes. This finding indicates that this virulent clone also acquires other drug resistance genes. We did not find ST131 in the chicken meat samples, which is reassuring at this time. However, other studies have recently identified ST131 in poultry and retail meat (*31*,*32*). These findings confirm that virulent clones of *E. coli* are capable of crossing species barriers between humans and animals. In addition, mobile drug resistance genes also cross this barrier and are likely to accelerate dissemination of drug resistance between animals and humans.

We conclude that the high rate of ESBL contamination of retail chicken meat in the Netherlands, which involves many of the same ESBL genes present in colonized and infected humans, is a plausible source of the recent increase of ESBL genes in the Netherlands. The similarity of *E. coli* strains and predominant drug resistance genes in meat and humans provides circumstantial evidence for an animal reservoir for a substantial part of ESBL genes found in humans. The threat of the high rate of antimicrobial drug use in food-production animals and associated emergence of drug resistance in zoonotic pathogens has been recognized for decades. Our group and others found that most samples of retail chicken meat contain transmissible drug resistance genes in bacterial species that are part of the normal human intestinal flora. This finding may have a profound effect on future treatment options for a wide range of infections with gram-negative bacteria.

Acknowledgments

We thank the laboratory technicians, consultant microbiologists and infection control nurses for assistance in obtaining data for the meat survey and the survey for fecal carriage among hospitalized patients.

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Asian Lineage of Peste des Petits Ruminants Virus, Africa

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Interest in peste des petits ruminants virus (PPRV) has been stimulated by recent changes in its host and geographic distribution. For this study, biological specimens were collected from camels, sheep, and goats clinically suspected of having PPRV infection in Sudan during 2000–2009 and from sheep soon after the first reported outbreaks in Morocco in 2008. Reverse transcription PCR analysis confirmed the wide distribution of PPRV throughout Sudan and spread of the virus in Morocco. Molecular typing of 32 samples positive for PPRV provided strong evidence of the introduction and broad spread of Asian lineage IV. This lineage was defined further by 2 subclusters; one consisted of camel and goat isolates and some of the sheep isolates, while the other contained only sheep isolates, a finding with suggests a genetic bias according to the host. This study provides evidence of the recent spread of PPRV lineage IV in Africa.

Peste des petits ruminants virus (PPRV) belongs to the genus *Morbillivirus*, in the family *Paramyxoviridae*. Like other members of the same genus, such as rinderpest virus, *Canine distemper virus*, *Measles virus*, and marine mammal viruses, PPRV is highly pathogenic for its natural hosts (*1*). As consistently reported by the World Organisation for Animal Health, PPRV causes high death rates in livestock. It has a major economic effect, particularly in the intertropical regions of Africa, on the Arabian Peninsula and in the Middle East and Asia (*2*–*5*). The main signs of the acute form of the disease are high fever, mouth ulceration, diarrhea, and pneumonia.

Although rinderpest virus was eradicated after intensive vaccination campaigns in the last quarter of the 20th century (*6*), PPRV has continued to spread in Africa and Asia. In East Africa, outbreaks occurred in 2007 in Uganda and Kenya and in 2009 in Tanzania (*7*); in North Africa, an outbreak occurred in 2008 in Morocco. Genotypic classification of PPRV has identified 4 lineages and appears to be an efficient tool to survey virus spread worldwide. Genetic variability is based on partial sequencing of the fusion (F) protein gene (*8*,*9*) and of the 3′ end of the nucleoprotein (N) gene (*10*). Both are well conserved genes with ≈10% nt mean variability between the most distantly related sequences (*11*); this variability can exceed 30% on some parts of the sequence. However, because the N gene is the most abundantly transcribed virus gene, sensitivity is better achieved with N mRNAs (*12*). Viruses of lineage I and II are restricted to western and central Africa; lineage III is common to eastern Africa and the southern part of the Middle East. In Asia, only viruses of lineage IV have been detected.

In Sudan, continuous outbreaks of PPRV have occurred for >30 years, mainly in sheep and goats (*2*,*13*). Although PPRV infection is well documented in small ruminants, data are rare for other species, such as cattle, buffalo, and camels. In 1997, a PPRV was isolated from pathologic samples collected during a rinderpest-like disease outbreak among buffalo in India (*14*). In September 2004, outbreaks

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DOI: 10.3201/eid1707.101216

of PPRV in Sudan affected both sheep and camels (*15*,*16*). In camels, a respiratory syndrome was the prominent disease characteristic observed, resembling a previous case reported in Ethiopia during 1995–1996 (*17*).

More recently, in summer 2008, Morocco reported outbreaks of PPRV for the first time (*18*). Because PPRV needs close contact for transmission, this new epizootic was likely the result of introduction of the virus into North Africa through the movement of live infected animals. To characterize PPRV strains identified in Sudan during 2000–2009 and in Morocco during the extensive 2008 outbreaks, a phylogenetic analysis was carried out on wild-type PPRV sequences obtained from biological samples collected from sheep, goats, and camels in these 2 countries.

Materials and Methods

Biological Materials

Biological specimens were collected in different parts of Sudan during 2000–2009 from camels, sheep, and goats that showed clinical signs of PPRV. Additional camel specimens were collected after outbreaks associated with high death rates were reported from the Kassala Region in 2004. A total of 80 field samples, including lung, liver, and spleen, were obtained from 49 camels, 26 sheep, and 5 goats with PPRV-like clinical signs. For most of the animals, virus detection and identification were performed directly on the tissue samples collected; however, virus isolation in tissue culture was attempted for some samples. A historical PPRV strain isolated in Sudan in 1971, the Gedarif PPRV strain, was included in this study.

In Morocco, a total of 36 samples were collected from sheep displaying PPRV signs during the 2008 outbreaks. These included oral and ocular swabs and mesenteric lymph nodes, spleen, lung, and whole blood samples.

Laboratory Investigations

Sample aliquots first were screened for PPRV antigen detection by immunocapture ELISA (ICE test; Biological Diagnostics Supplies, Ltd., Dreghorn, Scotland). A second aliquot was used for viral RNA extraction and virus isolation in tissue culture. Reverse transcription PCR (RT-PCR) amplified the N protein gene directly from tissue samples by using a set of pan-morbillivirus primers as described by Kwiatek et al. (*10*).

For the sequencing of the N gene, the 3′ 351 nt were obtained by employing primers NP3 and NP4 (*19*) and through a modification of the initial protocol by using a 1-step method (OneStep RT-PCR mix; QIAGEN, Courtaboeuf, France). For the F gene, 11 samples were selected from samples that previously tested positive by N amplification. For the specific and sensitive detection of PPRV from these clinical field samples, the initial method developed by Forsyth et al. in 1995 (*8*) was modified by using an additional pair of primer F1/FPPRrev in combination with F1/F2 in a nested PCR. Sequences positions of these primers were 5′-ATCACAGTGTTAAAGCCTGTAGAGG-3′ (PPRV-F1: 777 ± 801), 5′-GAGACTGAGTTTGTGACCTACAAGC-3′ (PPRV-F2: 1124 ± 1148) and 5′-ATATTAATGTGACAA GCCCTAGGGA-3′ (FPPRrev: 2055 ± 2079). The final amplicon was obtained at the expected length of 372 nt after 35 cycles. We analyzed 10 μL of the amplified products by electrophoresis on 1.5% agarose gel. For all positive results, 40 μL of the final product was used directly for sequencing (GATC Biotech, Constance, Germany). All sequences were deposited in GenBank under accession nos. HQ131917–HQ131958. FPPRrev and the study of the target sites of primers F1 and F2 (*8*) were designed by a comparative sequence analysis of various morbillivirus F sequences from the full genomes available in GenBank (accession nos. X74443, EU267273, EU267274, AY560591, FJ905304, and AJ849636).

Sequence Data, Alignment, and Phylogenetic Analysis

Sequencing reactions were performed by GATC Biotech. Each nucleic acid segment on the N and F genes of the Sudan and Morocco strains was aligned with other sequences from PPRV maintained in the database or retrieved from GenBank (Table 1) (*10*,*20*–*22*). Multiple alignments of 255 and 322 nt of the N and F genes, respectively, were made by using the ClustalW program (www.ebi.ac.uk/clustalw). Phylogenetic analysis was carried out by using the criterion of neighborhood based on the principle of parsimony (*23*). Dissimilarities and distances between the sequences first were determined with Darwin software (*24*). Tree construction was based on the unweighted neighbor-joining method proposed by Gascuel (*25*). Trees were generated with the TreeConMATRIXW program of Darwin (*26*). Bootstrap confidence intervals were calculated on 1,000 replicates.

Virus Isolation

For the samples from Sudan, isolation was successful on MDBK cells with lung samples from Cam_8, Cam_169, Cam_318, Ov_Soba, and Ov_Al Azaza, collected from 3 camels and 2 sheep, respectively. For the samples from Morocco, PPRV Morocco_08_02 virus was isolated successfully from a sheep lung sample after infection of Vero.DogSLAMtag cells (*27*). These isolates were sequenced and compared as described above.

Results

Detection of PPRV by N gene RT-PCR

A total of 80 animals from Sudan that initially tested positive in the ICE test were analyzed further by using RT-

Table 1. Peste des petits ruminant virus (genus *Morbillivirus*) strains and sequences retrieved from GenBank, Africa, 2000–2009*

Lineage	Origin	Year of isolation	Source	GenBank accession no.	
				N gene	F gene
I	Senegal	1968	ISRA/Senegal	DQ840165	NA
III	Sudan	1972	CVRL/Sudan	DQ840158	NA
II	Nigeria	1975	IAH/UK	DQ840161	NA
II	Nigeria	1975	IAH/UK	DQ840162	NA
II	Nigeria	1975	IAH/UK; CIRAD/France	DQ840160	X74443
II	Nigeria	1976	IAH/UK	DQ840163	NA
II	Nigeria	1976	IAH/UK	DQ840164	EU267274
II	Ghana	1978	IAH/UK	DQ840167	NA
II	Ghana	1978	IAH/UK	DQ840166	NA
III	Oman	1983	IAH/UK	DQ840168	NA
III	United Arab Emirates	1986	AAZA/UAE	DQ840169	NA
I	Burkina Faso	1988	CIRAD/France	DQ840172	NA
I	Guinea	1988	CIRAD/France	DQ840170	NA
I	Côte d'Ivoire	1989	CIRAD/France	DQ840199	EU267273
I	Guinea-Bissau	1989	CIRAD/France	DQ840171	NA
IV	Israel	1993	KVI/Israel	DQ840173	NA
I	Senegal	1994	ISRA/Senegal	DQ840174	NA
III	Ethiopia	1994	CIRAD/France	DQ840175	NA
IV	India	1994	NPRI/India	DQ840176	NA
IV	India	1994	CIRAD/France	DQ840179	NA
IV	India	1994	CIRAD/France	DQ840180	NA
IV	India	1995	CIRAD/France	DQ840177	NA
IV	India	1995	CIRAD/France	DQ840178	NA
IV	India	1995	CIRAD/France	DQ840182	NA
IV	Israel	1995	KVI/Israel	DQ840181	NA
III	Ethiopia	1996	CIRAD/France	DQ840183	NA
IV	Turkey	1996	CIRAD/France	DQ840184	NA
IV	India	1996	IVRI/India	AY560591	GQ452015
IV	Cameroon	1997	LANAVET/Cameroon	HQ131960	NA
IV	Iran	1998	CIRAD/France	DQ840185	NA
IV	Iran	1998	CIRAD/France	DQ840186	NA
IV	Israel	1998	KVI/Israel	DQ840191	NA
IV	Israel	1998	KVI/Israel	DQ840188	NA
IV	Israel	1998	KVI/Israel	DQ840189	NA
IV	Israel	1998	KVI/Israel	DQ840190	NA
II	Mali	1999	LCV/Mali	DQ840192	NA
IV	Saudi Arabia	1999	CIRAD/France	DQ840195	NA
IV	Saudi Arabia	1999	CIRAD/France	DQ840197	NA
IV	Tajikistan	2004	CIRAD/France	DQ840198	NA
IV	Central African Republic	2004	CIRAD/France	HQ131962	NA
IV	India	2005	CVSH/India	DQ267188	DQ267183
IV	India	2005	CVSH/India	DQ267191	DQ267186
IV	India	2005	CVSH/India	DQ267192	DQ267187
IV	India	2005	CVSH/India	DQ267189	DQ267184
IV	India	2005	CVSH/India	DQ267190	DQ267185
IV	China	2007	NEADDC/China	EU068731	EU816772
IV	China	2007	NEADDC/China	EU340363	EU815053
IV	Bangladesh	2009	DPBAU/Bangladesh	HQ131961	NA
II	Senegal	2010	ISRA/Senegal	HQ131963	NA
0	Kenya	2010	IAH/UK	Z30697	NA

*ISRA, Institut Sénégalais de Recherches Agricoles; NA, GenBank accession no. not available; CVRL, Central Veterinary Research Laboratory; IAH, Institute For Animal Health; CIRAD, Centre de Coopération Internationale en Recherche Agronomique pour le Développement; AAZA, Al Ain Zoo and Aquarium, Al Ain/Abu Dhabi, United Arab Emirates; KVI, Kimron Veterinary Institute; NPRE, National Project on Rinderpest Eradication; IVRI, Indian Veterinary Research Institute; LANAVET, Laboratoire National Veterinaire; LCV, Laboratoire Central Vétérinaire; CVSH, College of Veterinary Science and Husbandry; NEADDC, National Exotic Disease Diagnosis Center; DPBAU, Department of Pathology Bangladesh Agricultural University.

PCR. Results for 64 animals were positive, including 21 sheep (80.8%), 5 goats (100%), and 38 camels (77.6%) drawn from all of the regions studied: Khartoum, Blue Nile, Northern Sudan, Kassala, Kordofan, and Darfur (Table 2). Of the 36 samples tested from Morocco, 16 yielded a positive RT-PCR.

Characterization of Strains Involved in the Infection of Sheep, Goats, and Camels in Sudan, and in Sheep in Morocco

Of the tissue samples tested, lung and lymph nodes were the most suitable for sequencing because they had the highest viral load, thereby yielding a sufficient amount of PCR product. Sequence also was obtained from several virus isolates, including the historical Gedarif isolate from 1971. The partial N gene sequences were obtained for 26 of the 64 samples from Sudan and 6 of the 16 samples from Morocco. Sequences were aligned with an extended set of PPRV isolate sequences that either were in the database or were retrieved from GenBank where they were described by Kwiatek et al. (*10*) and Banyard et al. (*20*).

In contrast to what would have been expected for isolates from eastern Africa, most of the PPRV strains collected in Sudan during 2000–2009 were clustered in lineage IV (24); only a few remained in lineage III (2). Molecular typing also showed for the first time the presence of PPRV lineage IV in Morocco (Figure 1).

Lineage IV isolates from Sudan could be further distinguished into 2 clusters. The grouping of the sequences within the clusters was supported by bootstrap percentages >70%. In the first group, 17 sequences from all camel and goat isolates and some sheep isolates (camel Sudan Cam_1, Cam_3, Cam_8, Cam_169, Cam_223, Cam_264, Cam_268, Cam_304, Cam_330, Cam_352, Cam_318; sheep Ov_1, Ov_140, Ov_25, Ov_39300; goat Cap_9, Cap_1) showed 100% identity and matched with Saudi Arabia_1999_7 strain. Sheep Sudan Ov_41 differed from the previous sequences by 2 nt at position 68 (C to T) and 142 (A to G). The divergence of this cluster with consensus IV ranged from 1.2% (Cam_318) to 2%

Figure 1. Phylogenetic analysis of the 1232–1560 nt sequence of the N protein gene of sequenced peste des petits ruminant (PPR) virus strains. The phylogram was generated by analyzing 1,000 bootstrap replicates; clusters were supported by bootstrap values >70%. Strains from Sudan are represented by prefixes: cam, camel; cap, caprine; ov, ovine. The Kabete 0 strain of rinderpest (RBOK) vaccine strain of rinderpest virus retrieved in GenBank (accession no. Z30697) was used as an outgroup. Scale bar indicates nucleotide substitutions per site.

Table 2. Number of field samples with positive results by reverse transcription PCR for peste des petits ruminant virus, by animal and province, among 80 animals sampled in Sudan, 2000–2009*

	Sheep		Goats		Camels	
Region	No. tested	No. (%) positive	No. tested	No. (%) positive	No. tested	No. (%) positive
Khartoum	5	4 (80)	1	1 (100)	NS	NS
Blue Nile	10	7 (70)	3	3 (100)	18	13 (72.2)
Northern Sudan	4	4 (100)	1	1 (100)	21	16 (76.2)
Kassala	5	5 (100)	NS	NS	9	8 (88.9)
Kordofan	1	1 (100)	NS	NS	NS	NS
Darfur	1	0	NS	NS	1	1 (100)
Total	26	21 (80.8)	5	5 (100)	49	38 (77.6)

*Proportion of animals positive for peste des petits ruminant virus in sampling sites according to Sudan regions: Khartoum: 5/6; Blue Nile: Al Azaza, 1/1; Al Hilalia, 1/1; Gezira /Bashagra, 1/1; Tambool, 13/18; White Nile (Sennar/Rabak), 7/10; Northern: River Nile, Atbara, 16/21; Ed Damar, 4/4; Dongola, 1/1; Kassala: Gedarif, 3/4, Kassala, 7/7, Abudelaiq, 3/3. Darfur: Alfashir 0/1, Nyala, 1/1; Kordofan: El Nihood, 1/1. NS, species not sampled in region.

(Ov_41). Furthermore, the Saudi Arabia cluster was related closely to the 6 strains from Morocco collected from the 2008 outbreak, the closest differing by 4 nt.

A separate cluster containing only sheep samples collected during 2000–2008 (sheep Sudan Ov_10033, Ov_10034, Ov_23, Ov_Soba, Ov_Al Azaza) matched with 2 strains from central Africa (Cameroon_1997 [CAMER_1997] and Central African Republic_2004 [CAR_2004]) (*20*)**.** This cluster was less homogeneous than the cluster previously described; nucleotide variations from the lineage IV consensus sequence ranged from 1.6% (Ov_Al Azaza) to 3.9% (Ov_23).

Only 2 isolates collected at the start of the study (in mid-2000) from sheep in western and eastern Sudan fell into lineage III. Sequences of these 2 field isolates remained similar to the historical strains of Sinnar 72 that was isolated 40 years earlier from the Blue Nile region (*2*), and with the Gedarif PPRV strain that was isolated from sheep in 1971 (*13*). In addition, these isolate sequences were close to an Omani strain, Ibri_1983 (*28*) and a United Arab Emirates strain, Dorcas_1986 (*29*), but they differed from these strains with a nucleotide variation of 4.7%.

Because the dataset within the Saudi Arabia cluster was highly homogeneous, we compared an N phylogenetic tree (Figure 2, panel A) with an F phylogenetic tree (Figure 2, panel B) on a panel of viruses. These viruses were selected from the 2 lineage IV clusters defined in Figure 1 and from lineage III with additional PPRV from Asia, for which both gene sequences were available (*21*,*22*). The F phylogenetic tree confirmed the high homology within the Saudi Arabia cluster, and the cluster's closeness to strains from Morocco. It also allowed for assignment of strains from Sudan to the 2 described clusters represented by the Arabia_1999_7 and the Central Africa strains.

Unlike the N gene amplifications, amplications with F-specific primers (*8*) resulted in a number of negative samples by RT-PCR because of mismatches occurring mainly in the F2 reverse primer (Table 3). To circumvent this problem, another reverse primer (FPPRrev) was designed that matched with all lineages.

The sequences and the corresponding lineage classification generated in this study were collated to field information. Occurrence among species of the different lineages show that most of the isolates belong to lineage IV; all camel isolates grouped in the cluster Saudi Arabia, whereas only sheep isolates were found within the other cluster, Central Africa. Only sheep isolates were found in lineage III (Table 4). Geographic and times distribution according to animal species and lineage in Sudan during 2000–2009 show that lineage IV was circulating as early as mid-2000 and that progressive substitution of lineage III in a large zone encompassing the eastern, northern, Blue Nile, and Khartoum regions has taken place since this date (Figure 3).

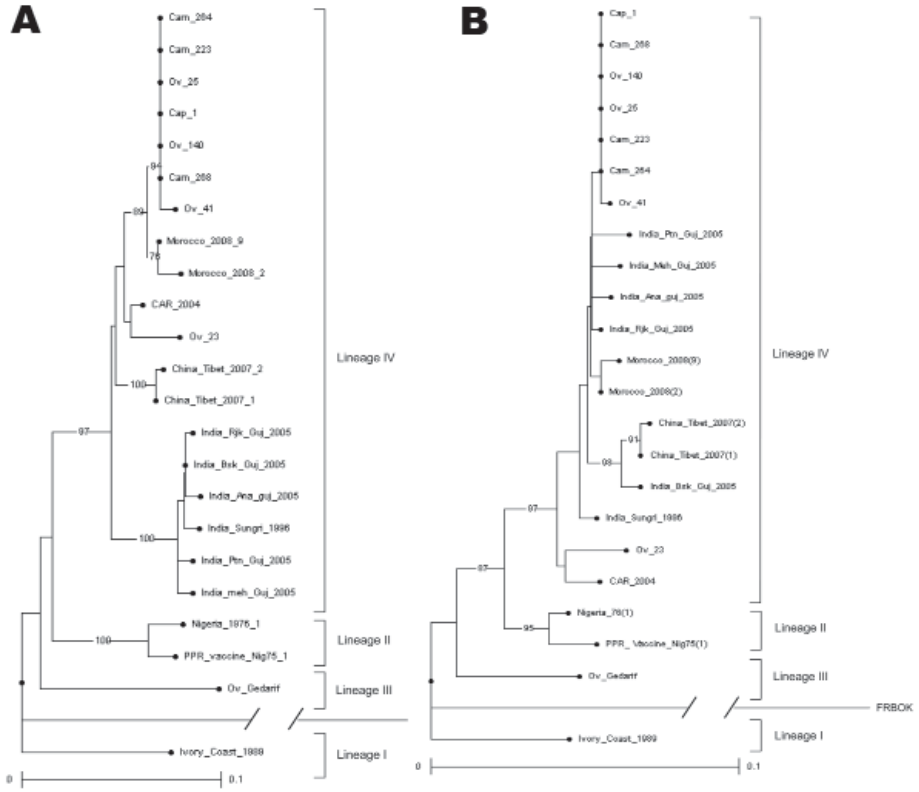

Figure 2. Phylogenetic analysis of the 3′ end nucleotide sequence of the N protein gene (A) and of the 777–1148 nt sequence of the F protein gene (B) of 11 peste des petits ruminant (PPR) virus samples selected from 2 lineage IV clusters and from lineage III as defined in Figure 1. Other designated strains were as published (*10*,*20*–*22*). The phylogram was generated by analyzing 1,000 bootstrap replicates; clusters were supported by bootstrap percentages >70%. Strains from Sudan are represented by prefixes: cam, camel; cap, caprine; ov, ovine. The Kabete 0 strain of rinderpest (RBOK) vaccine strain of rinderpest virus retrieved in GenBank (accession no. Z30697) was used as an outgroup. Scale bar indicates nucleotide substitutions per site.

Table 3. Multiple sequence alignment of F1/F2 primers and FPPRrev target sites from different PPRV isolates of lineage II compared with isolates of lineage IV*

PPRV isolate†	F1 (primer 5′), nt 777–801	F2 (primer 3′), nt 1124–1148	FPPRrev (primer 3′), nt 2055–2079
NIGERIA 75_1‡	ATCACAGTGTTAAAGCCTGTAGAGG	GCTTGTAGGTCACAAACTCAGTCTC	TCCCTAGGGCTTGTCACATTAATAT
NIGERIA 76_1	------------------------	---G--------------------	------------------------
ICV 89	------------------------	------------------------	------------------------
SUNGRI 96	------------------------	---G----------G---G-C---	------------------------
China/TibetGeg07-30	----------A----------	---G-----C-----G---G-C---	------------------------
TURKEY 00	------------------------	---G----------G---G-C---	------------------------

*FPPRrev, new reverse primer designed in this study; PPRV, peste des petits ruminants virus. Nucleotide position (numbered according to GenBank accession no. X74443 sequence). The consensus sequence corresponds to the F gene of the PPRV Nigeria 75_1 vaccine strain. F1/F2 primers from (*8*).
†Lineage and GenBank accession nos.: NIGERIA 75_1, lineage II, X74443; NIGERIA 76_1, lineage II, EU267274; ICV 89, lineage I, EU267273; SUNGRI 96, lineage IV, AY560591, China/TibetGeg07-30, lineage IV, FJ905304; TURKEY 00, lineage IV, AJ849636.
‡Vaccine strain.

Table 4. PPRV sequences analyzed from tissue samples of sheep, goats, and camels in Sudan and from sheep in Morocco, with lineage classifications, 1971 and 2000–2009*

Sample no.	Sequence available	Coordinates		Lineage classification	GenBank accession nos.		Source	Year
		X	Y		N gene	F gene		
Ov_39300	PPRV/Kuku/Khartoum/KHSUD00-1	15.617	32.6	IV-SA	HQ131933	–	Lung	2000
Cam_1	PPRV/Kassala/KSUD04-1	15.45	36.4	IV-SA	HQ131935	–	Lung	2004
Cam_3	PPRV/Kassala/KSUD04-2	15.45	36.4	IV-SA	HQ131947	–	Lung	2004
Cam_223	PPRV/Atbara/NSUD05-1	17.701	33.99	IV-SA	HQ131934	HQ131949	Lung	2005
Cam_268	PPRV/Atbara/NSUD05-2	17.701	33.99	IV-SA	HQ131936	HQ131951	Lung	2005
Cam_264	PPRV/Atbara/NSUD05-3	17.701	33.99	IV-SA	HQ131948	HQ131950	Lung	2005
Cam_304	PPRV/Tambool/BNSUD06-1	14.933	33.4	IV-SA	HQ131937	–	Lung	2006
Cam_330	PPRV/Tambool/BNUD06-2	14.933	33.4	IV-SA	HQ131938	–	Lung	2006
Cam_8	PPRV/Kassala/KSUD07	15.45	36.4	IV-SA	HQ131939	–	Isolate	2007
Cam_169	PPRV/Tambool/BNSUD07-1	14.933	33.4	IV-SA	HQ131940	–	Isolate	2007
Cam_318	PPRV/Tambool/BNSUD0-2	14.933	33.4	IV-SA	HQ131941	–	Isolate	2007
Cam_352	PPRV/Atbara/NSUD08	17.701	33.99	IV-SA	HQ131942	–	Lung	2008
Ov_1	PPRV/Abudelaiq/KSUD08	14.967	35.92	IV-SA	HQ131922	–	Lung	2008
Ov_25	PPRV/Bashagra/Gezira/BNSUD08	14.912	33.24	IV-SA	HQ131943	HQ131955	Lung	2008
Ov_140	PPRV/Gedarif/KSUD08	14.033	35.38	IV-SA	HQ131944	HQ131953	Lung	2008
Cap_1	PPRV/Rabak/BNSUD09	13.18	32.74	IV-SA	HQ131945	HQ131954	Lung/liver	2009
Cap_9	PPRV/Dongola/NSUD09	19.169	30.47	IV-SA	HQ131932	NS	Lung	2009
Ov_41	PPRV/Ed Damar/NSUD08	17.593	33.96	IV-SA + 2 mut	HQ131931	HQ131959	Lung	2008
Ov_10033	PPRV/Ed Damar/NSUD00-1	17.593	33.96	IV-SA	HQ131929	NS	Lung	2000
Ov_10034	PPRV/Ed Damar/NSUD00-2	17.593	33.96	IV-SA	HQ131930	NS	Lung	2000
Ov_Soba	PPRV/Soba/Khartoum/KHSUD00-2	15.51	32.63	IV-SA	HQ131920	NS	Isolate	2000
Ov_ Al Azaza	PPRV/Al Azaza/BNSUD00	14.204	35.54	IV-SA	HQ131917	NS	Isolate	2000
Ov_23	PPRV/Soba/Khartoum/KHSUD08	15.51	32.63	IV-SA	HQ131921	HQ131952	Spleen	2008
Ov_Gedarif	PPRV/Gedarif/KSUD71	14.033	35.38	III	HQ131918	HQ131956	Isolate	1971
Ov_Al Hilalia	PPRV/Al Hilalia/BNSUD00	14.921	33.23	III	HQ131919	NS	Isolate	2000
Ov_67496	PPRV/Abudelaiq/KSUD00	14.967	35.92	III	HQ131946	NS	Isolate	2000
Morocco_2008_1	PPRV/Morocco08-01	33.56	–6.89	IV	HQ131923	NS	Lymph	2008
Morocco_2008_2	PPRV/Morocco08-02	33.56	–6.89	IV	HQ131924	HQ131957	Lung	2008
Morocco_2008_3	PPRV/Morocco08-03	33.56	–6.89	IV	HQ131925	NS	Lymph	2008
Morocco_2008_4	PPRV/Morocco08-04	33.56	–6.89	IV	HQ131926	NS	Lymph	2008
Morocco_2008_9	PPRV/Morocco08-09	34.03	–6.8	IV	HQ131927	HQ131958	Lung	2008
Morocco_2008_11	PPRV/Morocco08-11	30.4	–9.6	IV	HQ131928	NS	Lung	2008

*PPRV, peste des petits ruminants virus; ov, ovine; IV-SA, lineage IV Saudi Arabia cluster; lung, lung tissue sample; KHSUD, Khartoum Region Sudan; cam, camel; KSUD, Kassala Sudan; NSUD, Northern Sudan; BNSUD, Blue Nile Sudan; isolate, virus isolate; cap, caprine; liver, liver tissue sample; NS, not sequenced; IV-SA + 2 mut, lineage IV, differing from Saudi Arabia cluster by 2 mutations; IV-SA, lineage IV Central Africa cluster; spleen, spleen tissue sample; lymph, lymph node sample.

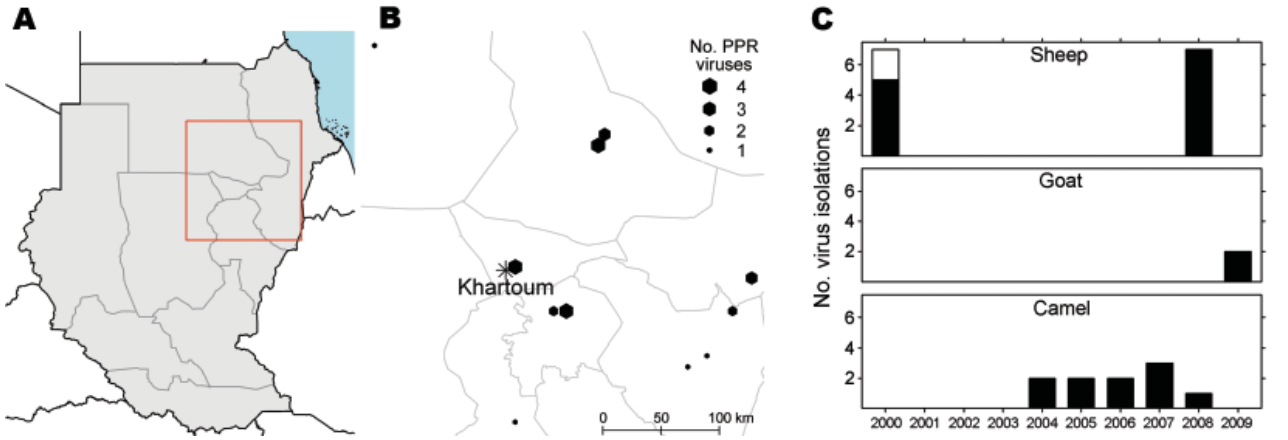

Figure 3. Distribution of samples positive for peste des petits ruminant (PPR) virus by reverse transcription PCR in Sudan for which lineage identification could be done, Sudan. A) Location of samples (red box) in Sudan. B) Locations and numbers of positive samples. C) Time distribution of virus isolations, by animal species.

Discussion

This study presents findings from PPRV surveillance in Sudan over a 10-year period and the results of tests conducted in Sudan and in Morocco during Morocco's 2008 outbreaks (*18*). Analysis of 80 samples confirmed the wide distribution of PPRV throughout Sudan, which has been known since 1971 (*2*,*13*,*15*). Genetic characterization of 26 samples positive for PPRV also provided strong evidence of the introduction and spread of Asia PPRV lineage IV in the country. Surprisingly, the number of samples containing the indigenous lineage III decreased dramatically during the study.

Viruses collected in Morocco also were classified as lineage IV. The origin of the outbreaks in Morocco remains unknown, although Ayari-Fakhfakh et al. (*30*) recently reported a PPRV seroprevalence in Tunisia of up to 7.45%, indicating that the virus is present across a wider area of northern Africa.

Analysis of the N and F genes showed that 2 clusters were identified in strains from Sudan: 1 related to a strain from Saudi Arabia, the other related to central Africa viruses. The 6 strains from Morocco collected during the 2008 outbreaks were related closely to the Saudi Arabia cluster.

Within the Saudi Arabia cluster, a remarkable genetic stability over a decade was observed; notably, all camel isolates fell into this cluster. The variability and nucleotide sequence was slightly higher for isolates in the central Africa cluster, within which only sheep were found. The inconsistent low genetic variability of isolates from Sudan may be a consequence of a species bias because of the limited contact between camels and sheep. When replicated in a single host that has a limited exposure to new variants, the viral genome thus may remain highly conservative. This hypothesis was verified previously for the measles virus over a shorter period (1997–2000). For the measles virus, it appears that the lower the level of circulation, the higher the sequence conservation (*31*).

Camels were not regarded as possible hosts for PPRV until 1992, when a number of authors reported PPRV seroconversion in these animals (*32*–*36*). The first documented outbreak of PPRV in camels, reported from Ethiopia in 1996, consisted of highly contagious respiratory syndromes with high illness rates but low death rates (*17*,*37*). The causative agent was confirmed to be a lineage III PPRV (A. Diallo, unpub. data). This present study confirmed the etiology of the disease in camels through the virologic and epidemiologic investigations and the isolation of PPRV. Surveillance of camels furthermore allowed the virus to be detected in consecutive outbreaks in Kassala, eastern Sudan (2004); Atbara, northern Sudan (2005); and Tambool, Blue Nile region, Sudan (2007) (*16*). Viruses from the same cluster also were recovered from sick sheep and goats in a large zone encompassing the eastern, northern, Blue Nile, and Khartoum regions as early as mid-2000. Under extensive pastoral farming conditions in these regions, camels may have served as a bridge with areas of northern Africa and contributed to the spread of a camel-derived strain of lineage IV, as seen in Morocco. In contrast, none of the camel and goat isolates were distributed in the central Africa cluster, although viruses of this cluster were cocirculating with camel viruses during the same period and in the same areas of northern Sudan, Khartoum, and Blue Nile.

The reason that isolates of lineage IV became predominant in Sudan, progressively replacing lineage III viruses, during the last decade remains unclear. Lineage IV has been present in Asia and in part of the Middle East for a long time, probably as low virulent strains (*5*). However, a constant rise of disease incidence recently has

been associated with this lineage and suggests increased virulence (*3*). A virulent lineage IV strain may have been introduced in Africa during the 1990s, resulting in outbreaks in both camels and small ruminants. In parallel, rinderpest virus control and eradication may have favored the decline of cross-immunity in small ruminants and their increased risk for PPRV as predicted earlier by Taylor (*38*).

Acknowledgments

We thank staff at the French Embassy of Sudan for their assistance in funding the scholarship of Y.H.A. to Centre International de Recherche Agronomique pour le Développement (CIRAD).

This study was partially supported by grants from the European Union Network of Excellence, EPIZONE (contract no. FOOD-CT-2006-016236).

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Co-infections of *Plasmodium knowlesi*, *P. falciparum*, and *P. vivax* among Humans and *Anopheles dirus* Mosquitoes, Southern Vietnam

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A single *Anopheles dirus* mosquito carrying sporozoites of *Plasmodium knowlesi, P. falciparum,* and *P. vivax* was recently discovered in Khanh Phu, southern Vietnam. Further sampling of humans and mosquitoes in this area during 2009–2010 showed *P. knowlesi* infections in 32 (26%) persons with malaria (n = 125) and in 31 (43%) sporozoite-positive *An. dirus* mosquitoes (n = 73). Co-infections of *P. knowlesi* and *P. vivax* were predominant in mosquitoes and humans, while single *P. knowlesi* infections were found only in mosquitoes. *P. knowlesi*–co-infected patients were largely asymptomatic and were concentrated among ethnic minority families who commonly spend nights in the forest. *P. knowlesi* carriers were significantly younger than those infected with other malaria parasite species. These results imply that even if human malaria could be eliminated, forests that harbor *An. dirus* mosquitoes and macaque monkeys will remain a reservoir for the zoonotic transmission of *P. knowlesi.*

Concerted control measures have considerably reduced the prevalence of malaria in Vietnam, and the parasites that cause it are now mostly restricted to forested rural areas (*1*). Forest malaria poses a special challenge for control because the exophilic and early biting habits of the mosquito vector *Anopheles dirus* render conventional vector control methods such as indoor residual spraying and insecticide treated mosquito nets difficult to apply as well as ineffective (*2–4*). The possibility of zoonotic malaria in Southeast Asian forests, because of the transmission of *Plasmodium knowlesi* from monkeys to humans (*5–10*), may form an additional complication.

Since surveys began in 2002, the forest populations of *An. dirus* mosquitoes in Khanh Phu, south-central Vietnam, have shown consistently high sporozoite infection rates (1%–2% of the thousands of specimens collected and dissected annually for microscopic examination of salivary glands), raising the question of whether all the sporozoites detected belong to species capable of infecting humans. In 2008, evidence was found for the co-infection of *P. knowlesi*, *P. falciparum,* and *P. vivax* in the salivary glands of 1 mosquito among 17 that had been processed by PCR with malaria parasite species–specific primers (*11*). Here we report the results of the PCR analysis of 72 additional sporozoite–positive salivary glands of *An. dirus* mosquitoes from the forest in Khanh Phu and of 211 blood samples from the local human population.

Study Population and Methods

Study Area

Khanh Phu (12°14′N; 108°56′E) is a commune with ≈3,000 inhabitants, mainly of the Raglai ethnic minority, who live between the forested foothills on the east side of the Truong Son mountain range in south-central Vietnam (Khanh Hoa Province), an area where malaria was previously hyper- to holo-endemic (*3*). Since 1993, the Medical Committee Netherlands–Vietnam (a Dutch nongovernmental organization) has cooperated

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DOI: 10.3201/eid1707.101551

with the National Institute of Malariology, Parasitology and Entomology in Hanoi; the Institute of Malariology, Parasitology and Entomology in Qui Nhon; and the Malaria Control Centre of Khanh Hoa Province to set up and operate the Khanh Phu Malaria Field Research Unit to provide Vietnamese malaria researchers with the opportunity to study local malaria epidemiology and develop and test improved control methods. *An. dirus* species A is currently the only malaria-transmitting species of mosquitoes in Khanh Phu. *Anopheles minimus* mosquitoes, previously the major malaria vector in the region, disappeared from Khanh Phu after 1998, following the wide-scale use of insecticide-treated mosquito nets (*3*,*12*). While the average malaria prevalence in the human population has been greatly reduced (from >50% before 1998 to 2%–3% during 2003–2009), persons who sleep overnight in the forest still run a high risk for infection. Analysis of samples from infected persons by thin-smear microscopy showed that approximately two thirds were caused by *P. falciparum*, one third by *P. vivax,* and a very small number by *P. malariae* during the 2003–2009 study period. This malaria prevalence typically affects the poorest members of the local Raglai ethnic community, whose livelihood partly depends on excursions into the forest to collect products such as bamboo and rattan, or to cultivate their plots on the mountain slopes. All residences within the commune were mapped, and all persons were registered at the research station and assigned unique code numbers based on residential location and family relations. (All methods in this study that involved human participants in the field were certified as permitted standard procedures by the National Institute of Malariology, Parasitology and Entomology in Hanoi.)

Mosquito Collection and Salivary Gland Examination

Mosquitoes were collected by human-baited landing catches at 4 collection sites in the forest and forest fringe areas near Nga Hai village in the southern part of Khanh Phu commune, from January 2008 through February 2010. Mosquito collectors were adult men of the Raglai ethnic group. They were intensively screened for malaria and promptly treated with artemisinin combination therapy if infected. The collectors worked in teams of 2 over the whole night, 1 person collected from 6:00 PM to 12:00 PM and the other from 12:01 AM to 6:00 AM. The monthly collection effort ranged between 40 and 60 person-nights per month, a total 1,285 person-nights over the 26-month period.

Anopheles species were determined on the basis of morphologic features (*13*). All *An. dirus* group mosquitoes were assumed to be *An. dirus* species A on the basis of previous accurate identifications and the known distribution of this species (*14*,*15*). Female anopheline mosquitoes were dissected for salivary glands, midgets, and ovaries, and these were examined by microscopy for the presence of sporozoites, oocysts, and parity, respectively. Sporozoite-infected glands were applied to filter paper and dried in an ambient atmosphere before storage in closed vials at 4°C–6°C.

Collection of Human Blood Samples

Blood samples were collected by 2 methods. First, to detect as many parasite-infected persons as possible, from March 8, 2009, through February 28, 2010, blood was collected by targeted active case detection from persons who had frequently worked in the forest or had reported fever. A total of 549 blood samples from 305 persons from 156 families were collected; 183 persons were sampled once, and no one was sampled >8 times; the sex ratio had a male bias (64% male). Sixty-nine of the 121 blood samples that were positive by microscopy, and an additional randomly selected 105 of the negative samples were processed by PCR to determine *Plasmodium* species. Second, from March 11, 2010, through March 30, 2010, a cross-sectional survey was undertaken in the 2 villages nearest to the mosquito collection sites. A single blood sample was taken from each of 624 residents, irrespective of symptoms or work place. One hundred and thirty-five persons in this sample had previously given samples as part of the targeted active case detection (ACD) survey; 489 had not given samples previously. From the 49 blood samples found positive by microscopy, 37 were randomly chosen for PCR processing.

Sixty-eight percent of all blood slides in both samples were from persons in the 2 southernmost villages of Khanh Phu (Nga Hai and Da Trai). This represents an area where ≈1,100 persons are living, spread over an area of 1.7 km^2 at 12°12.5′N and 108°55.5′E. All their houses are located <1 km from the nearest forest and 1–3 km away from the mosquito collection sites. *An. dirus* mosquitoes were rarely caught in these villages.

Blood was collected by finger-prick; thick and thin blood films were made for diagnosis, and blood was applied to filter paper for downstream molecular analyses. All adult volunteers provided informed consent and for children, consent was obtained from close relatives. All persons found to be infected with parasites (as diagnosed by microscopy) received treatment, according to the policy of the Vietnam Ministry of Health.

DNA Extraction and Parasite Species Identification by PCR

DNA was extracted from dried blood samples on filter paper and from sporozoite-infected glands, and subsequent malaria parasite species identification by PCR that targeted the 18S rRNA gene was carried out as previously described (*11*,*16*). Briefly, DNA was extracted by using the QIAamp

DNA micro kit (QIAGEN, Tokyo, Japan). Extracted DNA samples were stored at –20°C until use. *Plasmodium* species–specific nested PCR assays to detect and identify human malaria parasites were performed as described (*17*). For detection of the *P. knowlesi* 18S rRNA gene, the primers Pmk8 and Pmk9 were used (*5*). Because this primer set can occasionally cross-react with *P. vivax* DNA and produce false-positive results (*18*), samples were also subject to a further PCR targeting of the *P. knowlesi* circumsporozoite protein (CSP) gene as described (*8*). PCR products were separated by electrophoresis on 1.5% agarose gels and stained with ethidium bromide. Primer sequences for the gene of human *Plasmodium* species (*17*), 18S rRNA of *P. knowlesi* (*5*), and the CSP gene of *P. knowlesi* (*8*) were as previously described. *P. knowlesi* H strain (American Type Culture Collection no. 30158) (kindly donated by Satoru Kawai, Dokkyou University, Japan) was used as a positive control. We verified that no cross-reaction occurred between the primer sets used to amplify *P. vivax* CSP and *P. knowlesi* CSP by using DNA extracted from single infections of both species (data not shown).

Statistical Analysis

Student 1- or 2-tailed *t* tests were used for comparing means. Chi-square tests for association and confidence limits were calculated with Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Results

Plasmodium Infections of *An. dirus* Mosquitoes

From January 1, 2008, through February 22, 2010, a total of 6,834 female anopheline mosquitoes were captured, of which 83.2% belonged to *An. dirus s.s.*, 8.6% to *An. maculatus*, and 5.4% to *An. peditaeniatus*; the remaining 2.8% were divided between a further 11 anopheline species in very small numbers. Of the 8,331 *An. maculatus* mosquitoes dissected in Khanh Phu over >15 years, only 1 mosquito was ever found to be infected with sporozoites. None of the other *Anopheles* species, with the exception of *An. dirus*, were ever found to be infected with sporozoites. Thus *An. dirus* mosquitoes are regarded as the only malaria vectors in the Khanh Phu forest.

An. dirus mosquitoes were found during every month of sampling with an average human-biting density of 4.4 bites/person-night (range 0.3–17.4; online Appendix Table, www.cdc.gov/EID/content/17/7/1232-appT.htm). As in previous years in Khanh Phu (*3*), these mosquitoes usually reached peak densities in the dry season (February–April). Numbers were lowest during the hottest part of the year (May–August), climbed to a second peak with the onset of the rains during September–November, and usually decline again following heavy rains during October–December. The monthly average parous rate fluctuated between 65% and 90% (average 77%).

Sporozoites were detected by microscopic screening in 89 (1.57%) of 5,663 dissected *An. dirus* mosquitoes, and oocysts were observed on 0.94% of the midguts. The sporozoite rate showed some seasonal fluctuations, but these did not clearly correlate with the fluctuations in biting density. Multiplying the sporozoite rate by the human-biting rate gave an average annual entomological inoculation rate of 25.4 infective bites per person per year. This is comparable with the average rate estimated during the previous 8 years in the forest of Khanh Phu (22 infective bites per person per year, unpub. data). However, both the sporozoite rate (4.6%) and human-biting density (12 bites/person-night) reached a maximum in the last 2 months of the survey (January and February 2010). This finding implies that persons who slept unprotected in the forest during this period would have had a >50% chance of being bitten by an infected *An. dirus* mosquito during any 1 night.

Of the 86 sporozoite-positive mosquitoes, 73 underwent PCR analysis for malaria parasite detection, of which 72 were successfully assayed. Thirty-one (43%) of these 72 salivary glands were PCR positive for *P. knowlesi csp* and for *P. knowlesi* 18S rRNA. Five additional specimens were positive for *P. knowlesi* 18S rRNA but negative for *P. knowlesi* CSP and were therefore regarded as *P. knowlesi* negative (Figure 1). One specimen was only positive for *P. knowlesi* CSP and not by any other test, and therefore the species could not be confidently determined. The frequencies of *P. falciparum*, *P. vivax,* and *P. malariae* were: 50%, 50%, and 6%, respectively. In 22 (71%) of 31 glands, *P. knowlesi* was found as a co-infection with one of these other species. The combination *P. knowlesi* + *P. vivax* (in 14 glands) was far more common than *P. knowlesi* + *P. falciparum* (1 case). The combination of *P. knowlesi* + *P. vivax* + *P. falciparum* was, however, also quite common (7 glands).

The frequency of *P. knowlesi*–positive mosquitoes did not significantly differ between the 4 collections sites, and *P. knowlesi* was detected during 8 months of the past year, except in August, September, November, and December when sporozoite-infected mosquitoes were few (Figure 2). However, the highest number of sporozoite-infected mosquitoes and 13 of the 31 *P. knowlesi* infections were found in January 2010.

Plasmodium Infections of Humans

Of the 549 blood samples collected by targeted ACD from March 2009 through February 2010, 121 (22.0%) were parasite positive by microscopy, including 94 (17.1%) that were positive for *P. falciparum*. The cross-sectional survey conducted in March 2010 showed a parasite prevalence

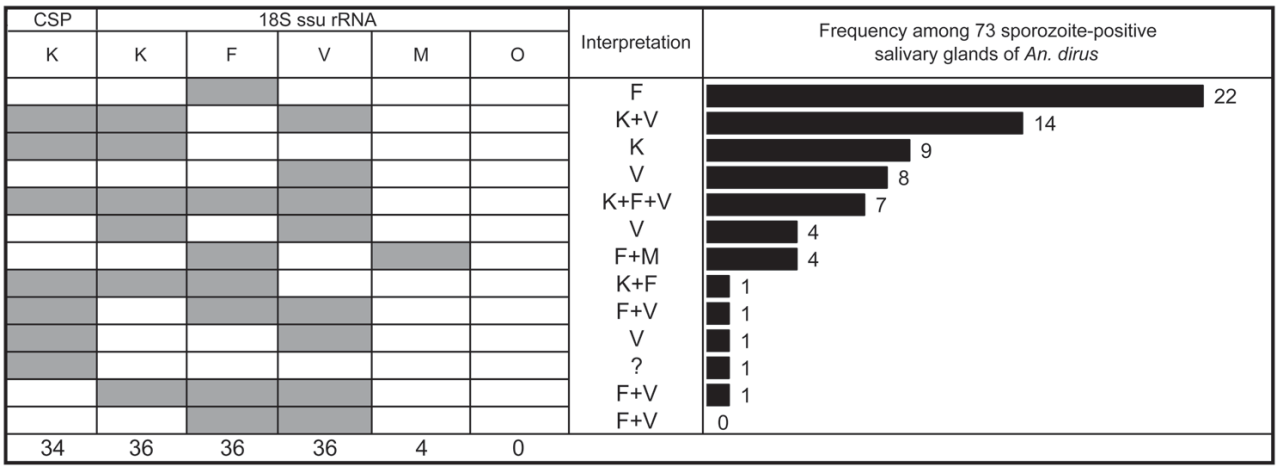

Figure 1. Results and interpretation of the PCR analyses of sporozoite-positive salivary glands of *Anopheles dirus* mosquitoes in Khanh Phu forest, Vietnam. CSP, circumsporozoite protein; ssu, small subunit; shaded cells, PCR products present; F, *Plasmodium falciparum*; V, *P. vivax*; K, *P. knowlesi*; M, *P. malariae*; ?, unknown.

of 7.9% (10.5% in male residents and 5.2% in female residents; χ^2 p = 0.013). The *P. falciparum* prevalence was 5.1% (7.3% in male residents and 2.9% in female residents; χ^2 p = 0.012).

The Table shows the results of parasite species identification by microscopy and PCR for both the targeted ACD and cross-sectional surveys. Both samples had a male bias. In the targeted ACD sample, this result reflected the skewed sex ratio in the total 549 blood samples. Among the 624 persons sampled during the cross-sectional survey the sex ratio was 50/50, but because infections were more common in men, the random subsample of 37 for PCR analysis of the 49 positive results by microscopy was also male biased. The mean age of women and men was similar, and the difference in mean age between findings of the targeted ACD and those of the cross-sectional survey was not significant (*t* test p>0.05). In the targeted ACD sample, 19 (18%) of 105 samples that were negative by microscopic examination were parasite positive by PCR. Five patients with positive results in the ACD were shown by PCR to have *P. malariae* as a co-infection, and 1 patient in the cross-sectional survey had a *P. malariae* single infection, all of which were not seen by microscopy. We were unable to determine the parasite densities of *P. knowlesi* in our samples because it was not possible to discriminate between parasite species in co-infections at low parasite densities.

A total of 32 blood samples were found to be infected with *P. knowlesi* on the basis of simultaneous positivity in the *P. knowlesi* 18S rRNA and *P. knowlesi csp* PCR tests: 19 (51%) of 37 malaria-infected persons in the cross-sectional survey, and 13 (15%) of 88 PCR-positive cases collected by targeted ACD. Four additional specimens in the ACD sample were positive for *P. knowlesi* 18S rRNA but negative for *P. knowlesi csp*, all in co-infections with other parasite species, most commonly *P. vivax*. Human blood samples positive for *P. knowlesi csp* and negative for *P. knowlesi* 18S rRNA were not found.

P. knowlesi infection was detected by targeted ACD in 8 of 12 months. On the basis of the PCR results of the samples from the ACD survey, the annual *P. knowlesi* incidence can be estimated at 10/1,000 person-years (95% confidence limit 4%–17%). The results from the cross-sectional survey imply a peak *P. knowlesi* prevalence in March 2010 among the population of the southern villages of 12.6% (95% confidence limit 6.3%–21.1%). The large increase in *P. knowlesi* co-infections in humans in March 2010 correlates with the increased frequency of this parasite in the mosquitoes in the preceding months.

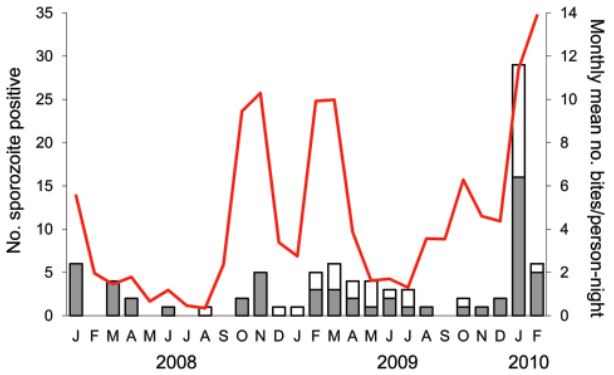

Figure 2. Dynamics of biting density and sporozoite positive salivary glands (including *Plasmodium knowlesi)* of *Anopheles dirus* mosquitoes in Khanh Phu forest, Vietnam. The solid red line connects the points of mean monthly *An. dirus* density (bites/person-night). Bars indicate the monthly number of sporozoite-positive salivary glands: white, *P. knowlesi*; gray, other species. The first mosquito found with *P. knowlesi* in August 2008 was described in Nakazawa et al. (*11*).

Table. Frequencies of malaria parasite species determined by microscopy and PCR in 2 samples of human blood from residents of Khanh Phu commune, Vietnam, 2009–2010*

Test categories	Active case detection†			Cross-sectional survey‡		
	Men	Women	Total	Men	Women	Total
No. tested	110	64	174	25	12	37
Patient mean age, y	25.7	25.1	25.5	20.7	20.0	20.4
By microscopy determination						
Total no. parasite positive	47 (43)	22 (34)	69 (40)	25 (100)	12 (100)	37 (100)
No. *Plasmodium falciparum* positive	38 (35)	15 (23)	53 (30)	16 (64)	4 (33)	20 (54)
No. *P. vivax* positive	14 (13)	8 (13)	22 (13)	13 (52)	8 (67)	21 (57)
By PCR determination						
Total no. parasite positive	60 (55)	28 (44)	88 (51)	25 (100)	12 (100)	37 (100)
No. *P. falciparum* positive	49 (45)	19 (30)	68 (39)	21 (84)	6 (50)	27 (73)
No. *P. vivax* positive	28 (25)	14 (22)	42 (24)	18 (72)	9 (75)	27 (73)
No. *P. knowlesi* positive	8 (7)	5 (8)	13 (7)	12 (48)	7 (58)	19 (51)
No. *P. malariae* positive	1	4	5	1	0	1
No. *P. ovale* positive	0	0	0	0	0	0

*Values are no. (%) except as indicated. All percentages are calculated over the total no. samples tested in each column.
†The active case detection sample consisted of 174 blood samples of 549, including 69 of 121 found positive by microscopy.
‡In the cross-sectional survey, blood was taken from 624 persons, 49 of which were found positive by microscopy. A random selection of 37 blood samples of these 49 positive samples was analyzed by PCR.

Twenty-eight of the 32 *P. knowlesi* infections were found in persons of Raglai ethnicity, the dominant group in the study area. However, 2 of the 5 persons of Trinh ethnicity and 2 of the 7 persons of Kinh ethnicity sampled also had a *P. knowlesi* co-infection; all had asymptomatic cases detected during the cross-sectional survey.

The average age of persons with *P. knowlesi* co-infections was 15.0 years in the targeted ACD survey and 16.9 years in the cross-sectional survey; both ages were significantly lower than the ages of *P. knowlesi*–infected persons in both samples (24.8 and 26.2 years, respectively; 2-tailed *t* test $p<0.001$; Figure 3, panel A) and those of all other positive case-patients (24.8 and 24.1 years, respectively; 2-tailed *t* test $p< 0.01$; Figure 3, panel B). *P. falciparum*–infected persons had a mean age of 23.4 years, which did not differ significantly from the mean age of 25.6 years for *P. falciparum*–negative persons (2-tailed *t* test $p>0.3$; Figure 3, panel C).

Six (19%) of the 32 *P. knowlesi* co-infected persons had fever. This finding was not significantly different from the 10% with fever among 86 uninfected persons in the PCR-analyzed sample (χ^2 $p = 0.23$). Only persons with single *P. falciparum* infections were significantly more often febrile (30%) than uninfected persons (χ^2 $p = 0.003$).

In the human blood samples, *P. knowlesi* was only ever found in a co-infection, almost always with *P. vivax* and often with *P. falciparum* in addition. The lack of the *P. knowlesi* and *P. falciparum* without *P. vivax* combination (only 1 case each in the mosquito and human samples; Figure 4, panels A and B) contributed most strongly to the significance of the nonrandom distribution (χ^2 $p<0.001$). *P. knowlesi* infection was equally frequent among PCR-positive women and men, whereas *P. falciparum* was significantly more frequent among the PCR-positive men (Table).

Conclusions

P. knowlesi occurs frequently in humans in Khanh Phu as well as in the *An. dirus* mosquito population in nearby forests. This finding, in combination with the increasing number of reports of *P. knowlesi* infections from Thailand (*6*) and Myanmar (*10*), in similar forested environments, highlights the wide range of this pathogen in humans in Southeast Asia.

Our results provide additional information about vector bionomics and the clinical manifestation and epidemiology of *P. knowlesi* in a native population. *P. knowlesi* occurs in high frequencies as co-infections in *An. dirus* species A mosquitoes, which is the only human malaria vector in this area (*3*). Other anopheline species thus far proven to transmit *P. knowlesi* between macaque monkeys and humans in Southeast Asia, *A. cracens* and *A. latens* (*8*,*19*), belong, together with *An. dirus* species, to the Leucosphyrus group. Due to the widespread distribution of several *An. dirus* species in Southeast Asia (*14*) a prominent role of *An. dirus* sensu lato in sylvatic and zoonotic malaria transmission seems likely.

In our study, *P. knowlesi* infections in humans were always associated with infections of other *Plasmodium* species. This finding was in contrast to infections in mosquitoes, in which *P. knowlesi* single infections were relatively common. Furthermore, the combination of *P. falciparum* + *P. knowlesi* was far less common in both humans and mosquitoes than would be expected by chance. This may suggest a degree of interaction between these species that precludes the establishment of co-infections. Such nonrandom association was, however, not found in studies from Thailand, Malaysia, and Myanmar (*6*,*8*,*10*).

Even though known to be potentially dangerous for humans (*20*–*22*), the *P. knowlesi* co-infections described

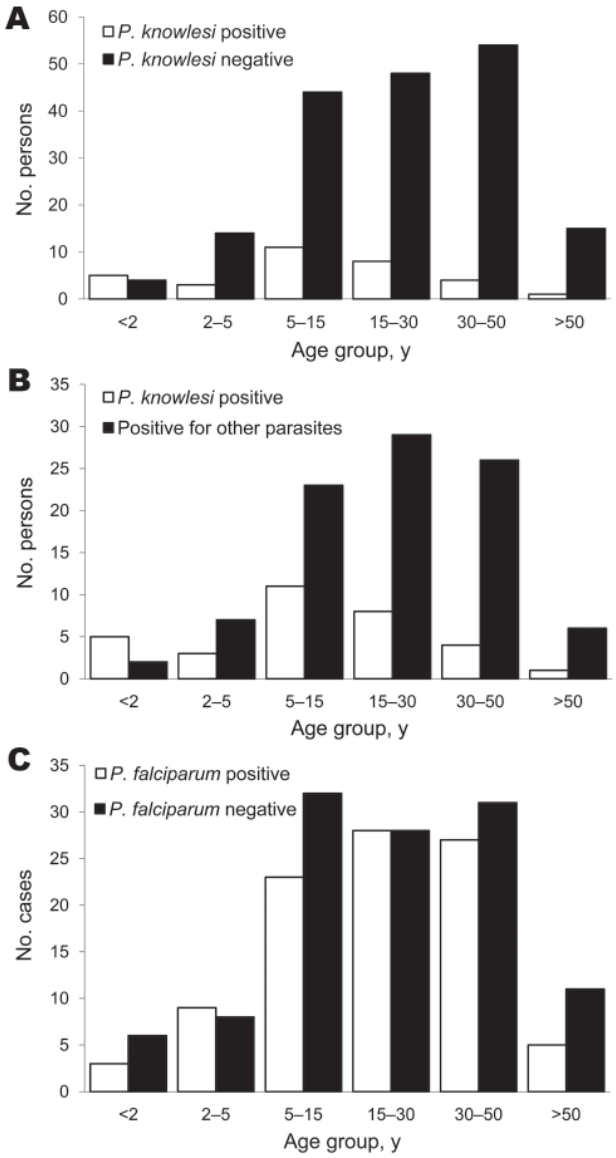

Figure 3. Age analysis of persons tested for *Plasmodium knowlesi* infection, Khanh Phu, Vietnam. A) Age groups of *P. knowlesi*–positive persons (n = 32; mean age 15.8 y) compared with *P. knowlesi*–negative persons (n = 179; mean age 26.2 y); p = 0.0004 (significant) by 2-tailed *t* test with unequal variance. B) Age groups of *P. knowlesi*–positive persons compared with ages of those positive for other parasites (n = 93; mean age 24.5 y); p = 0.004 (significant) by 2 tailed *t* test with unequal variance. C) Age groups of *P. falciparum*–positive persons (n = 95; mean age 23.4 y) compared with *P. falciparum*–negative persons (n = 116; mean age 25.6 y); p = 036 (not significant) by 2-tailed *t* test with unequal variance.

here did not lead to severe disease or fever and were concentrated in a group of ethnic minority families who often work in or near the forest, confirming a report from a neighboring area (*23*). The lack of symptoms, high sensitivity of the PCR method, and the fact that neither *P. knowlesi* nor *P. malariae* (which is often mistaken for *P. knowlesi*) was observed by microscopy suggest that the human *P. knowlesi* co-infections in this area cause very low levels of parasitemia. *P. knowlesi*–infected persons were on average significantly younger (15.8 years) than those infected with other species (23.9 years), which suggests that natural immunity is more easily acquired against *P. knowlesi* than against *P. falciparum* and *P. vivax*.

Macaques are common in the forests of Khanh Phu and are likely to be bitten by the same *An. dirus* population that bites humans. Whether these monkeys harbor a zoonotic malaria reservoir of *P. knowlesi* or if the parasite is also transmitted from person to person is currently unknown and requires further investigation. In this study, only *P. knowlesi, P. falciparum, P. vivax,* and *P. malariae* were investigated. Therefore whether *P. knowlesi* is the only malaria parasite in monkeys transmitted by *An. dirus* mosquitoes in this area is unknown.

Recent discoveries have demonstrated the relative ease and frequency at which malaria parasites may have switched between hosts (*24*–*29*). The factors that influence the probability of such host switching are likely to be many and varied, but the presence of a mosquito vector that brings

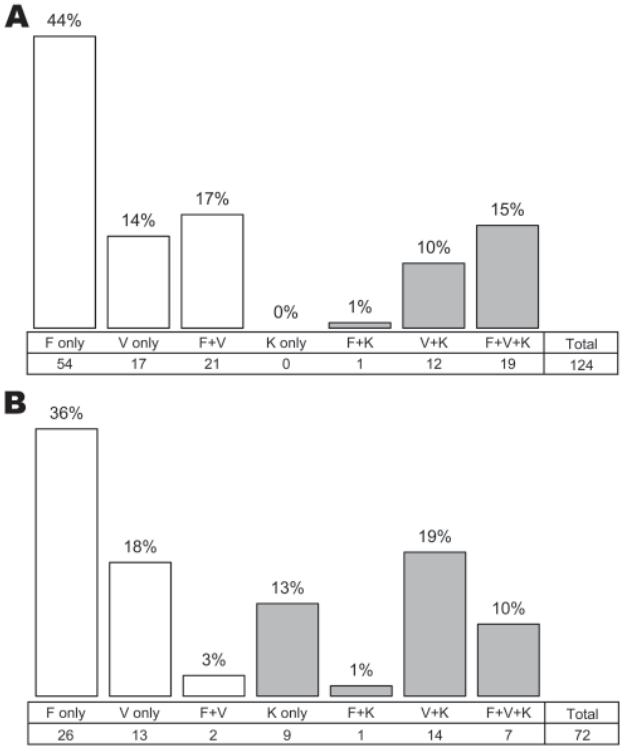

Figure 4. Frequency of single and co-infections among 124 human blood samples (A) and 73 mosquito salivary glands (B) positive for *Plasmodium* spp. infection by PCR. *P. malariae* was discarded. Gray bars indicate *P. knowlesi* infection or co-infection. F, *P. falciparum*; V, *P. vivax*; K, *P. knowlesi*.

the parasites in contact with different hosts must be a key precondition. *An. dirus* species A appears to fit this role.

Epidemiologic and Public Health Implications

These findings may fundamentally change the perspectives for the control of forest malaria. Previously, forest malaria may have been considered manageable because the parasite reservoir in the forest may be reduced through intensive case detection and treatment of human communities living in or near the forests. The likely presence of a nonhuman reservoir of *P. knowlesi* (and possibly other parasites) in monkeys and of a mosquito vector that intensively inoculates parasites among monkeys and humans reinforces the need to find methods of vector control or biting prevention that can be applied to *An. dirus* mosquitoes.

Acknowledgments

We thank Nguyen Son Hai, Phan Chau Do, Dang Duy Vu and Nguyen Le Dung for their help in collecting and processing the mosquito and blood samples; the mosquito collectors for their hard work and long nights in the forest; and the residents of Khanh Phu for their willingness to contribute blood samples over many years. We also thank the Provincial Health Service and Malaria Control Centre of Khanh Hoa Province as well as the authorities of Khanh Vinh district and Khanh Phu municipality for their administrative support.

The Khanh Malaria Research team and the field research costs were financially supported through private donations to the Medical Committee Netherlands–Vietnam. The laboratory work in Japan was partly supported by a grant-in-aid for the Japanese-Vietnam Collaborative Research Program from the Japan Society for the Promotion of Science and a grant-in-aid for Scientific Research from Fujita Health University.

Mr Marchand has been a senior advisor for the Medical Committee Netherlands–Vietnam for 20 years. He has developed and guided various projects in malaria and dengue operational research as well as in rural development among poor mountainous communities in Vietnam.

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Epidemiology of Influenza-like Illness during Pandemic (H1N1) 2009, New South Wales, Australia

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To rapidly describe the epidemiology of influenza-like illness (ILI) during the 2009 winter epidemic of pandemic (H1N1) 2009 virus in New South Wales, Australia, we used results of a continuous population health survey. During July–September 2009, ILI was experienced by 23% of the population. Among these persons, 51% were unable to undertake normal duties for ≤3 days, 55% sought care at a general practice, and 5% went to a hospital. Factors independently associated with ILI were younger age, daily smoking, and obesity. Effectiveness of prepandemic seasonal vaccine was ≈20%. The high prevalence of risk factors associated with a substantially increased risk for ILI deserves greater recognition.

During winter 2009, Australia experienced a strong influenza epidemic, caused by the pandemic (H1N1) 2009 virus. In New South Wales (NSW), the most populous state of Australia (≈7 million persons), the epidemic lasted from late June through early September (*1*). Despite intense surveillance and response efforts, determining the epidemiology of influenza at the whole-population level remains difficult, and considerable uncertainty about the disease remains because only a small proportion of infected persons are tested (*2*).

Survey methods have been infrequently used to assess the epidemiology of pandemic influenza virus infection in the general population. In 1919, a personal household interview survey using a sample of population census districts from large population centers was used to assess illness associated with the first wave of pandemic influenza in the Unites States. Persons called intelligent inspectors determined whether the household member was "sick since September 1, 1918, with influenza, pneumonia, or indefinitely diagnosed illness suspected to be influenza." The survey demonstrated substantial demographic, geographic, and socioeconomic variation in the apparent attack rate of influenza. Expressed as a percentage, the overall incidence rate of clinical infection was estimated to be ≈28% during the first wave. The incidence rate was ≈35% in children and declined with age to ≈30% in adults <35 years of age and to ≈10% in persons >75 years of age. Incidence was higher among women 15–35 years of age than among men in the same age group (*3*,*4*).

During the first epidemic wave of pandemic (H1N1) 2009, we used a continuous population health survey to better understand the epidemiology of the influenza (H1N1) virus in the general community. This situation also created an unprecedented opportunity to assess the prevalence of seasonal influenza vaccination among persons of all age groups in our population and its effectiveness against ILI during pandemic (H1N1) 2009.

Methods

Since 2002, the NSW Population Health Survey has been operating continuously to provide monthly estimates of health status and risk factors. The survey involves computer-assisted telephone interviews of a randomly selected member of randomly selected households. The target population is state residents in private households with private telephones. The sample is selected by using telephone number ranges obtained from an electronic telephone book that has been geocoded (spatial coordinates assigned to addresses) and stratified by 8 regional health

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DOI: 10.3201/eid1707.101173

service boundaries within the state. List-assisted random-digit dialing is then used to contact households. The target sample is ≈1,500 persons per regional health service per year, which equals 12,000 persons per year for the state. The survey covers all age groups, and interviews for children <16 years of age are reported by a parent or caregiver. Full details of sample selection and procedures are provided by Barr et al. (*5*). The survey has been approved by the NSW Population Health and Health Services Research Ethics Committee.

When circulation of pandemic (H1N1) 2009 virus in NSW became apparent, ethics approval was obtained to add supplementary questions to the survey to determine the incidence of influenza–like illness in the population and associated health care–seeking behavior and absence from normal duties; these questions were added on July 19, 2009. The questions were as follows: "In the last 4 weeks, did you have an illness with any of the following symptoms: fever or high temperature, cough, sore throat, runny nose, fatigue, chills or shakes, body aches and pains, shortness of breath, the flu or flu–like symptoms?" Responses were recorded for each sign or symptom. Respondents answering "yes" to any sign or symptom were asked the following: "Did you see a GP for this illness?"; "Did you go to a hospital or emergency department for this illness?"; and "How many days were you unable to work, study, or manage day-to-day activities because of the illness?" At the same time, we extended the age range for respondents routinely asked whether they had been "vaccinated or immunized against flu in the past 12 months" to persons ≥6 months of age. Previously, the question had been asked only of persons ≥50 years of age. The extended age range would enable assessment of vaccine effectiveness against ILI.

We defined ILI as self–reported fever or high temperature with cough and fatigue. In a range of general practice surveillance systems for seasonal influenza in Australia, this definition performed better than alternative definitions; positive predictive value was 23%–60% and negative predictive value was 64%–91% (*6*).

To obtain monthly estimates of ILI incidence, for any respondents reporting a symptom in the past 4 weeks we assigned their illness to the middle of the reference period, that is, 14 days before the interview date. This illness date was then assigned to a month of illness.

Answers to other questions routinely asked in the survey enabled analysis of additional factors that might be associated with ILI reporting (*7*), including age (0–15, 16–34, 35–49, 50–64, or ≥65 years); sex; household size (1–2 or ≥3 residents); number of children in household (<2 or ≥2); urban or rural location of residence; socioeconomic disadvantage at respondent's residential postal code, which is derived from the Australian Census and takes into account income, education, occupation, employment status, indigenous status, housing, and other variables (index of relative socioeconomic disadvantage [*8*]: lowest 2 quintiles = disadvantaged and upper 3 quintiles = not disadvantaged); current asthma (respondents ≥2 years of age, diagnosis made by a doctor, and symptoms or treatment in the past 12 months); nongestational diabetes or high blood glucose status (≥9 years, diagnosis made by a doctor); smoking status of persons ≥16 years of age (daily smoker, occasional smoker, ex-smoker, or nonsmoker); body mass index ([BMI] [weight in kilograms]/[height in meters]^2) of persons ≥2 years of age (≥30 = obese, 25 to <30 = overweight, and <25 = healthy or underweight); alcohol drinking at levels associated with health risk (>2 Australian standard drinks [1 standard drink = 10 g alcohol] on any day) (*9*); adequate physical activity for persons ≥16 years of age (at least 150 minutes exercising per week on ≥5 occasions, adequate or not adequate), psychological distress score for persons ≥16 years of age (Kessler–10 scale ([*10*] high or very high [score ≥22], moderate, or low); and vaccinated against pneumococcal disease in the past 5 years for persons ≥50 years of age (yes or no).

As is standard for household population surveys, the data record for each survey respondent was assigned a numeric weighting, which was used in all analyses to scale their results to the total NSW population. The weighting value takes into account the probability (by age, sex, and geographic region) of being selected for participation in the survey (*5*). Regression models were used to obtain the relative risk (RR) of reporting ILI for each of the factors listed in the previous paragraph. The dependent variable for each model was ILI, which was assigned 1 of 2 values: 1 if the respondent met the criteria for ILI and 0 otherwise. The independent variables were ≥1 factor.

Our modeling strategy was to individually test the association between each factor and ILI by using a regression model and then to develop a final model incorporating multiple factors to assess whether independent associations remained. Because age was strongly associated with ILI, we included it as an independent variable in the model for all single-factor assessments. Factors with $p<0.1$ for an individual association were included in the final model. Despite its nonsignificance, sex was included in the final model because the prevalence of risk factors in our population was known to differ by sex.

To estimate RRs from survey data instead of the more usual odds ratios, we used Poisson regression analysis with robust variance estimation. RRs were calculated by using the GENMOD procedure included in SAS Statistical Analysis Software version 9.1.3 for Windows (Cary, NC, USA) with the following programming statements: a model statement with options dist = Poisson and link = log; a class statement including the unique survey

respondent number variable; a repeated statement with an independent correlation structure (corr = ind) and specifying the unique survey respondent number variable as the subject parameter; and a weight statement specifying the respondent sample weighting normalized to sum to the total sample size (*11*–*13*). Because the Poisson model uses the natural logarithm as the link function, exponentiation of the parameter estimates was used to obtain the RR for the study factors.

Vaccine effectiveness for ILI was estimated by 1–RR. RR was the age-adjusted relative risk of reporting ILI among respondents reporting seasonal influenza vaccination in the past 12 months relative to that for unvaccinated respondents. RRs were obtained from the regression analysis (*14*).

Results

Incidence of ILI

From July 19 through October 14, 2009, completed interviews were obtained from 2,909 respondents from 5,017 eligible households contacted during that period. Participation rate was 58.0%.

During July 2009, estimated ILI incidence was 12.1% (95% confidence interval [CI] 9.1%–15.0%), representing 850,000 (95% CI 640,000–1,060,000) persons (Table 1). Incidence declined to 7.4% (95% CI 5.3%–9.5%) in August, and 3.6% (95% CI 1.2%–5.9%) in September. Assuming that during the 3-month window each person could only experience ILI 1 time, the monthly incidence can be summed to provide an estimate of the total proportion of the population experiencing ILI during that period. This calculation indicated that an estimated 23.1% (95% CI 18.8%–59.9%) of the NSW population, or 1,630,000 (95% CI 1,330,000–4,240,000) persons, experienced ILI during that period.

The only significant difference was the low incidence for persons ≥65 years of age (5.1%; 95% CI 2.4%–7.9%), compared with estimates of 19.1% (95% CI 11.9%–26.2%) for those 50–64 years of age and 33.3% (95% CI 24.7%–42.0%) for those 0–15 years of age (Table 2). Estimates were higher for female than male respondents and for residents of rural areas, but these differences were not significant.

Outcome of ILI

Inability to undertake normal duties for ≤3 days was reported by approximately half (51%, 95% CI 41%–61%) of those reporting ILI (Table 3). Another 12% (95% CI 6%–18%) were unable to continue their usual duties for at least 7 days. Approximately half (55%) sought care at a general practice (95% CI 46% – 65%), and 5% (95% CI 1%–9%) sought care at a hospital.

Table 1. Influenza-like illness cases, by time period, New South Wales, Australia, July–September 2009*

Period	Estimated no., millions	Estimated incidence, % (95% CI)
July	0.85 (0.64–1.06)	12.1 (9.1–15.0)
August	0.52 (0.38–0.67)	7.4 (5.3–9.5)
September	0.25 (0.09–0.42)	3.6 (1.2–5.9)
July–September	1.63 (1.33–1.94)	23.1 (18.8–27.4)

*Data from 2,909 New South Wales Population Health Survey respondents. Influenza-like illness defined as fever with cough and fatigue. CI, confidence interval.

Prevalence of Seasonal Influenza Vaccination

When we calculated prevalence of seasonal influenza vaccination for all age groups, we found that one quarter (25%, 95% CI 23%–28%) had been vaccinated against influenza in the previous 12 months. The proportion was highest among persons ≥65 years of age (74%, 95% CI 71%–78%), fell to 33% (95% CI 29%–38%) among those 50–64 years, and was <20% among those <50 years (Table 4). Previous estimates in NSW were based on persons ≥50 years of age because national immunization policy focused on this age group.

Factors Associated with Reporting ILI

Younger age was strongly associated with ILI (Table 5), which was ≈6× more likely to be reported for children <15 years of age than for persons ≥65 years of age. Therefore, for evaluation of all other individual factors, we adjusted for age. Only obesity and daily smoking were positively associated with reporting ILI. All remaining factors showed no significant association. In the final regression model, we included age, sex, BMI, and smoking status. Although sex was not associated with ILI, obesity and smoking in our population varied substantially by sex and were included in the model. Only persons ≥16 years of age were included because 16 years was the youngest age for which smoking questions were asked.

Table 2. Influenza–like illness incidence, New South Wales, Australia, July–September 2009*

Group	Incidence, % (95% CI)
Sex	
M	19.5 (13.8–25.2)
F	26.9 (20.4–33.4)
Age group, y	
0–15	33.3 (24.7–42.0)
16–34	27.6 (15.6–39.6)
35–49	21.3 (13.9–28.8)
50–64	19.1 (11.9–26.2)
≥65	5.1 (2.4–7.9)
Region	
Urban	22.3 (16.8–27.8)
Rural	26.2 (18.4–34.0)

*Data from 2,909 New South Wales Population Health Survey respondents. Influenza-like illness defined as fever with cough and fatigue. CI, confidence interval.

Table 3. Illness outcomes for persons reported influenza–like illness, New South Wales, Australia, July–September 2009*

Outcome	Estimated no. persons, millions (95% CI)†	Proportion, % (95% CI)
No. days unable to do usual activities		
0–3	0.83 (0.67–1.00)	51.3 (41.4–61.1)
4–7	0.60 (0.44–0.76)	36.7 (26.9–46.5)
>7	0.20 (0.09–0.30)	12.0 (5.7–18.4)
Type of health care sought		
General practice	0.90 (0.74–1.06)	55.4 (45.5-65.2)
Hospital	0.08 (0.02–0.15	5.2 (1.3–9.2)
General practice and hospital‡	0.04 (0.01–0.07	2.3 (0.5–4.1)
Neither general practice nor hospital	0.68 (0.53–0.83)	41.7 (32.3–51.1)

*CI, confidence interval.
†The estimated numbers were obtained by applying the proportions and 95% CIs in this table to the estimated 1.63 million persons with influenza–like illness (Table 1). Standard errors of that estimate were ignored.
‡Because this category overlaps the 2 categories above, total is not 100%.

The association with younger age remained in the final model (Table 6). Among the 13.0% (95% CI 10.8%–15.1%) of the population ≥16 years of age who reported daily smoking, the risk for ILI was 90% (95% CI 10%–226%) higher than that for less frequent smokers and nonsmokers combined. Among the 18.0% (95% CI 15.9%–20.1%) whose BMI was in the obese category, the risk for ILI was 132% (95% CI 30%–316%) greater than that for the combined group of persons whose BMI was healthy or underweight.

Effectiveness of Seasonal Influenza Vaccine

When the age-adjusted RR for ILI among persons reporting vaccination with the seasonal influenza vaccine in the past 12 months was used, the estimated vaccine effectiveness was 20.0% (95% CI –30.5% to 51.0%), indicating a possibly mild but nonsignificant benefit. Analysis of effectiveness in specific age groups and by sex, region, smoking status, or obesity did not indicate any significant benefit (Table 7).

Discussion

In NSW, during the first Southern Hemisphere winter in which pandemic (H1N1) 2009 virus was circulating, at least one quarter of the population and one third of children experienced ILI. Many infections other than influenza can cause ILI (*15*,*16*); however, this study was conducted during the peak months of the epidemic in NSW, when the predictive value of ILI for influenza infection would be optimal (*6*). The epidemic was recognized in Australia after mid-June and grew rapidly (*1*). We were able to obtain full monthly estimates of ILI from July only. Late June was part of the recall period of the survey questions for respondents interviewed in July, and some of that activity may have been included in the July estimate.

ILI incidence was similar in urban and rural regions and in each sex. Approximately half the persons who reported ILI had to limit their usual activities for <4 days. Approximately half sought care for their illness at a general practice, and 5% sought care at a hospital. Vaccination against seasonal influenza did not protect against ILI. Daily smoking and obesity each independently doubled the risk for ILI.

Consistent with the known epidemiology of pandemic (H1N1) 2009 virus infection (*1*,*17*–*19*), incidence of ILI decreased with age; the decline was sharp for those ≥65 years of age. The age-specific estimates of ILI incidence in NSW in 2009 were remarkably similar to those reported during the 1918 influenza pandemic in the United States (*4*). Our overall estimate of an ILI rate of 23% was higher than the overall population infection rate for pandemic (H1N1) 2009 of 16% estimated by a recent seroprevalence study from NSW (*20*). Although the CIs of both estimates overlapped and thus the estimates did not differ significantly, explanations for our higher estimate could be as follows: 1) some of the ILI in our study was caused by other influenza strains that circulated earlier in the season (*1*) and by pathogens other than influenza; 2) our study included information collected through the end of September, whereas the seroprevalence study included some specimens collected before the end of the epidemic

Table 4. Prevalence of seasonal influenza vaccination in past 12 months, New South Wales, Australia, July–September 2009*

Group	Prevalence, % (95% CI)
Total NSW population	25.4 (23.3–27.5)
Sex	
M	24.6 (21.3–27.9
F	26.3 (23.7–28.8
Age, y	
0–15	11.5 (7.5–15.5)
16–34	14.8 (10.2–19.3
35–49	16.0 (11.7–20.2
50–64	33.2 (29.0–37.5)
≥65	74.3 (70.7–77.9
Region	
Urban	24.7 (22.1–27.3)
Rural	27.2 (23.7–30.7)

*Data from 2,909 total New South Wales Population Health Survey respondents. CI, confidence interval; NSW, New South Wales.

Table 5. Risk factors evaluated for influenza-like illness, New South Wales, Australia, July–September 2009*

Factor	Factor prevalence, % (95% CI)	Reference category†	Age group analyzed (no. respondents)‡	Relative risk (95% CI)	p value§
Age, y		≥65 y	All ages (2,909)		
0–15	21.4 (19.4–23.5)			5.96 (3.25–10.93)	**<0.001**
16–34	26.0 (23.2–28.8)			4.76 (2.35–9.62)	**<0.001**
35–49	21.7 (19.4–23.9)			3.55 (1.86–6.79)	**<0.001**
50–64	17.7 (16.1–19.3)			3.00 (1.57–5.71)	**0.001**
Female sex¶	50.5 (47.8–53.2)	Male	All ages (2,909)	1.31 (0.89–1.94)	0.173
Rural region¶	29.9 (28.4–31.4)	Urban	All ages (2,909)	1.26 (0.87–1.84)	0.227
Socioeconomically disadvantaged¶ #	38.4 (35.9–40.9)	Not disadvantaged	All ages (2,909)	0.93 (0.63–1.38)	0.729
≥2 children in household¶	34.5 (31.9–37.2)	<2 children residing in household	All ages (2,909)	0.75 (0.51–1.10)	0.142
≥3 persons in household¶	67.7 (65.6–69.7)	1–2 persons in household	All ages (2,909)	1.14 (0.75–1.72)	0.536
Seasonal influenza vaccination in past 12 mo¶	25.6 (23.5–27.7)	No seasonal influenza vaccination in past 12 mo	≥6 mo (2,888)	0.80 (0.49–1.31)	0.372
Current asthma¶	11.4 (9.8–13.0)	No current asthma	≥2 y (2,831)	1.15 (0.69–1.92)	0.595
Body mass index¶			≥2 y (2,831)		
Obese	18.0 (15.9–20.1)	Healthy or underweight		2.14 (1.31–3.48)	**0.002**
Overweight	28.6 (26.3–31.0)	Healthy or underweight		1.06 (0.67–1.67)	0.816
Current diabetes or high blood glucose¶	8.0 (6.7–9.2)	No current diabetes or high blood glucose	≥9 y (2,636)	0.97 (0.50–1.91)	0.936
Daily smoker¶	13.0 (10.8–15.1)	Occasional/ex-/non-smoker	≥16 y (2,431)	1.94 (1.06–3.54)	**0.031**
Inadequate physical activity¶	48.6 (45.6–51.7)	Adequate physical activity	≥16 y (2,431)	1.04 (0.65–1.65)	0.881
Risky alcohol drinking¶	31.6 (28.6–34.7)	Low-risk alcohol drinking	≥16 y (2,431)	1.25 (0.76–2.05)	0.378
High or very high psychological distress score¶	11.2 (9.4–13.1)	Moderate or low psychological distress score	≥16 y (2,431)	1.41 (0.85–2.35)	0.182
Pneumococcal vaccination in past 5 y¶	28.3 (25.7–30.9)	No pneumococcal vaccination in the past 5 y (adjusted for age)	≥50 y (1,633)	1.20 (0.56–2.56)	0.640

*CI, confidence interval.
†Not stated and "Don't know" responses were included in the reference category. Among parameters with any such responses, the proportions were index of socioeconomic disadvantage 0.9%, vaccinated against influenza in the past 12 mo 1.0%, current asthma 0.3%, body mass index 5.2%, smoking status 0.1%, psychological distress score 3.0%, alcohol use 1%, and pneumococcal vaccination 7.0%.
‡Some questions are only collected on selected age groups, so sample sizes vary.
§Significant results at the 5% level are in **boldface**.
¶Adjusted for age.
#Disadvantaged = lowest 2 quintiles of the Australian index of relative socioeconomic disadvantage based on the respondent's residential postcode; not disadvantaged = upper 3 quintiles.

during August; and 3) population samples differed. However, the seroprevalence study detected mild and asymptomatic infections. Because the seroprevalence study used specimens requested from clinical chemistry laboratories without randomization, statistical biases may arise from the nonrandom sample selection and the disease factors leading to a clinical specimen being required. Age-specific comparisons between the 2 studies were broadly consistent. Serosurvey infection rate estimates were 10%–35% among children, 24% among adults 18–34 years of age, 20% among adults 35–64 years of age, and no infection among older adults. Our estimates for ILI were 33% among children, 28% among persons 16–34 years of age, 20% among adults 35–64 years of age, and 5% among older adults. The seroprevalence study estimated reduced infection rates outside the urban state capital of Sydney; our study found similar incidence rates in urban and rural areas.

Other studies that assessed household size and risk for transmission found mixed results: some found increased risk (*21*,*22*) and another found decreased risk (*23*) with increasing household size. A higher number of children in a household has also been identified as a risk factor for influenza transmission in households (*24*). However, our finding of no association with either household size or number of children in the household is consistent with the result of household transmission studies of the pandemic 2009 (H1N1) virus (*18*,*25*) and with results of another study of ILI among children during seasonal influenza season in Australia (*26*). This lack of effect may reflect improved living standards in this country.

The lack of protection from recent seasonal influenza vaccination has been reported elsewhere (*18*,*27*,*28*), but a study in Mexico found partial protection (*29*). The subtype H1N1 component of the Northern and Southern Hemisphere vaccines at that time was the same: A/Brisbane/59/2007–like.

Table 6. Final model of factors associated with reporting influenza-like illness, New South Wales, Australia, July–September 2009*

Factor, n = 2,431 respondents	Prevalence of factor, % (95% CI)	Reference category†	Relative risk (95% CI)	p value‡
Age, y		≥65 y		
16–34	26.0 (23.2–28.8)		4.74 (2.38–9.45)	**<0.001**
35–49	21.7 (19.4–23.9)		3.25 (1.68–6.26)	**<0.001**
50–64	17.7 (16.1–19.3)		2.60 (1.36–4.99)	**0.004**
Female sex	50.5 (47.8–53.2)	Male	1.25 (0.76–2.05)	0.375
Daily smoker	13.0 (10.8–15.1)	Occasional/ex-/nonsmoker	1.90 (1.10–3.26)	**0.021**
Body mass index				
Obese	18.0 (15.9–20.1)	Healthy or underweight	2.32 (1.30–4.16)	**0.005**
Overweight	28.6 (26.3–31.0)	Healthy or underweight	1.20 (0.68–2.12)	0.534

*Influenza-like illness defined as fever with cough and fatigue. Some questions are only collected on selected age groups, so sample sizes vary. CI, confidence interval.
†Not stated and "Don't know" responses were included in the reference category. Among parameters with any such responses, the proportions were body mass index 5.2%; smoking status 0.1%.
‡Statistically significant results at the 5% level are in **boldface.**

Obesity and smoking are 2 preventable risk factors we found to be strongly associated with ILI. Smoking has been frequently identified as a risk factor for influenza; the identified mechanisms are mechanical, structural, and immunity related (*30–32*). Although obesity has been frequently identified as a risk factor for severe outcomes of infection with pandemic 2009 (H1N1) virus (*33–35*), it has not been previously recognized as a risk factor for susceptibility to symptomatic influenza infection in humans. A recent study in mice found that an immune memory response to recent influenza infection was reduced among obese mice; this reduced memory led to more severe disease, lung pathology, and virus titers after a second exposure to the same mouse-adapted influenza strain (*36*).

Table 7. Effectiveness of seasonal influenza vaccine against influenza-like illness, New South Wales, Australia, July–September 2009*

Subgroup, n = 2,888	Vaccine effectiveness, % (95% CI)†
Age, y	
0.5–15	6.1 (–120.1 to 60.0)
16–34	44.6 (–166.8 to 88.5)
35–49	–0.9 (–137.2 to 57.1)
50–64	33.7 (–69.7 to 74.1)
≥65	–97.9 (–622.5 to 45.8)
Sex†	
M	34.5 (–71.7 to 75.0)
F	10.5 (–49.2 to 46.4)
Region†	
Urban	17.1 (–55.7 to 55.9)
Rural	25.5 (–49.7 to 62.9)
Smoking status†	
Daily smoker	1.4 (–175.3 to 64.7)
Not daily smoker	22.3 (–53.2 to 60.6)
Body mass index†	
Obese	25.0 (–81.6 to 69.1)
Not obese	9.2 (–66.0 to 50.4)
Overall†	20.0 (–30.5 to 51.0)

*For persons >6 mo of age vaccinated in past 12 mo. CI, confidence interval.
†Adjusted for age.

In addition to possibly excluding the early part of the epidemic, our study has other limitations. Influenza in respondents was not confirmed by testing; other common winter respiratory viruses, such as respiratory syncytial virus, can cause a similar syndrome (*16*). General practice surveillance in various regions of Australia, conducted during circulation of seasonal influenza virus, indicated that the syndrome definition we used had a positive predictive value of 23%–60% (*6*). Although these values are not high, positive predictive value is probably increased during a larger than usual epidemic (*37*). Pandemic concern may have prompted more persons than usual to get vaccinated for seasonal influenza. This concern and response would produce higher vaccination prevalence in our study than would have occurred in the absence of a pandemic. Evidence shows that publicity prompted increased vaccination among persons ≥65 years of age, from 68% in April 2009 to 77% in May 2009. Prevalence remained higher for several months (*38*). In our study, we were unable to include 2 frequently reported risk factors for poor outcomes of pandemic (H1N1) 2009 virus infection: pregnancy and indigenous status (*39*,*40*). Although indigenous status is included in the health survey, the number of Aboriginal and pregnant respondents in the period of time covered would be too small to obtain usable estimates for these risk factors.

Conclusions

When pandemic (H1N1) 2009 virus was circulating in the NSW population, ILI was experienced by at least one quarter of the population. Recent prepandemic seasonal vaccination was not protective. Although smoking is already known to increase susceptibility to influenza infection, obesity is not. The role of obesity in susceptibility needs further evaluation in studies in which influenza infection can be confirmed. The high prevalence of these preventable risk factors in our population, combined with a substantially increased risk for ILI, deserves greater recognition. Using an established health survey for monitoring ILI is

inexpensive and provides an opportunity to assess a broad range of risk factors. Continued monitoring will enable better assessment of the value of survey-based influenza surveillance through comparison with other influenza and respiratory illness surveillance systems and can provide continuous assessment of risk factors for ILI.

Acknowledgments

We thank Ray Ferguson for assembling and providing the data for analysis; the NSW Population Health and Health Services Research Ethics Committee for rapid assessment of the survey questions; and Janaki Amin, Jill Kaldor, and Baohui Yang for helpful comments on the analysis.

This work was partially supported by an Australian National Health and Medical Research Capacity Building grant (Health Evaluation and Research Outcomes Network) to D.J.M.

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Severe *Plasmodium knowlesi* Malaria in a Tertiary Care Hospital, Sabah, Malaysia

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The simian parasite *Plasmodium knowlesi* causes severe human malaria; the optimal treatment remains unknown. We describe the clinical features, disease spectrum, and response to antimalarial chemotherapy, including artemether-lumefantrine and artesunate, in patients with *P. knowlesi* malaria diagnosed by PCR during December 2007–November 2009 at a tertiary care hospital in Sabah, Malaysia. Fifty-six patients had PCR-confirmed *P. knowlesi* monoinfection and clinical records available for review. Twenty-two (39%) had severe malaria; of these, 6 (27%) died. Thirteen (59%) had respiratory distress; 12 (55%), acute renal failure; and 12, shock. None experienced coma. Patients with uncomplicated disease received chloroquine, quinine, or artemether-lumefantrine, and those with severe disease received intravenous quinine or artesunate. Parasite clearance times were 1–2 days shorter with either artemether-lumefantrine or artesunate treatment. *P. knowlesi* is a major cause of severe and fatal malaria in Sabah. Artemisinin derivatives rapidly clear parasitemia and are efficacious in treating uncomplicated and severe knowlesi malaria.

The simian parasite *Plasmodium knowlesi* has recently been found to be a major cause of human malaria in Malaysian Borneo (*1*,*2*), with the disease also reported from southern and eastern Asia (*3*). To our knowledge, the only large epidemiologic and clinical studies have been from Sarawak State, Malaysian Borneo, with case series or reports from persons or returning travelers from Myanmar (*4*), Thailand (*5*,*6*), Vietnam (*7*), Philippines (*8*,*9*), Singapore (*10*), Sarawak (*11*), western Malaysia (*12*), and Indonesia (*13*).

The potential for *P. knowlesi* to cause severe disease has been suggested by experimental simian and human infections (*14*–*16*). The first description of naturally acquired severe human *P. knowlesi* infection was a retrospective study from Sarawak that detailed 4 fatal cases with multiorgan failure (*17*). Subsequently, a prospective study from the Kapit District Hospital in Sarawak enrolled 107 persons with *P. knowlesi* monoinfection and demonstrated that 10 patients had severe disease as defined by World Health Organization (WHO) criteria, resulting in 2 deaths (*2*).

The disease spectrum and clinical features of large numbers of patients infected with *P. knowlesi* have not been described outside Sarawak. To reliably differentiate *P. malariae* from *P. knowlesi* infections by using only microscopy is difficult (*18*); such differentiation requires molecular methods (*1*). In a random survey from several districts in Sabah, the state that borders Sarawak, 44 of 49 cases of microscopy-diagnosed *P. malariae* infection were confirmed by PCR to be *P. knowlesi*, indicating that knowlesi malaria was not confined to isolated areas (*17*). In recent years at Queen Elizabeth Hospital (QEH), a tertiary care referral hospital in Kota Kinabalu, Sabah State, patients with severe malaria by WHO criteria had received a diagnosis by microscopy as *P. malariae* infection, but *P. knowlesi* was suspected as the etiologic agent. We

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DOI: 3201/eid.1707.101017

conducted a retrospective review of the clinical spectrum of all case-patients with *P. malariae* malaria who were admitted to QEH during December 2007–November 2009 and confirmed the diagnosis of *P. malariae* or *P. knowlesi* infection by molecular methods.

The optimal management of knowlesi malaria is not known. *P. knowlesi* infection has been successfully treated with chloroquine (*2*) and quinine (*2*); however, the therapeutic efficacy of other antimalarial agents is not known. Artemisinin-derivative combination therapy is now the WHO treatment of choice for uncomplicated falciparum malaria (*19*) and is increasingly recommended for nonfalciparum malaria (*20*); its efficacy in knowlesi malaria is unknown. Similarly, intravenous artesunate is now the treatment of choice for severe falciparum malaria in adults (*19*,*21*), but the therapeutic response to this regimen in severe knowlesi malaria is unknown. As part of our study, we documented the therapeutic responses in uncomplicated and severe knowlesi malaria treated with artemisinin derivatives.

Methods

Study Site

QEH serves as a tertiary care hospital for the Malaysian state of Sabah, which has an estimated population of 3 million. It has a modern well-equipped intensive care unit with facilities for invasive ventilation, hemodynamic support, and renal replacement therapy.

Retrospective Case Review

All patients with microscopy-diagnosed malaria during December 2007–November 2009 were recorded from a prospective laboratory register, and those with *P. malariae* monoinfection or mixed infections were identified. Additional patients, for whom conditions had been diagnosed by microscopy as caused by other *Plasmodium* species but were identified as *P. knowlesi* infections by PCR, were also included. Case records were reviewed, and clinical information was entered into a standardized data collection form. Severe disease was classified on the basis of WHO criteria for severe falciparum malaria (*22*). National policy recommends that all patients with microscopy-diagnosed malaria be hospitalized until negative blood smears are obtained on 2 consecutive examinations. The study was approved by the Medical Research Ethics Sub-Committee of the Malaysian Ministry of Health and the Menzies School of Health Research, Australia.

Laboratory Procedures

Blood films were examined by experienced laboratory microscopists, and the parasite count was classified on a scale of 1 to 4 (1= 4–40 parasites/μL, 2 = 41–400 parasites/μL, 3 = 401–4,000 parasites/μL, 4 = >4,000 parasites/μL). Hematologic results (Sysmex XT1800 [Sysmex Corp., Mundelein, IL, USA] and CELL-DYN Sapphire [Abbott Diagnostics, Abbot Park, IL, USA]) and prothrombin and partial thromboplastin times (STA Compact Hemostasis Analyzer [Diagnostica Stago, Asnières sur Seine, France]) were obtained on site. Serum sodium, potassium, glucose, creatinine, bilirubin, albumin (Roche/Hitachi Modular Analytics EVO, Roche, Basel, Switzerland), and arterial blood gas levels (Radiometer ABL520, Radiometer, Brønchøj, Denmark) were also assayed on site. Blood cultures were performed with an automated system (Becton Dickinson, Franklin Lakes, NJ, USA) and dengue serology by ELISA (PanBio, Brisbane, Australia). In accordance with QEH policy, all slides indicating *P. malariae* monoinfection or mixed infections were sent for molecular testing at the Sabah State Reference Laboratory, along with ≈15% of other species. Parasite DNA was extracted, and nested PCR was performed for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* by methods previously published (*1*,*17*).

Statistical Analysis

Data were analyzed by using STATA version 9.2 (StataCorp LP, College Station, TX, USA). For continuous variables, intergroup differences were compared by Student *t* test or Mann-Whitney U test. For categorical outcome variables, intergroup differences were compared by using the χ^2 test or Fisher exact test. Logistic regression was used to determine the association between binary outcomes and other variables. A 2-sided value of $p<0.05$ was considered significant.

Results

Baseline Characteristics

Included in the final analysis were 56 patients with PCR-confirmed *P. knowlesi* malaria. On the basis of WHO severity criteria (*22*), 22 (39%) had severe malaria, and 34 (61%) had uncomplicated disease (Figure). These patients were identified from a group of 74 patients with documented *P. malariae* malaria listed in the laboratory microscopy register: 54 had *P. knowlesi* monoinfection shown by PCR and medical records available for review (Figure). In addition, another 2 patients received a diagnosis by microscopy as being infected with other *Plasmodium* sp. but were found to have *P. knowlesi* monoinfections on PCR testing (Figure). All 24 patients with severe malaria had PCR performed; of these, 22 had *P. knowlesi* monoinfection (Figure). In the group with uncomplicated infection, 41 had PCR; 34/41 had only *P. knowlesi* monofection; 4 cases were mixed with *P. knowlesi* and other species; 2 cases were non–*P. knowlesi*, and no *Plasmodium* sp. was detected in 1 patient (Figure).

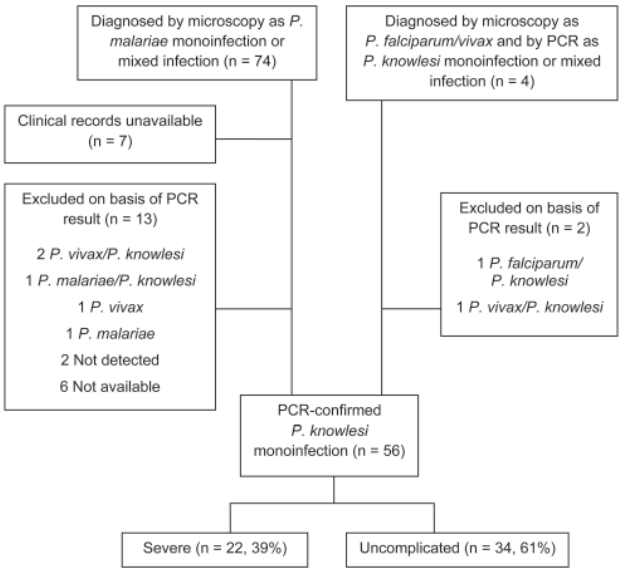

Figure. Flowchart of microscopy and PCR results of *Plasmodium malariae or P. knowlesi* infections by severity, Sabah, Malaysia, 2007–2009.

The baseline demographics and clinical symptoms of both groups with PCR-confirmed *P. knowlesi* monoinfections are detailed in Table 1. The mean age of the patients with severe malaria (57 years; 95% confidence interval [CI] 50–64 years) was significantly older than that of the uncomplicated group (37 years; 95% CI 34–42 years) ($p<0.001$); however, the proportion with chronic coexisting conditions did not differ significantly between groups (Table 1). In severe disease, the proportion of female patients (36%) was significantly higher than that in uncomplicated malaria (8.8%) (odds ratio [OR] 5.9, 95% CI 1.4–25.6; $p = 0.02$). Overall, 8 of 11 female patients had severe disease. Two women were pregnant; 1 had severe malaria, and the other had uncomplicated malaria. Two male patients had second uncomplicated infections during the study period; 1 became infected 10 months after the first, and the other had a mixed *P. vivax/P. knowlesi* infection 6 weeks after initial infection. Both patients had received chloroquine for the first *P. knowlesi* infection. One patient with uncomplicated disease also had HIV infection.

Almost all patients with severe and uncomplicated disease had a history of fever, and no significant difference was found in duration of fever or other clinical symptoms before the patient sought treatment (Table 1). However, patients with severe complications had a lower mean arterial pressure and an increased respiratory rate. Of those not already intubated on transfer or admission to QEH, patients with severe disease also had lower oxygen saturation at room air than did those with uncomplicated disease (Table 1).

Laboratory and Radiologic Investigations

In patients with severe disease, hemoglobin concentrations and platelet counts were significantly lower, and leukocyte counts, prothrombin time, and partial thromboplastin time were elevated, compared with results for patients with uncomplicated malaria (Table 2). Sodium and albumin concentrations were also significantly decreased, and creatinine and total bilirubin levels increased in patients with severe disease (Table 2). Parasite counts were significantly higher in severe disease (Table 2). Blood cultures, performed in 12/34 patients with uncomplicated disease, were all negative. Dengue serologic testing was performed on admission for 11 patients with uncomplicated disease; 10 were negative for immunoglobulin (Ig) M and IgG, and 1 patient was positive for IgM. Blood cultures were performed for 17 patients with severe disease; 1 patient had *Enterobacter cloacae* bacteremia, 5 had coagulase-negative *Staphylococcus* infection (attributed to contamination), and samples from the remainder were negative. Dengue serologic tests for 10 patients with severe disease were all negative. Chest radiographs were obtained on admission for 30 patients (8 with uncomplicated disease, 17 with severe disease); none with uncomplicated disease were reported to have infiltrates, compared with 8/17 with severe disease ($p = 0.01$); all of those with severe disease had acute respiratory distress syndrome (ARDS) with hypoxemia.

Severe Malaria

Of patients with severe disease, 17 were referred from district hospitals and 5 were directly admitted. Twenty-one of these patients had complications on admission, and in 1 patient with acute renal failure, respiratory distress developed 3 days after the start of therapy. During the hospital course, 7 patients each had 1 WHO criterion for severity, and 15 each had ≥2 severity criteria (2, n = 6; 3, n = 5; 4, n = 1; 5, n = 1; and 6, n = 2) (Table 3). The mean age of the 6 patients who died was 64 years (95% CI 49–78 years), and the mean age of the 16 survivors (was 53 years (95% CI 45–61 years; $p = 0.1$). Decreased Glasgow Coma Scores (GCS 14 and 11) on initial hospital visit were seen in 2 patients who died, but other signs and symptoms did not satisfy WHO criteria for cerebral malaria. No cases of severe anemia (<6 g/dL) were found. Acute respiratory distress (n = 13), acute renal failure (n = 12), shock (n = 12), and hyperbilirubinemia (n = 9) were the most common manifestation of severe disease (Table 3). Seven patients had acidosis (on the basis of arterial blood gas analysis) (Table 3). Seven (32%) patients had significant elevations of the prothrombin and partial thromboplastin times, although none were reported to have clinically important bleeding. In patients with acute respiratory distress, the ratio of the partial pressure of oxygen to the fraction of inspired

Table 1. Baseline demographic characteristics and clinical features of patients with severe and uncomplicated *Plasmodium knowlesi* malaria, Sabah, Malaysia, 2007–2009

Characteristic	Uncomplicated disease, n = 34	Severe disease, n = 22
Mean age, y* (range)	37 (20–66)	57 (22–84)
No. (%) female patients†	3 (8.8)	8 (36.3)
Self-reported previous malaria, no. patients	1	0
History of chronic illness, %	18	10
Mean duration of illness, d (range)	5.0 (1–30)	5.2 (2–7)
Symptoms		
Fever, %	100	96
Headache, %	62	57
Diarrhea, %	21	24
Physical findings		
Mean temperature (range)	38.2°C (36.5–41°C)	37.6°C (36.7–39.2°C)
Mean respiratory rate, breaths/min* (range)	20 (14–26)	26 (15–50)
Mean pulse rate, beats/min (range)	95 (69–151)	100 (76–130)
Mean arterial pressure, mm Hg (range)*	85 (61–106)	74 (42–106)
Mean oxygen saturation, % (range)*	98 (92–100), n = 20	88 (56–100), n = 17

*p<0.05 by Mann Whitney U test.
†p<0.05 by χ^2 test.

oxygen (PaO_2:FiO_2) was available for 11/13 patients. In this group, the mean ratio was 165 (range 101–250), with 10 meeting the criteria for ARDS (PaO_2:FiO_2 <200). Eight patients had cardiac function evaluated by transthoracic echocardiogram, and all had normal results, except for a 76-year-old woman with a left ventricular ejection fraction of 30%.

Seventeen patients required intensive care unit management, 12 received inotrope support, 11 required hemodialysis, and 10 received mechanical ventilation (Table 3). The median duration of intensive care stay was 6 days (range 1–11 days); for hemodialysis, 3 days (range 1–6); and for mechanical ventilation, 3 days (range 1–9). Eleven patients were transfused with erythrocytes, 2 with fresh frozen plasma, and 4 with platelets (Table 3).

Malarial and Antimicrobial Drug Therapy

Of 34 patients with confirmed, uncomplicated *P. knowlesi* malaria, 15 received oral chloroquine, 11 received oral quinine, and 8 received artemether-lumefantrine. Two patients from the quinine group received intravenous quinine for ≈24 hours before treatment was changed to oral therapy. Daily peripheral blood films were available for 10 patients who received chloroquine (mean admission parasitemia 2+), for 8 who received quinine (mean admission parasitemia 3+), and for 6 who received artemether-lumefantrine (mean admission parasitemia 2+). When we excluded patients who received intravenous therapy initially, the difference was significant in median parasite clearance times between those who received artemether-lumefantrine (1 day; range 0–3) and those who received chloroquine (2.5 days; range 1–3) or quinine (2.5 days; range 1–3); p = 0.01. The proportion with negative results for parasitemia by day 1 was 4/6, 3/10, and 1/8 for artemether-lumefantrine, chloroquine, and quinine, respectively (p = 0.1), and 5/6, 5/10, and 4/8 for each group on day 2 (p = 0.2). Among patients with uncomplicated disease, 11/34 patients received doxycycline, and 7/34 received other antimicrobial drugs during their hospitalization.

In December 2008, hospital policy changed, and the recommendation was made that patients receive intravenous artesunate, when available, rather than quinine for treatment of severe malaria. Of the 22 patients with severe *P. knowlesi* malaria, 16 received intravenous quinine, and 6 received intravenous artesunate. Daily peripheral blood films were available for 11 of the patients in the quinine group (mean admission parasitemia 3+) and 4 of the artesunate group patients (mean admission parasitemia 3+), with median parasite clearance time significantly shorter with artesunate (2 days; range 1–3) than with quinine (4 days; range 2–7) (p = 0.02). Of the 6 patients who died, 5 received quinine (median severity criteria 2; case-fatality rate 31%), and 1 received artesunate (median severity criteria 2.5; case-fatality rate 16.6%). Of patients with severe malaria, 13/22 received doxycycline, and 16/22 received other antimicrobial drugs during their hospital course.

Outcome

Six (27%) of the 22 patients with severe malaria died; mean time from admission to QEH and death was 2.5 days (range 0–4). Of these, all had ≥3 severity criteria; 6 had ARDS, 5 had acute renal failure, and 4 had shock. All patients who died had a parasitemia level of 4+; survivors had a median level of 3+. None of the patients with uncomplicated disease died. The mean duration of hospital stay was 8.4 days (95% CI 6.3–10.5) for those with severe disease and 5.3 days (95% CI 4.1–7.4) for those with uncomplicated malaria.

Table 2. Laboratory results for patients with severe and uncomplicated *Plasmodium knowlesi* malaria, Sabah, Malaysia, 2007–2009*

Laboratory result	Uncomplicated disease, n = 34	Severe disease, n = 22
Parasite count†	3 (1–4)	4 (2–4)
Hemoglobin concentration, gm/dL†	13.4 (9.1–17.6)	11.3 (6.2–16.8)
Leukocyte count, $\times 10^3$ cells/μL†	6.3 (3.4–15.3)	12.7 (3.5–21.6)
Neutrophil count, $\times 10^3$ cells/μL†	3.9 (1.4–10.1)	9.0 (2.73–16.9)
Lymphocyte count $\times 10^3$ cells/μL†	1.6 (0.6–3.3)	2.3 (0.24–6.4)
Prothrombin time, s†	13.8 (10.6–17.5)‡	14.9 (11.7–21)§
Partial thromboplastin time, s†	40 (29.9–57.6)‡	57 (31–136)§
Platelet count $\times 10^3$ cells/μL†	72 (21–227)	40 (12–130)
Serum creatinine, μmol/L†	92.6 (57–168)	289 (53–819)
Serum sodium concentration, mmol/L†	136 (128–146)	131 (123–140)
Serum total bilirubin, μmol/L	25.2 (4–48)¶	173 (11–660)
Serum albumin, gm/dL†	38 (26–73)#	22 (18–27)

*All values are mean (range).
†p<0.05 by Student *t* test or Mann Whitney U test.
‡n = 23.
§n = 19.
¶n = 15.
#n = 13.

Discussion

Studies from Sarawak have shown that *P. knowlesi* infections can result in severe and fatal disease (*2*,*17*). The present 2-year case series from Sabah indicates that in a tertiary referral hospital setting, the proportion of severe *P. knowlesi* malaria is higher than reported previously, with 39% of patients having severe malaria according to WHO criteria. The increased frequency of severe disease likely reflects referral bias because a large proportion of patients were referred from surrounding district hospitals. The case-fatality rate for severe malaria of 27% in this study is comparable to that of a previous study (*2*) and at least as high as that seen with *P. falciparum* malaria (*21*). The main demographic factors for severe malaria were increasing age (mean 57 years) and female gender. The former is consistent with a study of falciparum malaria where age was an independent risk factor for development of severe disease and death (*23*). The reason(s) for the large proportion of severe disease in female patients, noted previously in knowlesi malaria (*2*) and vivax malaria (*24*), remain unclear.

For 12 of the 13 patients with respiratory distress, the diagnosis was confirmed by a low arterial partial pressure of oxygen and decreased oxygen saturation with a need for mechanical ventilation. One patient with an increased respiratory rate alone had metabolic acidosis; the 4 other patients had ARDS and hypoxemia. This finding suggests that hypoxemia from acute lung injury is the major cause of respiratory distress in *P. knowlesi* malaria, although metabolic acidosis can also contribute. Shock occurred in more than half of patients who had severe malaria; however, repeated blood cultures showed clinically significant bacteremia in <10% of patients, which suggests that in most cases of severe knowlesi malaria, concurrent bacteremia does not contribute to hypotension. A previous report with smaller numbers of severe knowlesi malaria found metabolic acidosis in only 10% (*2*), compared with 30% of severe patients in this study for whom arterial blood gas results showed clear metabolic acidosis. The cause in 6 of 7 patients with acidosis may have been shock and hypoxemia; only 1 patient had neither. Although 30% of patients with severe disease had elevated prothrombin time or partial thromboplastin time, no clinically notable bleeding episodes were observed.

The susceptibility of pregnant women to severe disease in falciparum malaria (*22*) may also be the case in knowlesi malaria; 18% of women admitted with *P. knowlesi* malaria were pregnant. One patient in the third trimester of pregnancy survived acute renal failure and shock, but the fetus died.

The multiorgan failure experienced by patients with severe knowlesi malaria is similar to that reported in adults with severe falciparum malaria in areas where the transmission rate is low and unstable (*22*). However, as seen in a smaller series of severe knowlesi malaria, the ≈50% proportion with ARDS and shock is higher than that reported in series of severe falciparum malaria (*22*,*23*,*25*–*29*). Furthermore, 2 of the major clinical syndromes of severe falciparum malaria—unarousable coma and severe anemia—were absent (*2*). The absence of severe anemia may reflect the lower malaria transmission rate, the relatively short duration of illness, and the exclusion from this adult referral hospital of children, an age group prone to this complication in falciparum malaria (*23*). Severe anemia has been described in knowlesi malaria in children elsewhere in Sabah (*30*). The reasons for the lack of coma are less clear and may reflect differences in pathophysiology between knowlesi and falciparum malaria. In the only detailed autopsy study of fatal knowlesi malaria, widespread microvascular parasite accumulation was

Table 3. Details of patients with severe *Plasmodium knowlesi* malaria, Sabah, Malaysia, 2007–2009*

Patient no.	Age, y/ sex	Severity†	Parasite count	Platelets/ µL	Blood products	ICU	Inotropes	Ventilation	Dialysis	Treatment	Outcome
P. knowlesi only											
1	76/M	1, 6, 7	3	17,000	Plt	Y	N	N	N	Artesunate	S
2	22/M	4, 7	3	38,000	Bld	Y	Y	Y	N	Quinine	S
3	29/M	2	4	16,000	Bld, Plt	Y	N	Y	Y	Quinine	S
4	76/F	1, 2, 3, 4, 6, 7	4	42,000	Bld	Y	Y	Y	Y	Quinine	D
5	50/M	2, 4	2	60,000	Bld	Y	Y	N	Y	Quinine	S
6	55/M	2, 4, 7	4	42,000	N	Y	Y	N	N	Quinine	S
7	49/M	1	4	78,000	N	Y	N	N	N	Quinine	S
8	38/F	2	4	29,000	Bld	Y	N	N	Y	Quinine	S
9	65/M	3, 4	3	17,000	N	N	N	N	N	Quinine	S
10	70/F	2, 4, 6, 7	4	48,000	Bld	Y	Y	Y	Y	Quinine	S
11	65/F	1, 2, 4, 6, 7	4	26,000	Bld, FFP	Y	Y	Y	Y	Quinine	D
12	75/F	4, 6	3	22,000	N	N	Y	N	N	Artesunate	S
13	69/F	7	2	130,000	N	N	N	N	N	Artesunate	S
14	57/M	1, 2, 3, 4, 6, 7	4	35,000	Bld, Plt, FFP	Y	Y	Y	Y	Artesunate	D
15	60/M	1	4	37,000	N	N	N	N	N	Quinine	S
16	44/M	1	3	61,000	N	N	N	N	N	Artesunate	S
17	54/M	4, 7	2	72,000	N	Y	Y	N	N	Quinine	S
18	69/M	1, 2	3	41,000	Bld	Y	N	N	Y	Quinine	S
19	84/F	2, 5, 7	2	34,000	N	N	N	Y	N	Quinine	D
20	54/F	2, 4, 7	3	12,000	Bld, Plt	Y	Y	Y	Y	Artesunate	S
21	56/M	4, 7	4	28,000	N	N	Y	Y	N	Quinine	D
22	46/M	1, 2, 7	4	16,000	N	Y	Y	Y	Y	Quinine	D
P. vivax + *P. knowlesi*	48/M	1, 2, 6	2	24,000	N	Y	N	N	Y	Artesunate	S
ND	28/M	7	3	80,000	N	Y	N	Y	N	Artesunate	S

*ICU, intensive care unit; Bld, blood; Plt, platelets; Y, yes; N, no; S, survived; D, died; FFP, fresh frozen plasma; ND, none detected.
†Severity criteria: 1, hyperbilirubinemia; 2, acute renal failure; 3, hypoglycemia; 4, shock; 5, blackwater fever; 6, acidosis; 7, respiratory distress.

found, including within the brain, but no features to suggest cytoadherence of parasitized red cells to endothelial cells, a hallmark of the pathophysiology of severe falciparum malaria (*31*). Additional causes of impaired microvascular flow and organ dysfunction in falciparum malaria include dysregulated immune responses (*32*), endothelial activation with elevated angiopoietin-2 and von Willebrand factor (*33*,*34*), and decreased vascular nitric oxide bioavailability (*25*) and red cell deformability (*35*), but their roles in knowlesi malaria remain unknown.

Thrombocytopenia is nearly universal in *P. knowlesi* infections; platelet counts are lowest in cases of severe disease when no evidence suggests concurrent dengue. In contrast to platelet counts, leukocyte counts were higher in severe malaria than in uncomplicated malaria. Secondary bacterial infection was uncommon, which suggests that severe *P. knowlesi* infection itself may account for the neutrophilia.

The optimal management of knowlesi malaria is not known, and the 2010 WHO Malaria Treatment Guidelines do not provide recommendations for its treatment (*19*). Artemisinin-derivative combination therapy is recommended as first-line treatment of falciparum malaria in Africa and Asia, but there are no reports of artemisinin-derivative combination therapy efficacy in knowlesi malaria. In previous reports, uncomplicated *P. knowlesi* malaria was treated with chloroquine and primaquine (*2*,*36*), whereas severe disease was treated with intravenous quinine (*1*,*2*,*17*). Past studies in Sarawak have shown that although most patients with microscopy-diagnosed *P. malariae* infection had *P. knowlesi*, ≈10% were actually infected with *P. falciparum* (*17*). In settings such as Malaysia, with a high prevalence of chloroquine-resistant falciparum malaria (*37*,*38*), inadvertent use of chloroquine for misdiagnosed falciparum malaria may have deleterious consequences. In uncomplicated knowlesi malaria, we found that chloroquine, quinine, and artemether-lumefantrine were all efficacious, and although comparisons were uncontrolled, those receiving artemether-lumefantrine had faster parasite clearance times. The 1-day median parasite clearance time after using artemether-lumefantrine in our hospitalized patients was similar to that seen in a community study in which chloroquine was used (*2*), though parasite densities may not have been comparable.

Intravenous artesunate (compared with quinine) reduces the proportion of deaths in severe falciparum

malaria (*21*), but its efficacy is unknown in severe knowlesi malaria. In our study, artesunate-treated patients had faster parasite clearance, and the case-fatality rate (17%) was lower than for those who received quinine (31%). However, the retrospective design and small number of severe cases and deaths do not enable us to assess a possible survival benefit. Current treatment policy at QEH for uncomplicated knowlesi malaria is oral artemether-lumefantrine and for severe knowlesi malaria, intravenous artesunate.

Our study has several limitations. The main ones are the retrospective design and inability to review 10% of the charts. Although samples from most *P. malariae* patients were sent for molecular confirmation, 8% of the results were unavailable. Several cases microscopically diagnosed as falciparum or vivax malaria were PCR-positive for *P. knowlesi*. Because only ≈15% of non–*P. malariae* slides are sent for PCR, we may have underestimated the true proportion of patients hospitalized with knowlesi malaria. Because QEH is a hospital for adults, we were unable to describe the disease spectrum in children.

Our study further highlights the public health implications of *P. knowlesi*. A high proportion of knowlesi malaria patients admitted to a tertiary care referral hospital in a malaria-endemic area had severe and fatal disease characterized by multiorgan failure, a high proportion with ARDS and shock, and a notable absence of coma. The pathogenic mechanisms underlying this disease spectrum remain unknown. Artemisinin derivatives result in rapid parasite clearance and are efficacious in both uncomplicated and severe knowlesi malaria. Prospective studies to further define the epidemiology, pathogenesis, and optimal treatment for knowlesi malaria are needed.

Acknowledgments

We thank Frederick Andreos, Joseph Benedict, Damica Kunsiong, and Juhanah Gimboh for their help in the study. We also thank the Director General of Health (Malaysia) for permission to publish this article.

N.M.A. and T.W.Y. are supported by Australian National Health and Medical Research Council Fellowships

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Age as Risk Factor for Death from Pandemic (H1N1) 2009, Chile

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Pandemic (H1N1) 2009 affected Chile during the winter of 2009. The hospitalization rate was 0.56% overall and 3.47% for persons ≥60 years of age at risk for severe disease and death independent of concurrent conditions. Age ≥60 years was the major risk factor for death from pandemic (H1N1) 2009.

On April 23, 2009, the World Health Organization issued alerts about the emergence of pandemic (H1N1) 2009. On May 17, during fall in the Southern Hemisphere, the first case of this disease in Chile was identified (*1*). Pandemic (H1N1) 2009 then replaced seasonal influenza, which had accounted for <1% of confirmed influenza cases in Chile (*2*). Immediately after the alerts, a national mandatory notification system was started in Chile for influenza-like illness (ILI) cases and hospitalized persons with pandemic (H1N1) 2009 in public and private institutions. We analyzed data for patients with confirmed pandemic (H1N1) 2009 hospitalized during epidemiologic weeks 20–32 (May 19–August 3), 2009.

The Study

ILI was defined as fever >38.5°C and cough plus ≥1 of the following: sore throat, headache, and myalgia. Severe influenza was defined as any case confirmed by reverse transcription PCR in a hospitalized person. The National Ministry of Health provided oseltavimir to every patient >5 years of age who had ILI. Approximately 80% of ILI cases corresponded to pandemic (H1N1) 2009 confirmed by PCR in a pilot study.

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DOI: 10.3201/eid1707.101398

All fatal cases included in the analysis occurred in persons whose deaths were directly attributable to influenza. Demographic data, clinical features, concurrent conditions, and number of consultations before hospitalization were recorded (*3*).

Denominators used for determining overall incidence rates and incidence rates by age group were based on the national census of 2002. Denominators used for determining rates for hospitalized case-patients and case-fatality rates (CFRs) were 80% of ILI cases reported to the Ministry of Health during the study. We conducted statistical analysis by using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and Epi Info version 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

During May 19–August 3, 2009, a total of 342,588 ILI cases were reported. Median age of case-patients was 20.4 years (range <1–109 years). The overall attack rates were 1.2% for pandemic (H1N1) 2009 and 0.4% for persons ≥60 years of age. During the same period, 651,416 treatments with oseltamivir were reported. Treatment data indicated that the attack rate was 4%.

A total of 1,585 persons confirmed to have ILI were hospitalized. Median age was 33 years (range 11 days–94 years), and 52% were women. Overall rate of hospitalization was 9.4/100,000 persons. Case-hospitalization rate was 0.6% (3.5% for persons ≥60 years of age and 1.6% for children <5 years of age) (Figure 1). Pneumonia was the most common diagnosis at admission (77.3%). Underlying diseases were present in 560 (56.6%) of 989 case-patients, and 217 (22%) had ≥2 concurrent conditions.

A total of 130 hospitalized patients died (overall mortality rate 0.79/100,000 persons). Among these patients were 117 who died during the study period. Median age was 47 years (range 4 months–89 years) for persons who died and 30 years (range <1–94 years) for persons who survived ($p<0.0001$). Forty-seven percent of patients who died were ≥49 years of age. CFR was 0.04%: the highest rate was for patients ≥60 years of age (0.44%; $p<0.0000001$), followed by persons 15–59 years of age (0.045%). CFR was 0.02% for children <5 years of age and 0.008% for children 5–14 years of age (Figure 2). Fifty percent of all deaths were caused by severe respiratory failure.

Age was a risk factor for severe influenza and death. Patients ≥60 years of age and <5 years of age were at a higher risk for severe disease, and patients ≥60 years of age were at a higher risk for death. At least 1 concurrent condition was identified as a risk factor for death from pandemic (H1N1) 2009. Eighty-four (87.5%) of 96 patients who died and 485 (54.3%) of 893 of patients who survived

[1]These authors contributed equally to this article.

[2]Additional members of this committee who contributed data to this study are listed at the end of this article.

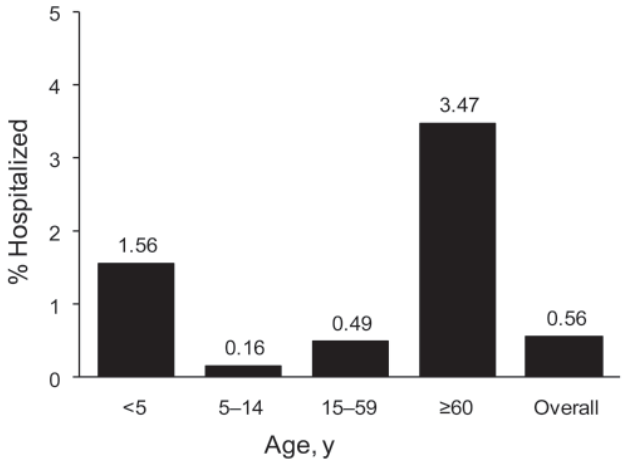

Figure 1. Hospitalization rate for patients with pandemic (H1N1) 2009, by age group among reported case-patients with influenza-like illness, Chile, 2009.

had an underlying disease (odds ratio 5.89, 95% confidence interval 3.08–11.52; p<0.00001). A concurrent condition was a risk only for persons 15–49 years of age (Table).

Mean time from onset of symptoms to hospitalization was 4.3 days (range 0–20 days) for persons who died and 3.6 days (range 0–20 days) for persons who survived (p = 0.03). Median time from onset of symptoms to beginning of treatment with oseltamivir was 3.0 days for persons who died and for those who survived.

Conclusions

The strength of this study was inclusion of nearly all patients with confirmed severe pandemic (H1N1) 2009 in Chile because of timely implementation of a national notification system. The population studied included 97% of hospitalized patients with pandemic (H1N1) 2009.

Differences in attack rate (1.2% by reporting of ILI cases and 4% by amount of oseltamivir provided) might be explained by the fact that not all ILI cases were captured (≈20% were lost because of underreporting) by the notification system and oseltamivir was prescribed to persons with illness not included in the ILI case definition. We demonstrated in a pilot study that ≈60%–70% of oseltamivir used complied with the case definition for ILI.

The first wave of pandemic (H1N1) 2009 in Chile showed the highest incidence rate in children 5–14 years of age; persons ≥60 years of age were least affected (*2*). However, our study identified persons ≥60 years of age as at greatest risk for severe respiratory disease and death, despite the lower incidence rates. During the influenza pandemic, risk for illness in this age group was low but risk for severe disease and death was higher than in the other groups, independent of underlying diseases. This finding differs from results of a study in California, USA, in which persons ≥60 years of age did not have a high hospitalization rate (*4*). Our finding is consistent with those of a report on seasonal influenza in which risk for severe infection and hospitalization was highest for elderly persons (*5*).

Before April 2009, influenza was rarely reported as the cause of death in Chile. Therefore, only CFRs for severe respiratory infection are available for comparison. The CFRs for patients >65 years during winter 2009 was similar to that during previous winters. We conclude that elderly persons' risk for a severe outcome during pandemic (H1N1) 2009 did not exceed this risk for a severe outcome during seasonal influenza. Previous reports have documented increased risk for severe outcomes in younger persons during pandemic (H1N1) 2009 and the absence of increased risk for disease severity among elderly persons (*4*,*6*,*7*). On the basis of these findings, some institutions made policy decisions to exclude anyone ≥65 years of age without concurrent conditions from satisfying recommendations for use of vaccine against pandemic (H1N1) 2009 because of absence of identified increased risk for infection. Our study indicates an age ≥60 is the greatest risk factor for a severe outcome during pandemic (H1N1) 2009 and seasonal influenza.

Delay in medical care was another risk factor for death in this study. The number of consultations before admission did not differ between the groups, suggesting that patients who died sought medical care later than patients who survived. Thus, timely medical consultation affected patient outcome.

This study indicates that an age ≥60 years was the greatest risk for death associated with pandemic (H1N1) 2009 influenza, similar to that for seasonal influenza. These results can be used for future planning strategies for influenza, strengthening the need for influenza vaccination, opportune medical evaluation, and timely therapy specific for this age group.

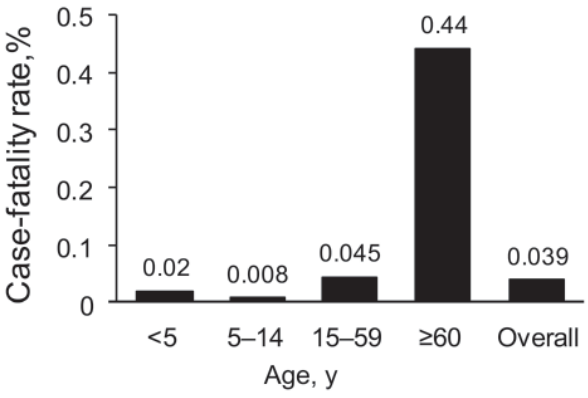

Figure 2. Case-fatality rate for pandemic (H1N1) 2009 by age group among reported case-patients with influenza-like illness, Chile, 2009.

Table. Risk factors for severe disease or death among patients with pandemic (H1N1) 2009, Chile, 2009*

Risk factor	OR (95% CI)
Severe disease	
Age group, y	
<5	3.66 (3.27–4.11)†
5–14	0.22 (0.18–0.25)
15–59	0.89 (0.81–0.98)
≥60	7.94 (6.93–9.10)†
Death	
Age group, y	
<15	0.17 (0.1–0.3)
15–59	1.44 (0.99–2.09)
≥60	15.06 (9.94–22.72)†
Concurrent condition	
All patients	5.89 (3.08–11.52)†
Age group, y	
<1	ND (inexact)‡
1–4	3.53 (0.24–101.6)
5–14	ND (inexact)‡
15–49	6.69 (2.4–20.0)†
50–64	2.39 (0.77–8.5)
>64	1.0 (0.18–7.0)

*OR, odds ratio; CI, confidence interval; ND, not defined.
†p<0.05.
‡Not significant.

Additional members of the Advisory Committee of the Chilean Ministry of Health who contributed data to this study are Jorge Jimenez, Enrique Paris, and Carlos Pérez (Pontificia Universidad Católica de Chile, Santiago); Miguel O'Ryan (Universidad de Chile, Santiago); and Luis Miguel Noriega and Pablo Vial (Universidad del Desarrollo, Santiago).

Acknowledgments

We thank Winston Andrade, Patricia Bustos, Graciela Torres, and Judith Mora for helping with influenza diagnosis of all hospitalized patients with suspected influenza during the outbreak, and the physicians for reporting ambulatory and hospitalized patients with influenza.

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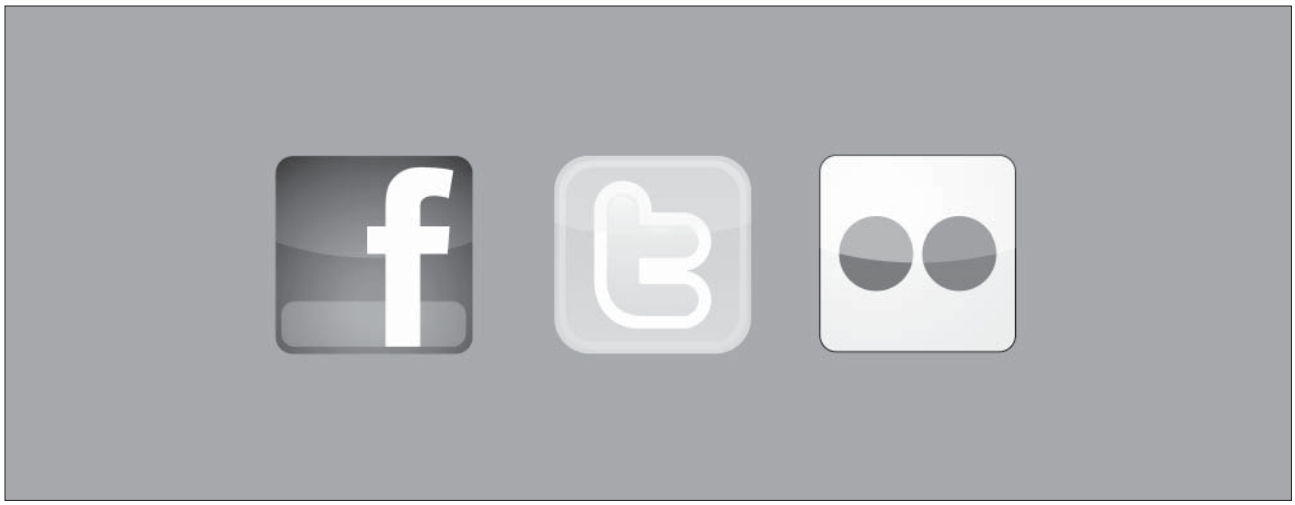

Multidrug-Resistant *Mycobacterium tuberculosis*, Southwestern Colombia

Beatriz E. Ferro, Luisa Maria Nieto, Juan C. Rozo, Liliana Forero, and Dick van Soolingen

Using spoligotyping, we identified 13 genotypes and 17 orphan types among 160 *Mycobacterium tuberculosis* isolates from patients in Valle del Cauca, Colombia. The Beijing genotype represented 15.6% of the isolates and was correlated with multidrug-resistant tuberculosis, female sex of the patients, and residence in Buenaventura and may represent a new public health threat.

The state of Valle del Cauca in southwestern Colombia has a higher incidence of tuberculosis (TB) than the rest of the country (47 vs. 24 cases per 100,000 inhabitants per year) (*1*,*2*). One of its largest cities, Buenaventura, the main port of Colombia on the Pacific Ocean, has multidrug-resistant TB (MDR TB; resistance to at least isoniazid and rifampin) and an MDR TB rate of 6% (*3*).

Several genotypes of *Mycobacterium tuberculosis* have been reported in Colombia, but Latin American Mediterranean (LAM) and Haarlem (H) strains predominate (*4*,*5*). In Colombia, the Beijing genotype was first detected in 1998 in 11 of 111 isolates from new and previously treated patients in Buenaventura (*6*). Further detection of this strain has been restricted to Valle del Cauca (www.ins.gov.co/index.php?idcategoria=8304).

The Beijing genotype was originally reported in China in 1995 and is associated with higher virulence and resistance to antituberculosis drugs in many areas (*7*–*10*). Therefore, the Beijing genotype of *M. tuberculosis* is likely to have had a strong effect on development of the worldwide TB epidemic and the current emergence of MDR TB and extensively drug-resistant TB (XDR TB) (*9*).

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DOI: 10.3201/eid1707.101797

South America has been relatively free of Beijing strains (*10*), and drug-resistance rates have not been extremely high in comparison with other regions. However, in this study, we report that the Beijing genotype is associated with MDR isolates in Colombia.

The Study

During January 2007–December 2008, health institutions in Valle del Cauca sent *M. tuberculosis* isolates to the Centro Internacional de Entrenamiento e Investigaciones Médicas in Cali, Colombia, for drug susceptibility testing. A total of 324 *M. tuberculosis* isolates from individual patients (new and previously treated) were subjected to first-line drug susceptibility testing by using the agar proportion method with 7H10 medium.

In 2009, with approval from the Centro Internacional de Entrenamiento e Investigaciones Médicas Institutional Review Board, we thawed 160 (49%) of 324 isolates (76 MDR and 84 drug susceptible) and cultured them on Löwenstein-Jensen agar slants. Isolates with other susceptibility profiles were excluded from this analysis. Isolates were obtained from 9 municipalities in Valle del Cauca: Buenaventura (n = 113), Cali (n = 36), and 7 other locations (n = 11). Eighty-seven patients (54.4%) had new cases. There were more male patients (57.5%) than female patients, and the median age of all patients was 32 years (range 1–82 years).

DNA extraction was performed by using the hexadecyltrimethylammonium bromide method (*11*), and isolates were subjected to spoligotyping (*12*). Spoligopatterns obtained were analyzed independently by 2 readers and compared with SpolDB4.0 (Pasteur Institute of Guadeloupe, Les Abymes, Guadeloupe) and MIRU-VNTRplus (www.miru-vntrplus.org) to assign them to a known genotype family.

Statistical analysis was conducted by using Stata version 9.0 (StataCorp LP, College Station, TX, USA) and the Openepi (www.openepi.com). Odds ratios (ORs) and 95% confidence intervals were calculated by using the Fisher exact test method. Chi-square test was used to determine statistical significance.

Thirteen genotypes were identified among the 160 isolates: LAM9 (32; 20%), H1 (32; 20%), Beijing (25; 15.6%), T1 (9; 5.6%), U (9; 5.6%), LAM2 (6; 3.8%), LAM3 (5; 3.1%), LAM1 (4; 2.5%), X1 (4; 2.5%), H3 (4; 2.5%), U (LAM30) (2; 1.3%), X3 (2; 1.3%), and LAM6 (1; 0.6%). Orphan genotypes accounted for 25 (15.6%) isolates, and 12 of these isolates were grouped in 4 patterns (Table 1).

Overall, the LAM and H families were the most common among isolates evaluated, particularly among susceptible ones. H strains represented a homogeneous group and were distributed in 4 spoligo-international types

Table 1. Frequency of genotypes by family in *Mycobacterium tuberculosis* isolates from Valle del Cauca, Colombia, 2007–2008*

Family	No. (%) isolates	Patient data		
		M/F	No. with susceptible isolates/ no. with MDR isolates	No. with new treatment/ no. previously treated
Beijing	25 (15.6)	9/16	1/24	13/11†
LAM1	4 (2.5)	4/0	4/0	4/0
LAM2	6 (3.8)	4/2	3/3	3/2†
LAM3	5 (3.1)	2/3	3/2	3/2
LAM6	1 (0.6)	1/0	1/0	0/1
LAM9	32 (20)	21/11	24/8	19/12†
H1	32 (20)	17/15	22/10	15/17
H3	4 (2.5)	2/2	4/0	4/0
T1	9 (5.6)	4/5	9/0	8/0†
U	9 (5.6)	6/3	2/7	3/6
U (LAM30)	2 (1.3)	2/0	2/0	1/1
X1	4 (2.5)	3/1	1/3	2/2
X3	2 (1.3)	2/0	2/0	2/0
Orphan1	1 (0.6)	0/1	1/0	1/0
Orphan2	2 (1.3)	0/2	0/2	1/1
Orphan3	1 (0.6)	0/1	1/0	1/0
Orphan4	2 (1.3)	1/1	0/2	2/0
Orphan5	1 (0.6)	1/0	0/1	0/1
Orphan6	1 (0.6)	0/1	0/1	1/0
Orphan7	1 (0.6)	1/0	0/1	0/1
Orphan8	1 (0.6)	0/1	0/1	1/0
Orphan9	1 (0.6)	0/1	0/1	0/1
Orphan10	1 (0.6)	1/0	0/1	0/1
Orphan11	1 (0.6)	1/0	0/1	0/1
Orphan12	6 (3.8)	5/1	0/6	2/4
Orphan13	1 (0.6)	1/0	1/0	1/0
Orphan14	1 (0.6)	1/0	0/1	0/1
Orphan15	1 (0.6)	1/0	1/0	1/0
Orphan16	1 (0.6)	1/0	1/0	0/0†
Orphan17	2 (1.3)	2/0	2/0	0/2
Total	160 (100)	93/67	84/76	87/68

*MDR, multidrug resistant.
†Complete data were not available for 5 genotypes.

(SITs 47, 49, 50, and 62). LAM isolates were distributed in 10 SITs (17, 20, 42, 64, 130, 162, 469, 545, 1711, and 1803).

Three results were obtained regarding the Beijing genotype. First, a Beijing family strain (SIT 190) caused the largest cluster among the MDR isolates, comprising 24 cases. The remaining Beijing strain (SIT 1) corresponded to a susceptible isolate from Buenaventura (Table 1). Second, the Beijing genotype showed a strong correlation with female patients and patients residing in Buenaventura (Table 2). Third, Beijing SIT 190 was found in 2 of 4 XDR TB isolates, as confirmed in susceptibility testing by the Instituto de Salud Pública de Chile (Santiago, Chile). These 2 isolates were found in 2 women, 16 and 24 years of age. The other 2 XDR TB isolates were an H strain and an orphan genotype strain.

Conclusions

Although our study used a convenience sample, it identified a high frequency of Beijing strains among MDR TB isolates and showed an association between the Beijing genotype and MDR TB in Latin America. In Buenaventura, where a high rate of primary drug resistance has been observed (*3*,*6*), the Beijing genotype is associated with MDR TB and thus transmission. Moreover, given the limited number of MDR isolates tested, the emergence of Beijing strains in Colombia may already be much larger than what we observed.

In multiple areas worldwide, the Beijing genotype has been associated with young patients and active and recent transmission (*8*). In our study, the same tendency was observed, and this may suggest the emergence of this genotype family in Colombia.

The high frequency of the Beijing strain might be caused by bacteriologic factors, host factors, or both (*8*). Valle del Cauca has a high proportion of persons of African descent (27.2% according to the national census in 2005); especially in Buenaventura, where 23 of the 25 Beijing isolates were found. Also, Buenaventura, which has >300,000 inhabitants, has a high TB incidence (72

Table 2. Characteristics of patients infected with Beijing and non-Beijing isolates of *Mycobacterium tuberculosis*, Valle del Cauca, Colombia, 2007–2008*

Characteristic	No. (%) isolates		OR (95% CI)	p value
	Beijing, n = 25	Non-Beijing, n = 135		
Drug resistance profile				
MDR	24 (96.0)†	52 (38.5)	38.31 (5.79–1,593.47)	<0.001
Susceptible	1 (4.0)‡	83 (61.5)		
Sex				
F	16 (64.0)	51(37.8)	2.93 (1.11–8.06)	0.02
M	9 (36.0)	84 (62.2)		
Treatment status				
New	13 (54.2)	74 (56.5)	0.91 (0.35–2.43)	0.83
Previously treated	11 (45.8)	57 (43.5)		
Unknown	1 (4.0)	4 (2.1)		
Age, y				
≤30	14 (63.6)	54 (42.9)	2.33 (0.84–6.87)	0.07
>30	8 (36.4)	72 (57.1)		
Unknown	3 (12.0)	9 (6.6)		
Place of residence				
Buenaventura	23 (92.0)	90 (66.7)	5.75 (1.31–52.07)	0.007
Other cities	2 (8.0)	45 (33.3)		

*OR, odds ratio; CI, confidence interval; MDR, multidrug resistant.
†All were spoligo-international type (SIT) 190.
‡SIT 1.

cases/100,000 inhabitants/year) (*13*). Commercial and tourism activities and high population mobility in this city may contribute to dissemination of the Beijing genotype to other regions of Colombia. Two persons infected with the Beijing strain found in Cali, Colombia, in this study and a recently described 15-year-old person with MDR TB infected with a Beijing-like genotype, who died in Bogotá shortly after initiation of treatment, may represent preliminary evidence of the mentioned risk (*14*).

Our results differ from those of a report that described a low frequency of Beijing strains in Latin America and no association with drug resistance (*10*). However, Colombia might have recently become a port of entry for Beijing strains and this entry may have started earlier without being detected. Additional epidemiologic and clinical information is necessary to correlate our findings with other factors, such as *M. bovis* BCG vaccination, ethnic group, disease severity, and outcome.

More discriminative typing methods such as mycobacterial interspersed repetitive unit–variable number tandem repeat analysis (*15*) or whole genome sequencing would enable typing to the strain level. This typing would shed light on epidemiologic links between the cases we reported and worldwide spread of the Beijing strain and on the location of these Beijing strains in a worldwide phylogenetic tree. Nevertheless, spoligotyping used in this study is sufficient to conclude that drug-resistant Beijing strains have become a public health problem in Buenaventura, Colombia.

Acknowledgments

We thank Mauricio Perez for contributing to the biostatistical analysis, and Sonia Villegas, Neal Alexander, and Daniel Rodríguez for valuable comments regarding the manuscript.

This study was supported by Centro Internacional de Entrenamiento e Investigaciones Médicas, Secretaría Departamental de Salud del Valle del Cauca, and Young Researcher Program of Colciencias (contract nos. 159-2008 and 797-2009).

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Visceral Larva Migrans in Immigrants from Latin America

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To determine whether increased migration is associated with an increase in incidence of toxocariasis (visceral larva migrans), we analyzed clinical data obtained from immigrants from Latin America. Although infection with *Toxocara* sp. roundworm larvae is distributed worldwide, seroprevalence is highest in tropical and subtropical areas.

Human toxocariasis is a zoonosis caused by the larvae of *Toxocara* sp. roundworms. Although distribution is worldwide, seroprevalence is highest in tropical countries, including in Latin America. Immigration from tropical areas to Europe continues to increase, with Spain a frequent destination. Data on visceral larva migrans (VLM) among immigrants from Latin America in western countries (primarily European countries, the United States, and Canada) are scarce. To determine whether increased migration is associated with increased VLM incidence, we analyzed clinical and epidemiologic data from immigrants from Latin America.

The Study

We analyzed information about 634 immigrants from Latin America seen at the Tropical Medicine Unit of the Ramón y Cajal Hospital in Madrid, Spain, during April 1989–June 2008. Immigrants who were visiting friends and relatives were excluded. Patients with VLM were identified.

We used 5 strict criteria for diagnosing VLM: 1) positive serologic test for *Toxocara* sp. roundworm infection, performed by using a commercial ELISA *Toxocara* immunoglobulin (Ig) G Ridascreen (R-Biopharm GmbH, Darmstadt, Germany), following the manufacturer's recommendations; 2) absolute peripheral blood eosinophil count >500 cells/mm^3; 3) exclusion of other parasites causing eosinophilia, such as intestinal nematodes, particularly *Strongyloides stercoralis* (excluded by larval culture and serology by ELISA IgG), *Schistosoma* sp., *Fasciola hepatica*, *Trichinella spiralis*, *Taenia solium*, *Echinococcus granulosus*, and cutaneous and blood microfilariae; 4) symptoms associated with VLM (respiratory signs, such as asthma, dyspnea, and eosinophilic pneumonia; dermatologic symptoms, including pruritus and recurrent urticaria; and abdominal symptoms, including abdominal pain and hepatomegaly); and 5) response to treatment with albendazole (10–15 mg/kg/d in 2 doses orally for 5 days) assessed 6 months after treatment, decreased titers to *Toxocara* sp. roundworm infection, decreased eosinophil count, and clinical improvement or resolution of symptoms.

The most frequent countries of origin for patients were Ecuador 221/634 (34.9%), Bolivia 176/634 (27.8%), Peru 71/634 (11.2%), and Colombia 56/634 (8.8%). Median age was 32 years (range 4–40 years); 421 (66.4%) patients were male. The median number of months from arrival in Spain to first consultation at the Tropical Medicine Unit was 19 months.

Eosinophilia was present in 135 (21.3%) patients. *Toxocara* antibodies were detected by ELISA in 31 (4.9%) patients. Concomitant serologic results positive for *Toxocara* sp. roundworm infection and eosinophilia were found in 28 (4.4%) patients; 606 patients were excluded. Of these 28 patients, 11 were excluded because of other concomitant parasitic infections that also can cause eosinophilia: 8 patients had positive ELISA results for *S. stercoralis* nematodes (not detected in fecal samples or larval culture); 1 had *Ascaris lumbricoides* eggs in feces; 1 had a positive indirect hemagglutination result but negative ELISA result for *E. granulosus* tapeworm; and 1 had a positive ELISA serologic result for *T. spiralis* nematodes. Another 12 patients were not included because detection of *Strongyloides* antibodies was not attempted. Only 4 of the 5 remaining cases fulfilled the strict inclusion criteria (Table); 1 patient was asymptomatic. After 6 months of treatment with albendazole, titers for *Toxocara* sp. roundworm infection and eosinophil count decreased, and symptoms improved or resolved for the 4 patients. Symptoms developed 3–18 months after arrival in Spain.

Clinical toxocariasis is rarely diagnosed in western countries as previously described despite evidence of environmental exposure (*1*). Results of seroprevalence surveys performed in healthy adults in France were positive for 2%–5% of persons in urban areas, compared with 14%–37% in rural areas (*2*). In Latin America, rates vary from 1.8% to 51.6% (*3*,*4*). However, literature references to VLM imported by immigrants are scarce (*5*), and the disease may be underdiagnosed in the immigrant population, partly because of nonspecific symptoms and the limitations of serologic diagnosis. In our study, serologic prevalence of

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DOI: 10.3201/eid1707.101204

Table. Descriptions of 4 cases of visceral larva migrans in immigrants from Latin America, Spain, April 1989–June 2008*

Case no.	Age, y/sex	Origin	Clinical signs and symptoms	Chest radiograph results	Eosinophil count, absolute/mm^3 (%)	6-mo follow-up		
						Eosinophil count/mm^3	Decrease in antibody titers	Symptoms
1	28/M	Bolivia	Asthma-like syndrome†	Slight right parahiliar infiltrate	700 (17.0)	500	Yes	None
2	29/F	Dominican Republic	Dry cough, dyspnea, chest pain, eosinophilic pneumonia	Bilateral alveolar infiltrates	1,400 (10.5)	600	Yes	None
3	5/F	Ecuador	Asthma-like syndrome, abdominal pain	No findings	1,050 (15.0)	700	Yes	None
4	40/F	Colombia	Abdominal pain	Not done	1,500 (14.8)	400	Yes	Clinical improvement

*All patients were treated with albendazole (10–15 mg/kg/d in 2 doses orally for 5 days).
†Wheezing and dry cough.

Toxocara antibodies was 4.9% (31/634).

Toxocariasis is a common cause of eosinophilia in peripheral blood, although its absence does not exclude infection by *Toxocara* sp. roundworms. In other studies, 27% of patients had reactive serologic results for *Toxocara* sp. roundworm infection without eosinophilia (*6*); similarly, 27% of patients with high antibody titers had eosinophil counts within the reference range (*7*). By including only patients with eosinophilia, our study applied more stringent criteria. Thus, 28 (90%) of 31 patients who had positive serologic results showed an elevated eosinophil count, in accordance with previously described high *Toxocara* sp. roundworm seroprevalence (<68%) in patients with eosinophilia of unknown cause (*8*).

Eleven of the 28 patients with positive serologic results for *Toxocara* sp. roundworm and eosinophilia also had positive serologic results for other parasites that cause eosinophilia. One patient who was infected with *A. lumbricoides* roundworm had asthma, hepatomegaly, and pruritus. The latter is not usually associated with this parasite, which suggests possible co-infection.

Serologic tests for *Toxocara* sp. roundworm infection should be interpreted with caution because commercial ELISA kits that use excretory and secretory antigens derived from second-stage larvae of *Toxocara* sp. roundworms exhibit a sensitivity of 91% and a specificity of 86%; cross-reactivity has also been described with other nematode infections. The positive serologic results for *T. spiralis* nematodes and *E. granulosus* tapeworms may have been caused by cross-reactivity (*9*). These patients had asthenia and asthma, respectively, and symptoms resolved after treatment with albendazole. Eight patients with *Strongyloides* antibodies were also excluded; however, this finding does not exclude co-infection by both parasites. Finally, a limitation of the study was that we could not definitively exclude cryptic strongyloidiasis for 12 patients because of the difficulty in finding *S. stercoralis* threadworms in feces and because detection of *Strongyloides* antibodies was not possible.

Other authors have already recommended caution when interpreting positive *Toxocara* sp. roundworm serologic results in asymptomatic persons or persons with equivocal symptoms (*10*). Therefore, the asymptomatic patient with positive serologic and eosinophilia results was also excluded.

This study illustrates the difficulties in diagnosing VLM in immigrants from tropical and subtropical areas of Latin America because only a very small proportion of patients in the series (n = 4) had VLM. The most common symptoms were respiratory (3/4); 2 patients had asthma-like syndrome and 1 had chest pain followed by abdominal pain (2/4). Typical manifestations of VLM are abdominal symptoms (pain, hepatomegaly) and respiratory symptoms (severe asthma, eosinophilic infiltrates). In addition to this, evidence points to *Toxocara* sp. roundworm infection as a risk factor for asthma in some populations (*11*,*12*).

Albendazole is the treatment of choice for VLM; for practical purposes, it could be recommended for presumptive treatment in immigrants from Latin America with eosinophilia in whom strongyloidiasis is suspected (*13*). However, the superiority of ivermectin over albendazole has been documented in the treatment of chronic strongyloidiasis (*14*).

Conclusions

VLM may be difficult to diagnose, especially in immigrants from regions in Latin America where polyparasitism is endemic. Positive serologic test results, marked eosinophilia, absence of other helminthic infections, compatible clinical signs, and disappearance of symptoms after specific treatment can help establish a VLM diagnosis, especially in areas of low parasitism. VLM should be included in the differential diagnosis of eosinophilia in immigrants (children and adults) from tropical areas if respiratory or abdominal symptoms are evident. Albendazole is an effective and relatively safe

drug that could be used to treat suspected VLM and other concomitant nematode infections, including cryptic *S. stercoralis* threadworm infections. Empirically described treatment may lead to resolution of clinical symptoms, even though ivermectin is a better treatment for chronic strongyloidiasis.

The Red de Investigación de Centros de Enfermedades Tropicales (RED: RD06/0021/0020) provided funding for this study.

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Pandemic (H1N1) 2009 and Hajj Pilgrims Who Received Predeparture Vaccination, Egypt

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In Egypt, vaccination against pandemic (H1N1) 2009 virus was required of pilgrims departing for the 2009 Hajj. A survey of 551 pilgrims as they returned to Egypt found 542 (98.1% [weighted]) reported receiving the vaccine; 6 (1.0% [weighted]) were infected with influenza virus A (H3N2) but none with pandemic (H1N1) 2009 virus.

The annual Hajj pilgrimage brings >2.5 million pilgrims from >160 countries to Mecca and Medina in Saudi Arabia during a 1-week period (*1*). In the past, 6.0%–9.8% of Hajj pilgrims with acute respiratory tract infection have been found to have influenza (*2*–*4*). Seasonal influenza vaccine is recommended for pilgrims by Saudi Arabia's Ministry of Health (*5*), but vaccination coverage has been low (*4*,*6*).

The Study

The Hajj occurs during the twelfth month of the Islamic calendar, a lunar calendar that shifts 11 days earlier each year. The 2009 Hajj took place during November 25–28, (1430 AH), when the Northern Hemisphere was experiencing high pandemic (H1N1) 2009 virus activity, raising concern that the gathering could further contribute to the global spread of pandemic (H1N1) 2009. Health experts meeting in Jeddah in June 2009 made recommendations for reducing the pandemic's effects during the Hajj (*7*). The value of predeparture vaccination was recognized, but it was thought unlikely that pandemic vaccine would be available in sufficient quantities to have a major effect on transmission during the Hajj. Egypt received the vaccine in time to begin vaccinating pilgrims on November 3, 2009, and required predeparture vaccination for all pilgrims to protect them against illness and reduce the importation of pandemic (H1N1) 2009 into Egypt when they returned. Egypt also enforced the Jeddah group's recommendation that the 2009 Hajj pilgrimage be made only by persons 12–65 years of age.

Pandemic (H1N1) 2009 virus was first detected in Egypt in June 2009. It did not become the predominant influenza virus causing influenza-like illness (ILI) in Egypt's ILI sentinel surveillance system until mid-November 2009 (*8*).

Approximately 80,000 Egyptians make the Hajj pilgrimage each year, usually returning to Egypt a few days to a few weeks after it. In all, 70%–80% of pilgrims arrive at Cairo International Airport or Port Tawfiq, near Suez (Ministry of Health, Egypt, unpub. data). During the peak return period, 7 or 8 flights arrive each day from Jeddah, with an average of 200–250 pilgrims per flight, and Port Tawfiq receives 1 ship per day from Jeddah with ≈1,000–1,200 pilgrims. The goal of the survey was to measure the prevalence of pandemic (H1N1) 2009 virus infection among returning pilgrims.

The survey was conducted by a team of 2 epidemiologists, 2 health workers, and 2 laboratory technicians from the Preventive Sector, Egyptian Ministry of Health, during the peak return period. Every tenth pilgrim on the ship from Jeddah arriving at Port Tawfiq on December 14, 2009, was selected for the survey sample without regard to illness status. At Cairo International Airport, pilgrims were selected from among all pilgrims on the 9 flights from Jeddah arriving during 9 AM–9 PM on December 10–12, 2009. Because the survey was conducted at the baggage-claim area, probability sampling proved to be difficult. With instructions to choose pilgrims throughout the area around the carousel without regard to age, sex, or illness status, the team selected a convenience sample of ≈50 pilgrims from each flight.

After providing verbal consent, pilgrims were asked their age, in which governorate they lived, and whether they had been vaccinated against pandemic (H1N1) 2009 virus. Their oropharynx was then swabbed, and swab specimens were placed in viral transport medium and kept in liquid nitrogen until transfer to the Ministry of Health's Central Public Health Laboratory. All specimens were tested at the US Naval Medical Research Unit No. 3 in Cairo by real-time reverse transcription PCR (rtRT-PCR) for influenza A viruses, and all specimens positive for influenza A viruses were tested for influenza A subtypes, including pandemic (H1N1) 2009 virus, by rtRT-PCR, according to guidelines of the World Health Organization and the Centers for Disease Control and Prevention (Atlanta, Georgia, USA)

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DOI: 10.3201/eid1707.101484

(*9*). Testing was not performed for influenza B. A recent study found oropharyngeal swab samples to be sensitive than nasopharyngeal swab samples for detecting pandemic (H1N1) 2009 virus by rtRT-PCR (*10*).

Results were weighted according to probabilities of selection within the ship and each plane, which were considered separate strata. Data were analyzed with PROC SURVEYFREQ in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

In all, 559 pilgrims were selected for the survey sample. Seven pilgrims refused to participate, and interview data were missing for 1 pilgrim, leaving 551 pilgrims in the analysis: 206 pilgrims from 4 flights on December 10, 219 pilgrims from 5 flights on December 12, and 126 pilgrims from the ship arriving December 14. The stated age of 549 (99.6%) of pilgrims in the sample was in the allowed age range of 12–65 years. Most were from the Cairo metropolitan area (43.8%) or Lower Egypt (51.0%); these areas were overrepresented compared with the proportion of the national population living in them (Table). All but 9 (98.1%) pilgrims reported receiving a predeparture vaccination against pandemic (H1N1) 2009 virus. No association was found between predeparture vaccination status and sex ($p = 0.38$), age (<55 years vs. ≥55 years) ($p = 0.95$), or area of residence (Cairo metropolitan area vs. outside this area) ($p = 0.20$). In all, 6 (1.0%, 95% confidence interval 0.2%–1.7%) pilgrims tested positive for influenza A. All had subtype H3N2. No pilgrim had positive results for pandemic (H1N1) 2009 virus.

This finding supports the conclusion that returning pilgrims likely contributed little to ongoing pandemic (H1N1) 2009 transmission in Egypt and is consistent with the intended effects of the predeparture vaccination requirement. At the time of the 2009 Hajj, pandemic (H1N1) 2009 was overwhelmingly the most common influenza virus in the Northern and Southern Hemispheres (*12*), and to our knowledge, few countries required predeparture vaccination against it. Thus, we expect that pilgrims were exposed to this virus during the Hajj, but the extent of exposure is uncertain. The only data of which we are aware that have been released thus far on the extent of such exposure were provided by the Minister of Health, Saudi Arabia, who announced on the last day of the Hajj that pandemic (H1N1) 2009 had been diagnosed in only 73 persons during the Hajj (5 of whom died) (*13*). Haworth et al. have argued that a much larger number of cases is likely, on the basis of the expected case-fatality ratio among the Hajj pilgrims and on modeling results (*14*).

Our survey had several limitations. First, the results may not apply to pilgrims who returned to Egypt before or after the survey period. Second, pilgrims from Upper Egypt and coastal Egypt were underrepresented in the survey sample. Third, convenience sampling was used to select pilgrims arriving by plane. Fourth, unvaccinated pilgrims may have been reluctant to tell interviewers they had not been vaccinated because predeparture vaccination was required. Finally, some pilgrims may have been infected with pandemic (H1N1) 2009 virus shortly before swab samples were obtained but were not yet shedding virus.

Table. Demographic characteristics, vaccination history, and influenza A infection prevalence among 551 pilgrims returning from the Hajj, Egypt, 2009

Characteristic	No. pilgrims (weighted %)
Age group, y	
<30	11 (2.2)
30–39	66 (12.4)
40–49	141 (23.9)
50–59	219 (40.2)
60–69	114 (21.2)
≥70	0
<12*	1 (0.3)
>65*	1 (0.1)
Gender	
M	311 (57.8)
F	240 (42.2)
Area of residence†	
Coastal Egypt	12 (3.1)
Lower Egypt	298 (51.0)
Cairo metropolitan area	232 (43.8)
Upper Egypt	9 (2.1)
Verbal history of pandemic (H1N1) 2009 vaccination	
Yes	542 (98.1)
No	9 (1.9)
Pilgrims infected with influenza A virus, by subtype‡	
Seasonal (H1N1)	0
Seasonal (H3N2)	6 (1.0)
Pandemic (H1N1) 2009	0

*Outside the age limits recommended for Hajj pilgrims by the Ministry of Health, Saudi Arabia, and enforced by Egypt's Ministry of Health.
†The proportion of the national population living in these 4 areas according to the 2006 census was 11.3%, 34.3%, 19.4%, and 34.9%, respectively (*11*). The areas were formed by grouping Egypt's governorates as follows: Coastal Egypt: Alexandria, Damietta, Ismailia, Matrouh, North Sinai, Port-Said, Suez, Red Sea; Lower Egypt: Behera, Dakahlia, Gharbia, Kafr-ElSheikh, Menoufia, Sharkia; Cairo metropolitan area: Cairo, Giza, Kalyoubia; Upper Egypt: Aswan, Asyout, Beni-Suef, ElWadi ElGidid, Fayoum, Helwan, Luxor City, Menia, Qena, South Sinai, Suhag, and 6th October.
‡By real-time reverse transcription PCR testing.

Conclusions

Egypt demonstrated that it could implement a predeparture vaccination requirement despite late arrival of the vaccine, and our survey found no evidence that pilgrims returned to Egypt with pandemic (H1N1) 2009 virus infection during the peak return period. These results may prompt other countries to consider a similar influenza vaccination policy before the Hajj and other mass gatherings where amplification of influenza virus transmission is a major threat. Studies of vaccine effectiveness, cost-effectiveness, and cost-benefit in these settings would provide additional useful information.

Acknowledgments

We thank the members of the Ministry of Health who assisted with the survey: Amany El-Shaeer for supervising field work, Quarantine Department members who obtained authorization for the survey, sanitarians who interviewed pilgrims and obtained swab specimens, and Central Public Health Laboratory staff for managing and transferring specimens. We also thank Engy Habashy and Mary Younan for conducting rtRT-PCR testing, Adel Azabfor for assisting with data management, and Marc-Alain Widdowson for reviewing the manuscript.

This evaluation was part of Egypt's public health practice response to pandemic (H1N1) 2009. It was reviewed by appropriate Centers for Disease Control and Prevention authorities and deemed not to be research in accordance with the federal human subjects protection regulations (45 Code of Federal Regulations 46.101c and 46.102d) and the agency's Guidelines for Defining Public Health Research and Public Health Non-Research.

The Global Emerging Infections Surveillance and Response System of the US Department of Defense provided funding for laboratory testing.

Dr Kandeel is undersecretary of preventive affairs in the Egyptian Ministry of Health. He has a long-standing interest in communicable disease prevention and control, including infection control in health facilities.

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Bartonella spp. in Bats, Guatemala

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To better understand the role of bats as reservoirs of *Bartonella* spp., we estimated *Bartonella* spp. prevalence and genetic diversity in bats in Guatemala during 2009. We found prevalence of 33% and identified 21 genetic variants of 13 phylogroups. Vampire bat–associated *Bartonella* spp. may cause undiagnosed illnesses in humans.

Multiple studies have indicated that bats might serve as natural reservoirs to a variety of pathogens, including rabies virus and related lyssaviruses, Nipah and Hendra viruses, Marburg virus, and others (*1*,*2*). Bats' high mobility, broad distribution, social behavior (communal roosting, fission–fusion social structure), and longevity make them ideal reservoir hosts and sources of infection for various etiologic agents. In addition to viruses, bacteria and ectoparasites have been detected in bats (*3*–*5*) and can potentially cause human infection (*6*).

Bartonella spp. have been found in rodents, insectivores, carnivores, ungulates, and many other mammals. Naturally infected hematophagous arthropods, such as fleas, flies, lice, mites, and ticks are frequently implicated in transmitting *Bartonella* spp. (*3*–*5*,*7*). Detection of *Bartonella* DNA in the saliva of dogs suggests the possibility that *Bartonella* spp. can be transmitted through biting (*8*). Increasing numbers of *Bartonella* spp. have been identified as human pathogens (*9*,*10*). However, a mammalian reservoir has not been determined for some newly identified species, such as *B. tamiae* (*9*). Extensive surveillance for *Bartonella* spp. among diverse groups of animals, including bats, has become crucial.

To our knowledge, *Bartonella* spp. in bats have been studied only in the United Kingdom and Kenya (*11*,*12*). To better understand the role of bats as reservoir hosts of *Bartonella* spp. and their potential risk for infecting humans and animals, we looked for *Bartonella* spp. in bats in Guatemala, estimated prevalence, and evaluated the genetic diversity of the circulating *Bartonella* strains.

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DOI: 10.3201/eid1707.101867

The Study

In 2009, a total of 118 bats were collected from 5 sites in southern Guatemala (Figure 1). The bats represented 15 species of 10 genera; the most prevalent (26.3%) species was the common vampire bat (*Desmodus rotundus*); the other 14 species accounted for 0.8%–12.7% of the bats sampled. Diversity of bats was 6–8 species per site (Table 1). Blood specimens from the bats were collected and cultured for *Bartonella* spp., according to a published method (*12*). A total of 41 *Bartonella* isolates were obtained from 39 (33.1%) of the 118 bats; colonies with different morphologic characteristics were identified from blood of 2 *Pteronotus davyi* bats. Prevalence of *Bartonella* spp. in Conguaco (60%, 15/25) was significantly higher than that in Oratorio (11.8%, 2/17), San Lucas Tolimán (14.3%, 2/14), and Taxisco (22.6%, 7/31) but did not differ from that in Santa Lucía Cotzumalguapa (41.9%, 13/31). *Bartonella* spp. were cultured from 8 bat species. The *Bartonella* spp. prevalence among *Phyllostomus discolor* (88.8%, 9/8), *P. davyi* (70%, 7/10), and *D. rotundus* (48.4%, 15/31) bats was significantly higher than that among *Sturnira lilium* (8.3%, 1/12) and *Glossophaga soricina* (13.3%, 2/15) bats. No *Bartonella* spp. were

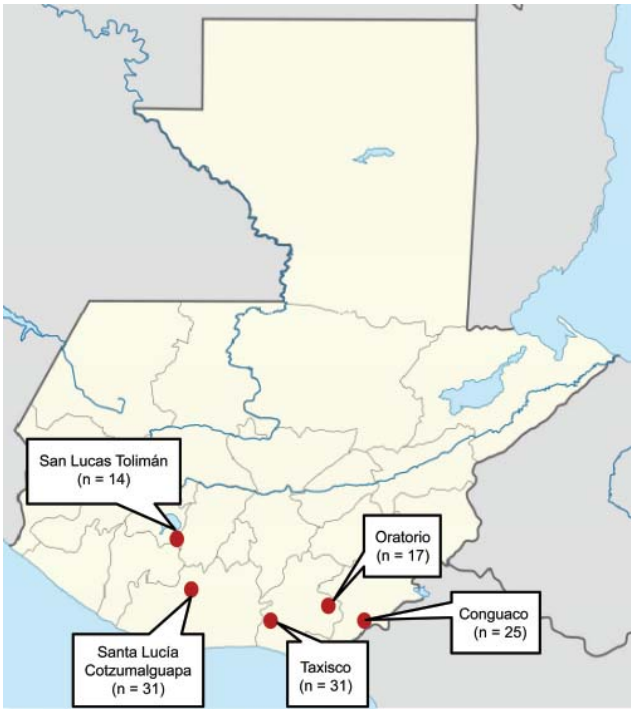

Figure 1. Sites of bat collection, showing number of bats collected from each site, Guatemala, 2009.

Table 1. Prevalence of *Bartonella* spp. in bats from 5 collection sites, Guatemala, 2009

Bat species	No. positive/no. cultured					Overall, no. positive/ no. cultured (%)
	Conguaco	Oratorio	San Lucas Tolimán	Santa Lucía Cotzumalguapa	Taxisco	
Artibeus jamaicensis	0/3	0/2	0	0/3	0/5	0/13
Artibeus lituratus	0/1	0/1	0/1	0	0	0/3
Artibeus toltecus	1/1	0	0	0	0	1/1 (100)
Carollia castanea	0	0	0	0/1	0	0/1
Carollia perspicillata	0	0/2	0/3	0	4/9	4/14 (28.6)
Desmodus rotundus	5/7	2/4	0/1	6/12	2/7	15/31 (48.4)
Glossophaga soricina	1/1	0/3	1/5	0/3	0/3	2/152 (13.3)
Micronycteris microtis	0	0	0	0	1/3	1/3 (33.3)
Myotis elegans	0	0	0	0/1	0/1	0/2
Myotis nigricans	0	0	0	0	0/1	0/1
Phyllostomus discolor	7/8	0	1/1	0	0	8/9 (88.9)
Platyrrhinus helleri	0	0	0	0/1	0	0/1
Pteronotus davyi	0	0	0	7/10	0	7/10 (70)
Sturnira lilium	1/3	0/5	0/2	0	0/2	1/12 (8.3)
Sturnira ludovici	0/1	0	0/1	0	0	0/2
Total	15/25	2/17	2/14	13/31	7/31	39/118 (33.1)

found in *Artibeus jamaicensis* (0/13) and 6 other bat species tested (Table 1).

Identity of 41 *Bartonella* isolates was confirmed by PCR amplification of a specific region in the citrate synthase gene by using primers BhCS781.p (5′-GGGGACCAGCTCATGGTGG-3′) and BhCS1137.n (5′-AATGCAAAAAGAACAGTAAACA-3′). Subsequent sequencing analyses of the 41 isolates revealed 21 genetic variants (Table 2) that clustered into 13 phylogroups (I–XIII) with 6.6%–24.7% divergence. The phylogroups were also distant from any previously described *Bartonella* species and genotypes identified in bats from the United Kingdom and Kenya (Figure 2). Each phylogroup contained 1–6 variants; similarities within phylogroups were 96.2%–99.7% (Table 2).

Of the 13 phylogroups, phylogroups I, IV, and VII were identified in isolates obtained from different bat species (Table 2), suggesting that bats of different species may share the same *Bartonella* strain; whereas 4 species of bats—*C. perspicillata*, *D. rotundus*, *P. discolor*, and *P. davyi*—were infected with 2–4 *Bartonella* strains (Table 2). *P. davyi* from 2 bats belonged to phylogroups II or VIII.

Conclusions

The high (≈33%) prevalence of *Bartonella* spp. in bat populations in southern Guatemala might suggest

Table 2. GenBank accession numbers and distribution of 21 genetic variants of *Bartonella* spp. in bats from Guatemala, 2009

Accession no.	Type strain	Host bat species	No. sequences	Distribution (no. isolates)	Phylogroup
HM597187	B29042	*Desmodus rotundus*	1	*D. rotundus* (1)	I
HM597188	B29043	*D . rotundus*	3	*D. rotundus* (3)	I
HM597189	B29044	*D . rotundus*	2	*D. rotundus* (2)	I
HM597190	B29107	*D . rotundus*	1	*D. rotundus* (1)	I
HM597191	B29108	*D. rotundus*	3	*D. rotundus* (2); *C. perspicillata* (1)	I
HM597192	B29114	*D. rotundus*	3	*D. rotundus* (2); *C. perspicillata* (1)	I
HM597193	B29102	*Pteronotus davyi*	3	*P. davyi* (3)	II
HM597194	B29109	*P. davyi*	1	*P. davyi* (1)	II
HM597195	B29119	*D. rotundus*	3	*D. rotundus* (3)	III
HM597196	B29122	*D. rotundus*	1	*D. rotundus* (1)	III
HM597198	B29116	*Phyllostomus discolor*	2	*P. discolor* (2)	V
HM597199	B29126	*Carollia perspicillata*	2	*C. perspicillata* (2)	IV
HM597200	B29230	*P. discolor*	1	*P. discolor* (1)	IV
HM597201	B29115	*P. discolor*	3	*P. discolor* (3)	VI
HM597202	B29110	*Glossophaga soricina*	3	*G. soricina* (2); *P. davyi* (1)	VII
HM597203	B29105	*P. davyi*	3	*P. davyi* (3)	VIII
HM597204	B29112	*P. discolor*	2	*P. discolor* (2)	IX
HM597205	B29134	*P. davyi*	1	*P. davyi* (1)	X
HM597206	B29137	*Sturnira lilium*	1	*S. lilium* (1)	XI
HM597207	B29172	*Micronycteris microtis*	1	*M. microtis* (1)	XII
HM597197	B29111	*Artibeus toltecus*	1	*A. toltecus* (1)	XIII

persistent infection of long-lived bats with *Bartonella* spp., similar to their infection with some viruses (*13*). Depending on the bat species, *Bartonella* spp. exhibit high, low, or no infectivity, which may explain the variation in *Bartonella* spp. prevalence between study sites because the assemblage of bat species differed at each site. Additional studies are needed to illustrate the distribution of *Bartonella* spp. among the bat fauna in Guatemala and throughout the region.

Further characterization is necessary to verify whether the *Bartonella* strains representing a variety of distinct phylogroups represent novel *Bartonella* species. Unlike the discovery in bats in Kenya (*12*), host specificity of *Bartonella* spp. was not found in bats in Guatemala. Such lack of specificity may be partly associated with the arthropod vectors that parasitize bats, although we were unable to attempt isolation of agents from the bat ectoparasites. Future studies of bat ectoparasites would enable testing of hypotheses about whether any arthropods may be vectors in the *Bartonella* spp. transmission cycle and whether ectoparasite specificity contributes to the lack of host specificity observed in this study.

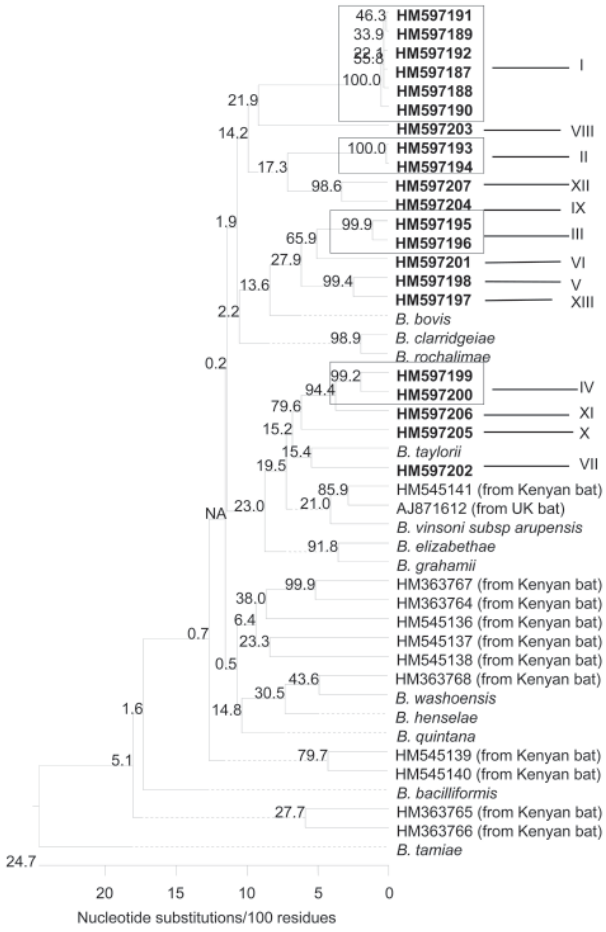

Figure 2. Phylogenetic relationships of the *Bartonella* spp. genotypes based on partial sequences of the citrate synthase gene detected in bats from Guatemala, Kenya, United Kingdom, and some reference *Bartonella* spp. The phylogenetic tree was constructed by the neighbor-joining method, and bootstrap values were calculated with 1,000 replicates. A total of 21 *Bartonella* genotypes, forming 13 *Bartonella* phylogroups, were identified in the bats from Guatemala. Each genotype is indicated by its GenBank accession number in **boldface**; the phylogroups are marked by Roman numerals I–XIII.

The tendency of some bat species to share roosts, reach large population densities, and roost crowded together creates the potential for dynamic intraspecies and interspecies transmission of infections (*14*). In accordance with this hypothesis, our finding that co-infection with multiple *Bartonella* strains in a single bat species, and even in an individual bat, indicate that active interspecies transmission of *Bartonella* spp. likely occurs among bats in Guatemala. The specificity of ectoparasite arthropod vectors among the bat fauna remains unclear and may contribute to interspecies transmission of *Bartonella* spp. among bats.

The long life spans of bats (average 10–20 years) may have made them major reservoirs that contribute to the maintenance and transmission of *Bartonella* spp. to other animals and humans. The bite of the common vampire bat has been long recognized to transmit rabies virus to humans throughout Latin America (*2*). These bats typically feed on the blood of mammals, including domestic animals and humans (*15*). Predation of vampire bats on humans is a major problem in Latin America (*2*). If *Bartonella* spp. can be transmitted to humans through the bite of bats, the need for further studies with vampire bats is imperative. *Bartonella* spp.–specific DNA has been detected in ectoparasites collected from bats (*3*–*5*). Presumably, if *Bartonella* spp. are transmitted through a bat ectoparasite vector, some, if not all, bat-associated *Bartonella* spp. could be transmitted to humans because bats are frequent hosts to a wide variety of ectoparasites, including bat flies, fleas, soft ticks, and mites. However, transmission potential might vary with the degree of synanthropic roosting or foraging behavior within the bat community.

Because an increasing number of *Bartonella* spp. are being associated with human illness, the need to identify the animal reservoirs of these novel *Bartonella* spp. and to understand their disease ecology is also increasing. Our study of *Bartonella* spp. in bats has enlarged our scope of this zoonotic potential as we search for the reservoirs that harbor novel and known *Bartonella* spp.

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Clonal Genotype of *Geomyces destructans* among Bats with White Nose Syndrome, New York, USA

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The dispersal mechanism of *Geomyces destructans*, which causes geomycosis (white nose syndrome) in hibernating bats, remains unknown. Multiple gene genealogic analyses were conducted on 16 fungal isolates from diverse sites in New York during 2008–2010. The results are consistent with the clonal dispersal of a single *G. destructans* genotype.

Geomycosis, or white nose syndrome, is a newly recognized fungal infection of hibernating bats. The etiologic agent, the psychrophilic fungus *Geomyces destructans*, was first recognized in caves and mines around Albany, New York, USA (*1*,*2*). The disease has spread rapidly in New York and other states in the northeastern United States. At least 1 affected bat species is predicted to face regional extinction in the near future (*3*). Much remains unknown about this fungus, including its ecology and geographic distribution. For example, although hibernacula are high on the list of suspected sites, where the bats acquire this infection is not known. Similarly, although strongly suspected, the role of humans and other animals in the dispersal of *G. destructans* and the effect of such dispersals in bat infections have not been confirmed. We recently showed that 6 *G. destructans* strains from sites near Albany were genetically similar (*2*), raising the possibility of a common source for the spread of this infection. Corollary to this observation and other opinions (*3*,*4*), the US Fish & Wildlife Service has made an administrative decision to bar human access to caves as a precautionary measure (www.fws.gov/whitenosesyndrome/pdf/NWRS_WNS_Guidance_Final1.pdf). Thus, an understanding of the dispersal mechanism of *G. destructans* is urgently needed to formulate effective strategies to control bat geomycosis.

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DOI: 10.3201/eid1707.102056

The Study

We applied multiple gene genealogic analyses in studying *G. destructans* isolates; this approach yields robust results that are easily reproduced by other laboratories (*5*). Sixteen *G. destructans* isolates recovered from infected bats during 2008–2010 were analyzed. These isolates originated from 7 counties in New York and an adjoining county in Vermont, all within a 500-mile radius (Table 1). The details of isolation and identification of *G. destructans* from bat samples have been described (*2*). One isolate of a closely related fungus *G. pannorum* M1372 (University of Alberta Mold Herbarium, Edmonton, Alberta, Canada) was included as a reference control. To generate molecular markers, 1 isolate, *G. destructans* (M1379), was grown in yeast extract peptone dextrose broth at 15°C, and high molecular weight genomic DNA was prepared according to Moller et al. (*6*). A cosmid DNA library was constructed by using pWEB kit (Epicenter Biotechnologies, Madison, WI, USA) by following protocols described elsewhere (*7*). One hundred cosmid clones, each with ≈40-Kb DNA insert, were partially sequenced in both directions by using primers M13 and T7. The nucleotide sequences were assembled with Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI, USA) and BLAST (www.ncbi.nlm.nih.gov/BLAST) homology searches identified 37 putative genes. Sequences of 10 genes, including open reading frames, 3′ and/or 5′ untranslated regions, and introns, were evaluated as potential markers for analyzing *G. pannorum* and *G. destructans*. Our screening approach indicated that 8 gene

Table 1. *Geomyces destructans* isolates studied, New York, USA

Isolate	Date obtained	Site, county*
M1379†	2008 Mar 28	Williams Hotel Mine, Ulster
M1380†	2008 Mar 28	Williams Hotel Mine, Ulster
M1381†	2008 Mar 28	Williams Hotel Mine, Ulster
M1383†	2008 Apr 11	Graphite Mine, Warren
M2325	2010 Jan 25	Westchester
M2327	2010 Feb 2	Dewitt, Onondaga
M2330	2009 Mar 5	Lancaster, Erie
M2331	2009 Mar 9	White Plains, Westchester
M2332	2009 Mar 11	Dannemora, Clinton
M2333	2009 Mar 11	Dannemora, Clinton
M2334	2009 Mar 12	Newstead, Erie
M2335	2009 Mar 16	Ithaca, Tompkins
M2336	2009 Oct 6	Bridgewater Mine, Windsor, VT
M2337	2010 Feb 9	Akron Mine, Erie
M2338	2010 Mar 4	Hailes Cave, Albany
M2339	2010 Mar 11	Letchworth Tunnel, Livingston

*All locations in New York state except Bridgewater Mine, Windsor, Vermont.
†Previously analyzed by randomly amplified polymorphic DNA typing.

targets could be amplified from both *G. destructans* and *G. pannorum* by PCR (Table 2).

To obtain DNA sequences from 1 *G. pannorum* and 16 *G. destructans* isolates, we prepared genomic DNA from mycelia grown in yeast extract peptone dextrose broth through conventional glass bead treatment and phenol-chloroform extraction and then ethanol precipitation (*7*). AccuTaq LA DNA Polymerase (Sigma-Aldrich, St. Louis, MO, USA) was used for PCR: 3 min initial denaturation at 94°C, 35 amplification cycles with a 15-sec denaturation at 94°C, 30-sec annealing at 55°C, and 1-min extension at 68°C and a 5-min final extension at 68°C. PCR products were treated with ExoSAP-IT (USB Corp., Cleveland, OH, USA) before sequencing. Both strands of amplicons were sequenced by the same primers used for PCR amplification (Table 2). A database was created by using Microsoft Access (Microsoft, Redmond, WA, USA) to deposit and analyze the sequences. Nucleotide sequences were aligned with ClustalW version 1.4 (www.clustal.org) and edited with MacVector 7.1.1 software (Accelrys, San Diego, CA, USA). Phylogenetic analyses were done by using PAUP 4.0 (*8*) and MEGA 4 (*9*).

We cloned and sequenced ≈200 Kb of the *G. destructans* genome and identified genes involved in a variety of cellular processes and metabolic pathways (Table 2). DNA sequence typing by using 8 gene fragments showed that all 16 *G. destructans* isolates had identical nucleotide sequences at all 8 sequenced gene fragments but were distinct from *G. pannorum* sequences. A maximum-parsimony tree generated from the 8 concatenated gene fragments indicated a single, clonal genotype for the 16 *G. destructans* strains (Figure 1). This consensus tree included 4,470 aligned nucleotides from all targeted gene sequences with 545 variable sites that separate the *G. destructans* clonal genotype from *G. pannorum*. Further analyses of the same concatenated gene fragments with exclusion of 50 insertions and deletions between *G. destructans* and *G. pannorum* yielded a tree with a shorter length (495 steps instead of 545 steps) but an identical topology (online Technical Appendix Figure 1, www.cdc.gov/EID/content/17/7/1273-Techapp.pdf). This pattern remained unchanged when different phylogenetics models were used for analysis (online Technical Appendix Figure 2). The lack of polymorphism among the 16 *G. destructans* isolates was unlikely because of evolutionary constraint at the sequenced gene fragments. We found many synonymous and nonsynonymous substitutions in target genes among a diversity of fungal species, including between *G. destructans* and *G. pannorum* (*10*) (online Technical Appendix Figure 3).

Conclusions

Our finding of a single clonal genotype in *G. destructans* population fits well with the rapid spread of geomycosis in New York (Figure 2). Our sampling population covered both spatial and temporal dimensions, and the numbers of isolates analyzed were adequate in view of difficulties encountered in obtaining pure isolations of *G. destructans* (*11*). Although the affected New York sites are separated by sizable distances and include geographic barriers, a role for the natural dissemination of the fungus through air, soil, and water cannot be ruled out. Indeed, several fungi with

Table 2. *Geomyces destructans* and *G. pannorum* target gene fragments used for multiple gene genealogic analyses, New York, USA

Gene*	Homology (GenBank accession no.)	Amplicon size/ sequence used for comparison, bp	Primer sequence, 5′ → 3′†	*G. destructans*/*G. pannorum* GenBank accession nos.
ALR	*Penicillium marneffei* (XP_002152078.1)	654/534	V1905 (f): CGGAGTGAGATTTATGACGGC V1904 (r): CGTCCATCCCAGACGTTCATC	HQ834314–HQ834329/HQ834330
Bpntase	*Glomerella graminicola* (EFQ33509.1)	921/745	V1869 (f): TCAGACGGACTCGGAGGGCAAG V1926 (r): TCGGTTACAGAGCCTCAGTCG	HQ834331–HQ834346/HQ834347
DHC1	*Sordaria macrospora* (CBI53717.1)	597/418	V1906 (f): GGATGATTCGGTCACCAAACAG V1907 (r): ACAGCAAACACAGCGCTGCAAG	HQ834348–HQ834363/HQ834364
GPHN	*Ajellomyces capsulatus* (EEH06836.1)	659/525	V1918 (f): CACTATTACATCGCCAGGCTC V1919 (r): CTAAACGCAGGCACTGCCTC	HQ834365–HQ834380/HQ834381
PCS	*A. capsulatus* (EEH08767.1)	920/749	V1929 (f): AGGCTGCGATTGCTGAGTGC V1873 (r): CCTTATCCAGCTTTCCTTGGTC	HQ834382–HQ834397/HQ834398
POB3	*Pyrenophora tritici-repentis* (XP_001937502.1)	653/417	V1908 (f): CACAGTGGAGCAAGGCATCC V1909 (r): ACATACCTAGGCGTCAAGTGC	HQ834399–HQ834414/HQ834415
SRP72	*A. dermatitidis* (EEQ90678.1)	941/640	V1927 (f): AAGGGAAGGTTGGAGAGACTC V1895 (r): CAAGCAGCATTGTACGCCGTC	HQ834416–HQ834431/HQ834432
VPS13	*Verticillium albo-atrum* (XP_003001174.1)	665/545	V1922 (f): GAGACAACGCTTGTTTGCAAGG V1923 (r): ACATGCGTCGTTCCAAGATCTG	HQ834433–HQ834448/HQ834449

*Genes: *ALR*, α-L-rhamnosidase; *Bpntase*, 3′(2′),5′-bisphosphate nucleotidase; *DHC1*, Dynein heavy chain; *GPHN*, Gephyrin, molybdenum cofactor biosynthesis protein; *PCS*, peroxisomal-coenzyme A synthetase; *POB3*, FACT complex subunit; *SRP72*, signal recognition particle protein 72; *VPS13*, vacuolar protein sorting-associated protein.
†f, forward; r, reverse.

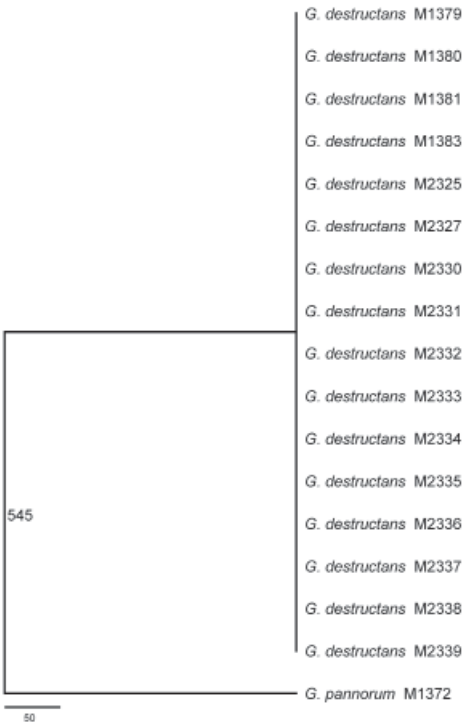

Figure 1. Consensus maximum-parsimony tree derived from analyzing 8 concatenated gene fragments including a total of 4,470 aligned nucleotides by using PAUP* 4.0 (*8*). The number 545 on the branch indicates the total number of variable nucleotide positions (out of the 4,470 nt) separating *Geomyces pannorum* M1372 from the clonal genotype of *G. destructans* identified here. Fifty of the 545 variable sites correspond to insertions and deletions. Scale bar indicates number of nucleotide substitutions per site.

geographic distributions similar to that in our study have shown major genetic variation among strains (*12*,*13*). It is also possible that humans and/or animals contributed to the rapid clonal dispersal. In such a scenario, the diseased or asymptomatic bats might act as carriers of the fungus by their migration into new hibernation sites where new animals get infected and the dissemination cycle continues (*4*). Similarly, the likely roles played by humans and/or other animals in the transfer of the fungal propagules from an affected site to a clean one cannot be ruled out from our data.

Virulent clones of human and plant pathogenic fungi that spread rapidly among affected populations have been recognized with increasing frequency in recent years (*12*,*14*). However, other pathogens, such as the frog-killing fungus *Batrachochytrium dendrobatidis*, have emerged with both clonal and recombining populations (*13*). Our data do not eliminate the possibility that the *G. destructans* population undergoes recombination in nature. This process to generate genetic variability would require some form of sexual reproduction, which remains unknown in *G. destructans.* In addition, the fungus might have both asexual and sexual modes in its saprobic life elsewhere in nature, but it exists only in asexual mode on bats (*15*).

In conclusion, our data suggest that a single clonal genotype of *G. destructans* has spread among affected bats in New York. This finding might be helpful for the professionals involved in devising control measures. Many outstanding questions remain about the origin of *G. destructans*, its migration, and reproduction, all of which will require concerted efforts if we are to save bats from predicted extinction (*3*).

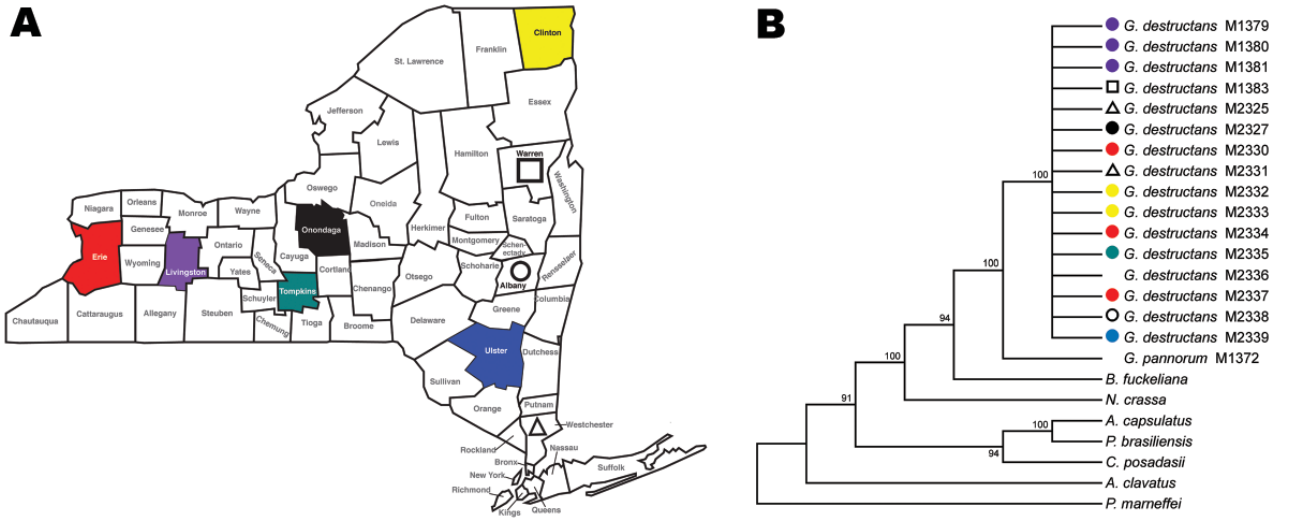

Figure 2. Collection sites in New York counties (A) are color-matched with respective *Geomyces destructans* isolates in maximum-parsimony tree based on nucleotide sequence of the VPS13 gene (B). The tree was constructed with MEGA4 (*9*) by using 450 nt and bootstrap test with 500 replicates. In addition to *G. destructans* and *G. pannorum*, fungi analyzed were *Ajellomyces capsulatus* (AAJI01000550.1), *Aspergillus clavatus* NRRL 1 (AAKD03000035.1), *Botryotinia fuckeliana* B05.10 (AAID01002173.1), *Coccidioides posadasii* C735 delta SOWgp (ACFW01000049.1), *Neurospora crassa* OR74A (AABX02000023.1), *Paracoccidioides brasiliensis* Pb01 (ABKH01000209.1), and *Penicillium marneffei* ATCC 18224 (ABAR01000009.1).

Acknowledgments

We acknowledge the Wadsworth Center Applied Genomic Technologies Core for DNA sequencing and Media, Glassware and Tissue Culture Unit for specialized culture media. We thank Jared Mayron for creating a Microsoft Access database.

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Natural *Burkholderia mallei* Infection in Dromedary, Bahrain

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We confirm a natural infection of dromedaries with glanders. Multilocus variable number tandem repeat analysis of a *Burkholderia mallei* strain isolated from a diseased dromedary in Bahrain revealed close genetic proximity to strain Dubai 7, which caused an outbreak of glanders in horses in the United Arab Emirates in 2004.

Glanders, a World Organisation for Animal Health (OIE)–listed disease, is a contagious, life-threatening disease of equids caused by the gram-negative bacterium *Burkholderia mallei* (*1*). Although eliminated in western Europe, glanders remains endemic to several Asian, African, and South American countries. It recently reappeared in Pakistan and Brazil in 2008 and 2009, respectively, and appeared for the first time in Kuwait and Bahrain in 2010 (*2*,*3*).

Natural *B. mallei* infections are known to occur in various mammals (e.g., cats, bears, wolves, and dogs). Camels are also susceptible to *B. mallei*, as experimental infection has demonstrated (*4*,*5*). We report a natural infection of dromedaries (*Camelus dromedarius*).

An outbreak of glanders is ongoing in equids in Bahrain (*6*). Most of the reported cases were found in Saar and Shakhoura in the Northern governorate. Samples from 4,843 horses and 120 donkeys were sent to the OIE Reference Laboratory at the Central Veterinary Research Laboratory in Dubai, United Arab Emirates. Of these samples, 45 horses with clinical signs consistent with glanders were positive by complement fixation test and were euthanized along with 4 donkeys that also had positive test results. In addition to horses and donkeys, dromedaries showed clinical signs of glanders, but *B. mallei* infection has not yet been confirmed. Here we provide evidence for a *B. mallei* infection in 1 of the diseased dromedaries.

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DOI: 10.3201/eid1707.110222

The Study

On a small private farm, 2 of 7 horses had positive serologic reactions and showed typical clinical signs of glanders. On the same premises, 6 dromedaries were kept several meters away from the sick horses in a separate enclosure. Three dromedaries that showed clinical signs of glanders, including severe mucopurulent discharge from both nostrils (Figure 1, panel A), fever, emaciation, and fatigue, died. One of these dromedaries underwent necropsy. Serum samples from this dromedary tested positive for glanders with both the OIE-acknowledged complement fixation test (titer 10+++) and with the Central Veterinary Research Laboratory–developed in-house competitive ELISA (*7*) with an inhibition of 57%.

An EDTA blood sample was incubated for 11 days in a blood culture system (Oxoid, Cambridge, UK) until it became positive. This fluid was then cultured on sheep blood agar at 37°C for 72 h. The isolate stained poorly gram-negative, was rod shaped, and tested oxidase positive. Suspected *B. mallei* colonies were analyzed with the API 20 NE-test (bioMérieux, Marcy l'Etoile, France) and were positive for nitrate, glucose assimilation, arginine dehydrolase (after 4 days of incubation), N–acetyl glucosamine, and potassium gluconate. The API 20 NE-test identified the colonies as *B. mallei* because the same API ID number (1140504) occurred as in the previously isolated Dubai 7 strain (*1*).

During necropsy, typical glanderous lesions in the lung, choanae, and nasal septae were observed. Golf ball–sized reddish-gray nodules resembling tubercles with a central gray necrotic zone were detected in the lungs. In the choanae and nasal septae, stellate scars, ulcers, and honeycomb necrotic patches covered with yellow pus (Figure 1, panel B) were seen. Glanderous lesions were absent from other organs.

The presence of *B. mallei* in lung and choanae specimens was examined by using standard culturing techniques as described by Wittig et al. (*1*). For bacterial growth, sheep blood agar plates were incubated at 37°C for 72 h. *B. mallei* was directly isolated from the pus, which had accumulated in the choanae, but not from nasal and eye swabs and not from the lung lesions. However, the tissue samples were stored at –20°C for >20 days before incubation.

For molecular analysis, cultivated bacteria were resuspended in saline and inactivated at 98°C for 20 min. Total DNA was extracted by using the DNA-purification Kit (QIAGEN, Hilden, Germany). Sequence analysis of the 16S rRNA (1,400 pb) gene displayed the *B. mallei*–

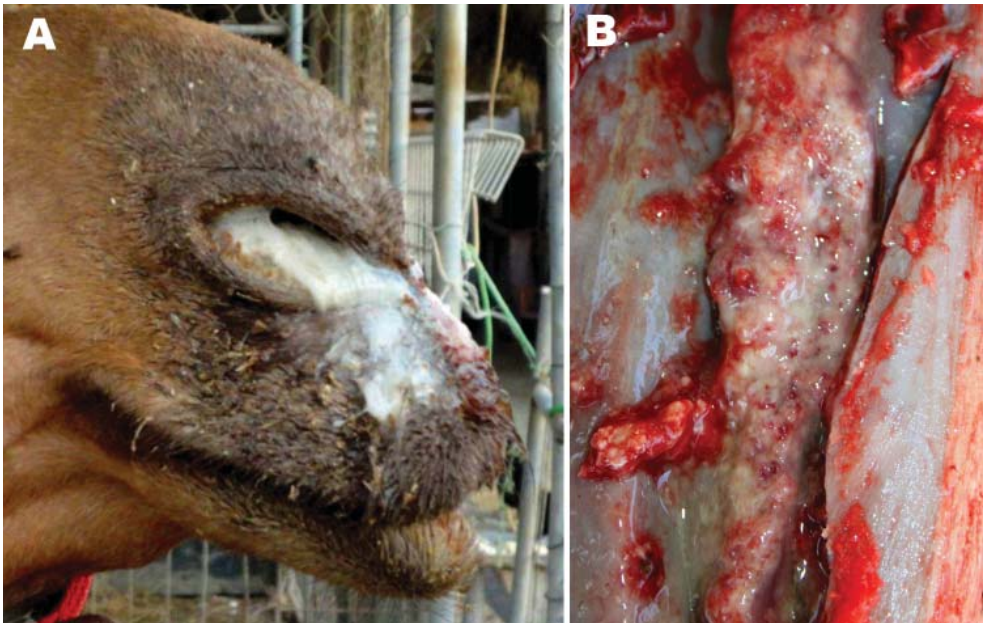

Figure 1. A) Severe mucopurulent discharge from both nostrils of a glanderous dromedary (*Camelus dromedarius*), Bahrain. B) Glanderous lesions in the choanae of a dromedary.

specific single nucleotide polymorphism that differentiates *B. mallei* from *B. pseudomallei* (*8*) (not shown). *B. mallei* was further confirmed by multilocus sequence typing displaying the *B. mallei*–specific sequence type 40 (alleles 1, 12, 3, 4, 1, 18, 1), as previously described by Godoy et al. (*9*).

Multilocus variable number tandem repeat analysis based on 23 different loci (*10*) was used for further subtyping through sequencing of the variable number tandem repeat regions (online Appendix Table, www.cdc.gov/EID/content/17/7/1277-appT.htm). Phylogenetic analysis of these data was performed as described by Hornstra et al. (*10*) and compared with existing *B. mallei* strains (Figure 2). In this analysis, the strain (THSK2) isolated from the dromedary clustered with *B. mallei* strain Dubai 7 (Figure 2) that had been isolated from a horse in the United Arab Emirates (*11*).

Conclusions

Old World camels, the dromedary (*C. dromedarius*), and the Bactrian camel (*C. bactrianus*) are susceptible to *B. mallei* (glanders) and *B. pseudomallei* (melioidosis) infection (*12*,*13*). However, reports of *B. mallei* infection in dromedaries have described artificial infections (*4*,*5*). We report natural *B. mallei* infection in a dromedary that occurred during a glanders outbreak in horses.

Clinical signs as well as gross pathologic and microscopic lesions of the diseased dromedary were similar to changes seen in equids. These changes were dominated by severe mucopurulent nasal discharge, nodules and ulcers with pus in the choanae and nasal septae, and granulomas in the lungs that resembled tubercle lesions (pseudo tubercles).

B. mallei was isolated from venous blood, indicating septicemia. The pathogen was also directly isolated from the pus, which had accumulated in the choanae, but not from nasal and eye swabs and, unexpectedly not from the lung lesions. A possible explanation for the failure to isolate *B. mallei* from the nasal swabs was the heavy growth of various other contaminating bacteria because no selective culture medium exists for *B. mallei*. It could also be explained by storage of the samples at –20°C for >20 days, which probably destroyed the bacteria.

The genetic relatedness of the strain isolated from the dromedary to the strain isolated in 2004 from horses in the United Arab Emirates suggests that this strain might be endemic to this region. It also appears to be genetically distinct from a recent outbreak in Pakistan, demonstrating the persistence of multiple strains on a larger geographic scale. Isolation of this pathogen from both camels and horses poses new challenges to the international trade of equids from and to countries where camels are raised.

Acknowledgments

We thank Rahime Terzioglou and Astrid Thomas for excellent technical assistance.

H.H. was supported by the Science & Technology Directorate, US Department of Homeland Security, under award 2010-ST-1080-000015.

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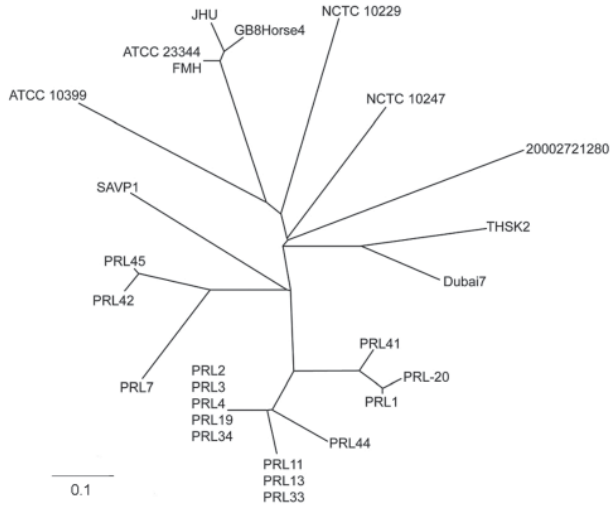

Figure 2. Unrooted neighbor-joining tree based on 23 variable number tandem repeat loci demonstrating the genetic relationship of the camel strain (THSK2) to other existing strains of *Burkholderia mallei*. The most closely related *B. mallei* strain to THSK2 is Dubai 7, which was isolated from a horse in the United Arab Emirates in 2004. Scale bar represents 0.1 changes.

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Plasmodium vivax Malaria among Military Personnel, French Guiana, 1998–2008

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We obtained health surveillance epidemiologic data on malaria among French military personnel deployed to French Guiana during 1998–2008. Incidence of *Plasmodium vivax* malaria increased and that of *P. falciparum* remained stable. This new epidemiologic situation has led to modification of malaria treatment for deployed military personnel.

French Guiana is a French Province located on the northern coast of South America that had 221,500 inhabitants in 2008 (*1*). Malaria is endemo-epidemic to the Amazon basin. Since 2000, the annual number of *Plasmodium falciparum* and *P. vivax* malaria cases in French Guiana has ranged from 3,500 to 4,500 (*2*). Approximately 3,000 French military personnel are deployed annually in French Guiana, and malaria occasionally affects their operational capabilities.

Only military personnel on duty in the Amazon basin are required to take malaria chemoprophylaxis; personnel deployed in coast regions are not. Until February 2001, the chemoprophylaxis regimen consisted of chloroquine (100 mg/d) and proguanil (200 mg/d). During March 2001–October 2003, mefloquine (250 mg/wk) was used. Since November, 2003 malaria chemoprophylaxis has been doxycycline (100 mg/d), which is initiated on arrival in the Amazon basin. All chemoprophylaxis is continued until 4 weeks after departure. Because of the absence of marketing authorization as chemoprophylaxis by the French Medicines Agency, primaquine was not used until recently. Other individual and collective protective measures did not change during 1998–2008.

Despite the availability of chemoprophylaxis, since 2003, several malaria outbreaks have been identified after operations against illegal mining in the Amazon basin (*3*,*4*). The purpose of those studies was to describe outbreaks and determine factors related to malaria cases. We report French military health surveillance epidemiologic data on malaria among military personnel deployed to French Guiana during 1998–2008.

The Study

Epidemiologic malaria surveillance in French Armed Forces consists of continuous and systematic collection, analysis, interpretation, and feedback of epidemiologic data from all military physicians (online Technical Appendix, www.cdc.gov/EID/content/17/7/1280-Techapp.pdf). Malaria is defined as any pathologic event or symptom associated with confirmed parasitologic evidence (*Plasmodium* spp. on a blood smear, a positive quantitative buffy coat malaria diagnosis test result, or a positive malaria rapid diagnosis test result) contracted in French Guiana. A case occurring in a person during or after a stay in French Guiana without a subsequent stay in another malaria-endemic area was assumed to be contracted in French Guiana. Each malaria attack was considered a separate case. Equal information was available for the entire 11-year study period. Data from weekly reports and malaria-specific forms were used for analysis.

Indicators are expressed as annual incidence and annual incidence rate. The denominator of the annual incidence rate is the average number of military personnel at risk for malaria during a given year.

Statistical analysis was performed by using Epi Info 6.04dfr (Centers for Disease Control and Prevention, Atlanta, GA, USA). Comparisons over time were made by using the χ^2 test for trend and between groups by using the Kruskal-Wallis test. A p value <0.05 was considered significant.

The incidence rate for malaria cases among French military personnel deployed to French Guiana has increased since 1998 ($p<0.001$). *P. falciparum* incidence has remained stable ($p = 0.10$), and *P. vivax* incidence has increased ($p<0.001$) (Figure). In 2007 and 2008, French military personnel in French Guiana represented only 23.0% and 22.2% of those deployed to malaria-endemic regions. However, most reported malaria cases were contracted in this region (50.0% and 62.9%, respectively, of all cases). *P. vivax* was responsible for most malaria attacks reported in French Guiana (Table). The proportion of malaria attacks

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DOI: 10.3201/eid1707.100009

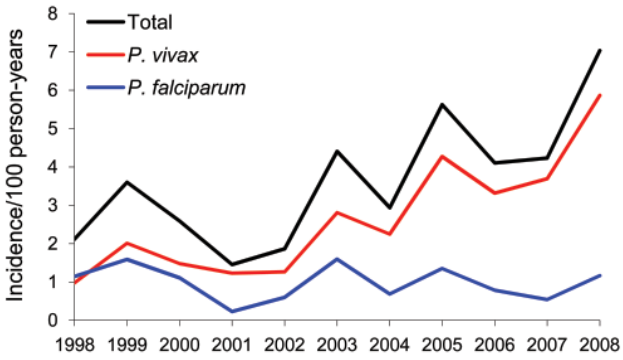

Figure. Incidence of malaria cases among French Armed Forces, by *Plasmodium* species, French Guiana, 1998–2008.

caused by *P. vivax* increased from 44% to 84% ($p<0.001$) during the study period.

In 2008, among the 264 reported cases, 221 were temporarily unavailable for duty. Median lost work days were lower for attacks of *P. vivax* malaria than for *P. falciparum* malaria (5 days/attack, interquartile range 4–7 days/attack vs. 7 days/attack, interquartile range 5–10 days/attack; $p = 0.006$)

Among 264 malaria cases contracted in French Guiana, 39.4% were in persons who had reported ≥1 malaria attack in the previous 6 months. Among the 221 *P. vivax* malaria cases, 45.5% were in persons who had already reported ≥1 *P. vivax* malaria attack in the previous 6 months. In 2008, among those required to take chemoprophylaxis (i.e., during a mission to the Amazon basin and 28 days after the mission), 45.0% admitted not taking their chemoprophylaxis within 8 days before onset of symptoms.

Conclusions

P. vivax malaria attacks have resulted in a substantial number of lost work days and have adversely affected operational readiness of military personnel. Despite availability of appropriate chemoprophylaxis, since 1998, French Armed Forces have been affected by an increase in incidence of *P. vivax* malaria. Several causes of this increase have been hypothesized.

First, epidemiologic trends for all-cause malaria in French Guiana and overall reported malaria incidence has not changed substantially since the end of the 20th century: ≈4 000 cases were reported annually during the 1990s (*5*) and 3,500–4,500 were reported during 2008 (*2*). However, the proportion of *P. vivax* malaria has increased from 20% of cases in the 1990s (*6*) to 56.1% during September 2003–February 2004 among patients at the Cayenne Public Hospital (*7*). Furthermore, 70% of malaria cases diagnosed in 2006 among French travelers returning from French Guiana were caused by *P. vivax* (*8*). One explanation for this parasitologic evolution may involve immigration from Brazil and Suriname to illegal gold-mining areas in the Amazon basin of French Guiana (*2*,*6*). These immigrating populations brought *P. vivax* from high-prevalence regions to an area where an efficient vector for malaria, *Anopheles darlingi* mosquitoes, was present (*9*). Changes in weather patterns and regional infrastructure could also explain this increase.

Second, military missions have intensified. In 2008, a police and military operation to reduce illegal gold-panning activities in the Amazon basin occurred in French Guiana. This operation might explain the 2008 peak in the incidence rate. Since 2002, these operations have resulted in several outbreaks among forces in French Guiana, especially in 2003 and 2005 (*2*,*3*).

Third, deficiencies have occurred in implementing individual and collective protective measures. These military operations were conducted by personnel from French Guiana or France, few had any rainforest experience. Despite extensive training, instructions were clearly not followed, as demonstrated by a 45% noncompliance rate for chemoprophylaxis.

P. vivax has accounted for >80% of reported malaria cases in French Guiana for the past 3 years. In addition, relapses of *P. vivax* malaria occur in the absence of radical treatment. In 2008, 45.5% of persons with *P. vivax* malaria had already reported ≥1 *P. vivax* malaria attack in the past 6 months. Although the *P. vivax* malaria mortality rate is low, the effect of *P. vivax* malaria on force operational readiness is high because relapses decrease the availability of military personnel. In addition, *P. vivax* malaria can be severe, despite its reputation as a mild form of malaria (*10*). Since 2009, to reduce the number of relapses, a French Ministry of Defense circular has recommended treatment with primaquine for 2 or 3 weeks after a first attack of *P. vivax* malaria. Studies of the use of primaquine chemoprophylaxis are ongoing (*11*–*13*).

Table. Cases of *Plasmodium* spp. malaria among French Armed Forces, French Guiana, 1998–2008*

	Species				
Year	*P. falciparum*	*P. vivax*	*P. malariae*	*P. ovale*	Unknown
1998	41	35	0	1	3
1999	56	71	1	0	4
2000	36	48	0	2	5
2001	7	38	0	3	1
2002	19	40	1	2	0
2003	54	95	0	0	0
2004	22	72	1	3	0
2005	50	158	3	1	0
2006	29	123	0	2	0
2007	20	137	1	0	0
2008	44	221	0	0	0

*Cases of malaria caused by 2 parasites (co-infections) were included for each involved species.

In conclusion, the incidence of *P. vivax* malaria is increasing in French Guiana, especially in French Armed Forces. The incidence of *P. falciparum* malaria remains stable. This new epidemiologic finding can affect the level of individual health and operational capabilities. Performance of vector evaluation studies and control in the regions could be another possible intervention.

Acknowledgments

We thank Mark Dearden for assistance with the preparation of this report.

This study was supported by the French Medical Forces.

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Burkholderia pseudomallei in Unchlorinated Domestic Bore Water, Tropical Northern Australia

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To determine whether unchlorinated bore water in northern Australia contained *Burkholderia pseudomallei* organisms, we sampled 55 bores; 18 (33%) were culture positive. Multilocus sequence typing identified 15 sequence types. The *B. pseudomallei* sequence type from 1 water sample matched a clinical isolate from a resident with melioidosis on the same property.

Burkholderia pseudomallei is an environmental bacterium that causes melioidosis (*1*), a disease that is endemic throughout much of southeastern Asia and tropical northern Australia and sporadically occurs in other regions (*2*). Most infection is thought to result from percutaneous inoculation, but inhalation, aspiration, and ingestion of soil or water containing *B. pseudomallei* bacteria are the most recognized routes of infection. Outbreaks of melioidosis in Australia after exposure to contaminated water have been described. An outbreak of 159 cases in intensive piggeries (hog lots, a type of factory farm that specializes in raising pigs up to slaughter weight) in Queensland was attributed to contamination of the water supply (*3*), and a clonal outbreak in pigs on a small farm outside Darwin, Northern Territory was linked to *B. pseudomallei* cultured from the farm's bore water (*4*). Two clonal clusters of human melioidosis have also been found in remote indigenous communities in northern Australia where molecular typing of recovered bacteria traced the source of infection to a contaminated community water supply. Fatalities occurred in both outbreaks. In 1 outbreak, the water supply was not chlorinated (*5*); in the other, the chlorination system was not adequately maintained (*6*).

Bore water can be contaminated with *B. pseudomallei* in our region (*4*,*7*). We surveyed a series of bores to ascertain how commonly such contamination occurs and whether *B. pseudomallei* is transient or persistent in positive bores. We then compared the genetic diversity of *B. pseudomallei* strains recovered from bores with strains from human melioidosis cases and other environmental strains from the region.

The Study

Darwin, capital of the Northern Territory, Australia, is a coastal tropical city at 12°S. It has 2 distinct seasons: a hot monsoonal wet season from October through May and a dry season with very little, if any, rain from June through September. The city has a population of ≈100,000. Outside the city are many rural blocks of land 1–20 acres in size. Most have a family house, with cultivated gardens or native bush; domestic animals; and sometimes small numbers of farm animals, such as goats, pigs, and chickens. Horticultural activities include planting of mangoes, Asian vegetables, and watermelons. Most residents use unchlorinated groundwater provided by deep bores that tap into the underlying aquifers. We estimate that >3,000 such bores are in the rural areas and provide unchlorinated water to as many as 10,000 persons. Each year, 25–50 human cases of melioidosis occur in the Northern Territory; 50% occur in Darwin residents and 10%–15% occur in those living in rural areas surrounding Darwin (M. Mayo et al., unpub. data). Melioidosis also occurs in domestic and farm animals in the region.

We sampled bore water from 55 blocks in the Darwin rural region. All blocks were within a 30-km radius of Darwin, and all used unchlorinated bore water for domestic and irrigation purposes. Water samples were collected from the bore head (initial outlet of groundwater at source), water storage tank, and other water exit points (taps, hoses). For each sample, 1 liter of water was filtered through 0.22-μm filters (Millipore Corporation, Bedford, MA, USA). Filters were then cultured separately in Ashdown broth (Oxoid, Melbourne, Victoria, Australia) and tryptone soy broth (Oxoid) with gentamicin 10 mg/mL. Broth was plated onto Ashdown agar (Oxoid) on days 2, 7, and 14. Bacterial colonies suggestive of *B. pseudomallei* by morphologic appearance on Ashdown agar were confirmed by Gram stain, oxidase test, agglutination with *B. pseudomallei* antiserum, and a specific PCR targeting *B. pseudomallei* type III secretion system (*8*). Confirmed *B. pseudomallei* bacteria were cultured on chocolate agar (Oxoid), and DNA was extracted by using a DNeasy tissue kit (QIAGEN, Hilden, Germany). Multilocus sequence typing (MLST) of

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DOI: 10.3201/eid1707.100614

bacterial DNA determined the sequence type (ST) for each isolate (*9*), allowing comparison with STs in the global MLST dataset (http://bpseudomallei.mlst.net).

B. pseudomallei was cultured from 18 (33%) of 55 water samples; 16 (36%) of 45 blocks tested during the wet season were positive, and 2 (20%) of 10 blocks tested during the dry season were positive. From 18 initial isolates, 9 distinct STs were identified; ST266 was found at 4 separate sites and ST109 at 3 (Table). Nine of the 18 positive sites were resampled 3 times during a 2-year period. In 5 (56%) of 9 sites, *B. pseudomallei* was recovered at least 1 additional time, and 3 sites were positive on 4 sampling occasions. STs of isolates from repeat sampling showed up to 3 different STs at the same location at the same time. At 1 site, the same ST (ST325) was present in all 4 samplings during the 2 years. Nevertheless, at each of the 5 sites with repeat positive cultures, including this site, an ST different from the original ST was recovered, even if the original ST was still present (Table).

During the sampling period, a total of 15 distinct STs were recovered from water samples; of these, 10 were found in *B. pseudomallei* isolates collected from humans with melioidosis in the rural area (Figure), including 2 STs from fatal cases (ST109 and ST132). Of the 5 other STs, ST243 and ST334 occurred in humans in urban Darwin, and ST328 was recovered from a goat with fatal melioidosis. Although we do not have data on bacterial load in these positive water sources, the strain recovered from bore water at 1 location (ST131) was an identical ST to the *B. pseudomallei* isolate recovered from the sputum of a resident of that property who had nonfatal melioidosis pneumonia.

Surveys of *B. pseudomallei* across northern Australia have shown a large genetic diversity among strains but distinct regional separations on MLST (*10*). Although the overall diversity of *B. pseudomallei* within Australia is considered greater than that seen in southeastern Asia (*11*), consistent with Australian *B. pseudomallei* lineages being ancestral to those elsewhere, environmental studies from Thailand have also shown enormous diversity in STs within a small geographic location (*12*). What remains unclear from these studies is whether differential virulence exists among environmental strains of *B. pseudomallei* and whether only a proportion of those isolates recovered from the environment have the potential to cause clinical disease (*13*).

Therefore, although STs found in this study were also represented in humans with melioidosis, the actual public health implications of the findings require further elucidation. Other variables require further investigation to determine the implications of our findings. These include bacterial load and differential bacterial virulence potential among the *B. pseudomallei* strains in water supplies.

Table. Sampling, culture, and MLST results from initial and repeat sampling of rural unchlorinated domestic water supplies, Northern Territory, Australia*

Site no.	1st sampling	2nd sampling	3rd sampling	4th sampling
1	109	Negative	Negative†	Negative
2	266	558, 326, 559	326, 559†	109
3	325	325	325, 328†	325†
4	109	Negative	334†	Negative
5	320	–	–	–
6	326	Negative	Negative†	Negative†
7	109	109	121†	109
8	132	–	–	–
9	325	Negative	Negative†	Negative
10	266	Negative	Negative†	Negative
11	266	–	–	–
12	330†	–	–	–
13	333†	333, 243†	Negative	Negative
14	132	–	–	–
15	266	–	–	–
16	132	–	–	–
17	109	–	–	–
18	131	–	–	–

*MLST, multilocus sequence typing; –, not resampled.
†Indicates sampling during the dry season (June–September); 2nd–4th samplings were conducted during a 2-year period.

Additional considerations would be to quantify the infection risk potential from exposure to culture-positive water through ingestion or after aspiration or inhalation of droplets or aerosols containing *B. pseudomallei* during, for instance, showering.

Conclusions

B. pseudomallei is common in unchlorinated domestic bore water supplies in the rural region of Darwin, Northern Territory, Australia. Initially, 33% of sites tested were

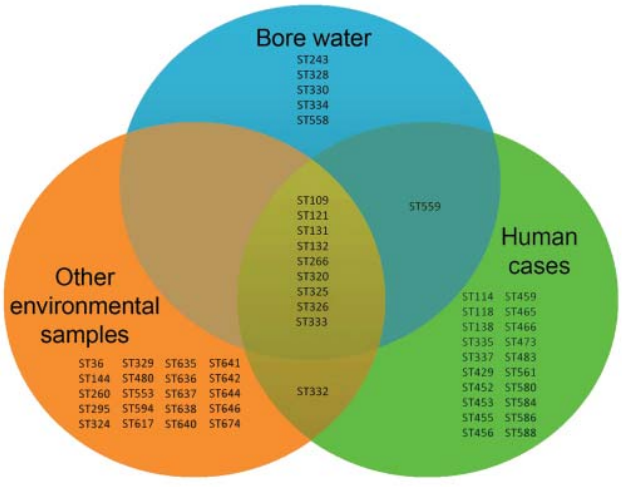

Figure. Venn diagram of sequence types (STs) determined by multilocus sequence typing found in *Burkholderia pseudomallei* strains from bore water (n = 15 STs), human cases (n = 31 STs), and other environmental samples (n = 30 STs) from the rural region of Darwin, Northern Territory, Australia.

positive for this bacterium, and more than half of these sites on at least 1 occasion were positive again when resampled. MLST showed a great diversity of STs, with persistence and variation in ST found on repeat sampling. STs often matched those found in humans with melioidosis from the same region. *B. pseudomallei* ST found in the sputum of 1 case-patient with melioidosis was a direct match to the ST of *B. pseudomallei* cultured from the bore water on the property on which this case-patient lived.

This work was supported by grants from the National Health and Medical Research Council of Australia, the Northern Territory Research and Innovation Fund, and the Wellcome Trust, UK.

Mr Mayo is manager of the Menzies School of Health Research melioidosis program. His areas of expertise include developing improved methods for isolation and identification of *B. pseudomallei* from environmental samples.

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Epidemiology and Investigation of Melioidosis, Southern Arizona

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Burkholderia pseudomallei is a bacterium endemic to Southeast Asia and northern Australia, but it has not been found to occur endemically in the United States. We report an ostensibly autochthonous case of melioidosis in the United States. Despite an extensive investigation, the source of exposure was not identified.

Burkholderia pseudomallei is endemic to Southeast Asia and northern Australia; the organism has also been identified on other continents and islands but not North America (*1*). *B. pseudomallei* is present in soil and water and can cause infection through inhalation, aspiration, ingestion, or percutaneous inoculation (*2*–*4*). Persons with certain chronic health conditions, particularly diabetes, are predisposed to melioidosis disease after exposure to this bacterium. Person-to-person transmission has been documented but is rare (*5*). Clinical signs of the disease vary, depending in part on the route of exposure, and can manifest as pneumonia, septicemia, or single or multiple abscesses (*2*). Treatment is prolonged and made more difficult by the bacterium's intrinsic resistance to antimicrobial drugs (*6*). No cases of *B. pseudomallei* infection have been documented in the United States in persons without a history of prior travel to a country where the disease is endemic (*7*).

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DOI: 10.3201/eid1707.100661

The Study

In October 2008, a 32-year-old man with a history of type II diabetes, hypertension, and obesity was admitted to a small community hospital (hospital A) in Arizona. He had severe right knee pain and fever. On 3 occasions the week before being admitted, he had been evaluated in hospital A's emergency department for severe right knee pain. The patient denied cough, chest pain, and swelling and pain of other joints. Over the course of 2–3 months, he lost weight and had exhaustion, intermittent right knee pain, and nightly fevers. The patient denied recent trauma.

Medications at admission included those common for control of diabetes. The patient did not smoke, drink alcohol, or use illicit drugs and was in a monogamous heterosexual relationship. He worked at an automobile body shop preparing and painting cars and had previously worked as a motorcycle and all-terrain vehicle mechanic. He reported gardening as a hobby and had many indoor and outdoor plants. He also had several dogs in the household but reported no unusual exposure to other animals.

After admission to hospital A, the patient underwent arthrocentesis of the right knee. Culture of the synovial fluid did not yield any bacterial growth, and evidence of infection or crystals was not apparent. A blood specimen was sent for culture and yielded what was initially identified as *Escherichia coli* by an automated in-house instrument. Sensitivity to antimicrobial drugs was consistent with this bacterium.

Chest radiograph and computerized axial tomography scan with contrast were unremarkable and did not demonstrate evidence of pneumonia. Laboratory tests indicated no sexually transmitted infections. The patient received clindamycin, imipenem, vancomycin, and metronidazole intravenously. After 8 days in hospital A and no resolution of fever or knee pain, he was transferred to hospital B, a large regional hospital, with an initial diagnosis of persistent *E. coli* sepsis and possible vegetative valve lesions. Transesophageal echocardiogram performed at hospital B did not show vegetative valve lesions.

After admission to hospital B, the patient underwent arthrocentesis of the right knee. Although there was evidence of infection, synovial fluid culture yielded no growth. Blood cultures grew *B. pseudomallei* identified by an automated in-house instrument. Because initial results were unexpected, blood samples were submitted to a reference laboratory and the Arizona State Public Health Laboratory (Phoenix, AZ, USA) where results confirmed the presence of *B. pseudomallei*. The bacteria continued to grow in blood cultures for 16 days after initial hospitalization at hospital A on October 7. Cultures of the knee fluid grew *B. pseudomallei* for 7 days; sputum cultures were positive for 6 days. All cultures were

negative 2 weeks before the patient was discharged from hospital B on December 5.

The patient's hospital course was complicated by respiratory failure that required intubation and ventilation, acute renal failure, pneumothorax and pneumoperitoneum, and anemia and hypotension. Fever resolved 21 days after admission to hospital A. Knee swelling persisted for ≈6 weeks. Antimicrobial therapy administered to the patient while he was an inpatient in hospital B included meropenem, moxifloxacin, vancomycin, ceftazidime, gentamicin, and trimethoprim/sulfamethoxazole. The patient was discharged with oral doxycycline and trimethoprim/sulfamethoxazole to a rehabilitation facility 7 weeks after his initial hospital admission.

Clinical isolates were analyzed to confirm *B. pseudomallei* infection and to determine the genetic origin of the isolate strain. Specimens from hospital A had been destroyed by the time the patient's melioidosis was diagnosed, which precluded the possibility of determining whether the presumptive *E. coli* infection was actual or misdiagnosed. After receipt at the Arizona State Public Health Laboratory, an isolate was submitted to the Centers for Disease Control and Prevention (Atlanta, GA, USA) for confirmation, and bacterial DNA was extracted and sent to the Translational Genomics Research Institute (Phoenix, AZ, USA) for genetic characterization. Molecular analyses determined that the isolate strain originated from Southeast Asia, most likely Malaysia, or a nearby country. Serologic testing performed 6 weeks post infection demonstrated a *B. pseudomallei* indirect hemagglutination assay titer of 160; any titer is considered positive in a person living in an area where the disease is nonendemic (*2*). Serum samples collected early in the course of illness were not available for testing.

The patient and his family were interviewed to determine travel history and possible sources of exposure. No lifetime travel outside of the United States and only limited intrastate and interstate travel were established. The epidemiologic investigation, therefore, focused on the patient's home and work sites. Possibilities for exposure included occupational exposure to imported vehicle parts, exposure to a person or object from a disease-endemic area, recreational exposure to imported soil or plants, or inoculation with contaminated medication.

Extensive investigation showed no evidence of exposure at the patient's worksite and no known exposure to any person or objects from a disease-endemic area. We conducted multiple on-site residential investigations, primarily focusing on the patient's self-reported history of exotic plant repotting. Plant soil and root samples were collected in and around the patient's home 6 weeks after diagnosis (winter), and 6 months later (summer) and were taken for analysis to the select agent laboratory of Northern Arizona University (Flagstaff, AZ, USA). *B. pseudomallei* could not be cultured from any of the samples tested.

Infection may have occurred from exposure to contaminated medical products. Because the patient was initially hospitalized with sepsis identified as *E. coli*, sepsis might have been the source of his knee pain, and he was subsequently inoculated with *B. pseudomallei* during knee arthrocentesis or from a contaminated oral or intravenous medication. However, investigation of possible medication contamination did not yield any remarkable results.

Conclusions

Despite extensive investigation, when, how, or where the patient was exposed to *B. pseudomallei* remains unclear. Although travel to a disease-endemic area including Southeast Asia was ruled out, molecular analysis of the etiologic agent showed that it was consistent with Southeast Asian origin.

This case demonstrates the difficulty in diagnosing a disease caused by a rare organism not endemic to the area and the complications that can ensue from delayed diagnosis. Unfortunately, we could not identify the source of exposure despite an aggressive epidemiologic, environmental, and laboratory investigation. Heightened awareness and surveillance by public health officials for this select agent is critical to learning more about the possible presence of *B. pseudomallei* in the United States.

Acknowledgments

We thank Gage Patterson for performing *B. pseudomallei* biochemical testing; Darla Hansen, Greg Moody, and Neil Karnes for assistance with the investigation; Kevin Freeman for performing the melioidosis serology; and Jay Gee, Mindy Glass, and Alex Hoffmaster for performing confirmatory tests.

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Melioidosis, Phnom Penh, Cambodia

Erika Vlieghe, Lim Kruy, Birgit De Smet, Chun Kham, Chhun Heng Veng, Thong Phe, Olivier Koole, Sopheak Thai, Lut Lynen, and Jan Jacobs

We describe 58 adult patients with melioidosis in Cambodia (2007–2010). Diabetes was the main risk factor (57%); 67% of infections occurred during the rainy season. Bloodstream infection was present in 67% of patients, which represents 12% of all bloodstream infections. The case-fatality rate was 52% and associated with inappropriate empiric treatment.

Melioidosis, an infectious disease caused by *Burkholderia pseudomallei*, is endemic to Southeast Asia and tropical Australia (*1*,*2*). *B. pseudomallei* is a gram-negative bacterium that causes lung or soft tissue infections with or without bloodstream infection (BSI) (*3*); the case-fatality rate can exceed 80%. Treatment includes third-generation cephalosporins or carbapenems, followed by maintenance courses of sulfamethoxazole/trimethoprim (SMX/TMP) with or without doxycycline.

In Cambodia, few microbiologically confirmed cases have been described (*4*–*7*). We describe 58 adult patients in whom melioidosis was diagnosed during July 1, 2007–January 31, 2010, at Sihanouk Hospital Centre of Hope, Phnom Penh, Cambodia.

The Study

Melioidosis was defined as growth of *B. pseudomallei* from any clinical specimen (blood, pus, or urine). Nonfermentative gram-negative rods suspected for *B. pseudomallei* (wrinkled colonies, oxidase positive, polymyxin and gentamicin resistant, amoxicillin/clavulanic acid susceptible [*8*]) were identified by the API 20NE system (bioMérieux, Marcy L'Etoile, France). MICs were determined with Etest (Biodisk, Solna, Sweden). Interpretive criteria were those defined for *B. pseudomallei* by the Clinical and Laboratory Standards Institute (*9*).

Recurrences were defined as the culture-confirmed reappearance of symptoms after initial response to therapy (*10*). Treatment was considered appropriate if it contained ceftazidime, a carbapenem, or amoxicillin/clavulanic acid with or without SMX/TMP.

Risk factors were assessed by univariate analysis. Ethical approval was granted by the University Hospital Antwerp and the National Ethical Committee in Phnom Penh.

Seventy-one isolates of *B. pseudomallei* were recovered from 58 patients (mean age 49 years, range 18–73 years); 34 (59%) were men. Seasonal patterns of infection are shown in Figure 1 and geographic distribution of patients' homes (56) in Figure 2. Melioidosis was diagnosed in 39 (67%) patients during the rainy season. In 39 patients, *B. pseudomallei* was recovered from blood samples, which represented 12.0% of the 328 clinically significant organisms from BSIs and 1.0% of the 3,976 systemic inflammatory response syndrome episodes during the study. In 2 patients, melioidosis was retrospectively considered a recurrence 137 and 231 days postinfection.

Fifty-four (52 initial and 2 successive) isolates were used for resistance testing (Table 1). No resistance was noted for ceftazidime, meropenem, amoxicillin/clavulanic acid or doxycycline, but 12 (22.2%) isolates had MICs equal to the susceptibility breakpoint for chloramphenicol.

Risk factor information available for 51 patients included diabetes mellitus (34 [59%] patients); alcoholism (7 [12%]); and corticosteroid use (3 [5%]). Most (39) patients had BSI with or without pneumonia. Median delay to growth of blood cultures was 4 days (range 2–8). During the study, *B. pseudomallei* was increasingly recovered from nonblood specimens, in line with growing laboratory expertise. Involvement of the lungs was noted in 28 (48%) patients. Other sites included skin and soft tissue (17 patients), bone and joints (8), urogenital tract (4), spleen (8), liver (5), and psoas muscle and thyroid gland (1 each). Infection was often multifocal. Seventeen (29%) patients had shock or multiorgan failure. The median delay from symptom onset to seeking treatment was 28 days (range 1–730 days).

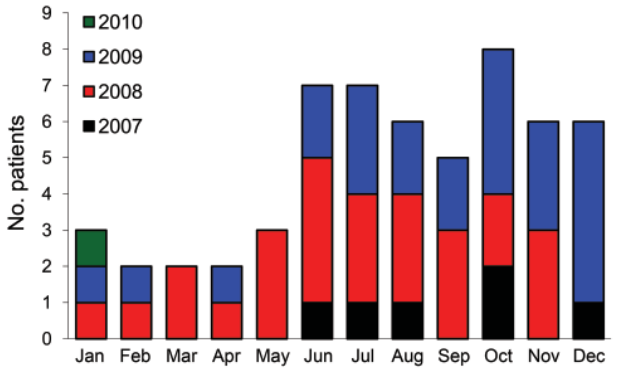

Figure 1. Number of patients in whom melioidosis was diagnosed, by season, Phnom Penh, Cambodia, July 1, 2007–January 31, 2010.

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DOI: 10.3201/eid1707.101069

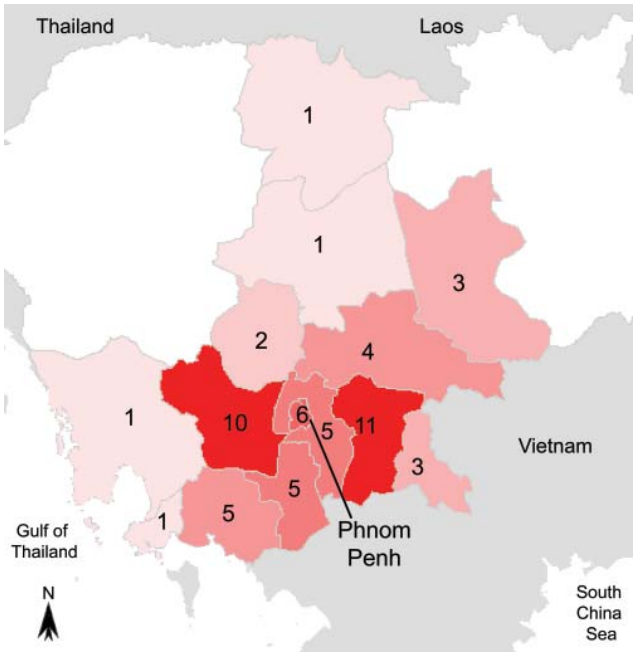

Figure 2. Map of Cambodia with geographic origin of the 58 patients with melioidosis diagnosed during July 1, 2007–January 31, 2010.

Thirty (52%) patients died; no outcome data were available for 3 patients. Death occurred early; 19 (63%) nonsurvivors died within 1 week after admission. In univariate analysis, risk factors for death were signs of shock, multiorgan failure, or BSI and not receiving appropriate empiric therapy (Table 2). Among the 25 survivors, 22 (88%) recovered without recurrence; the other 3 were lost to follow-up during maintenance treatment. The mean duration of follow-up was 12.8 months (range 3.5–28 months).

Treatment data were available for 53 patients; 18 (34%) received inappropriate empiric therapy; all died early. Thirty-five patients received appropriate treatment; 23 patients were given ceftazidime (2 g 3×/d for ≥14 days) with or without SMX/TMP (30 mg/kg 2×/d), 6 received amoxicillin–clavulanic acid (875–1,000 mg 3×/d) with or without SMX/TMP, and another 6 received SMX/TMP with doxycycline (200 mg 1×/d). Twenty-three patients continued maintenance therapy, primarily SMX/TMP with or without doxycycline (22 patients). Total treatment duration ranged from 3 to 6 months.

Conclusions

Our findings of melioidosis in 58 adults complement the recently published data on melioidosis in children in Cambodia (*7*). A limitation of our study is its retrospective nature; a small number of patients precluded detailed study of risk factors and calculation of population-based incidence data. In addition, we have not yet studied the isolates to the genetic level. However, presently used phenotypic characteristics have been validated against molecular reference standards as accurate tools for *B. pseudomallei* identification (*8*).

Risk factors for patients and epidemiologic profiles were similar to those observed in northeastern Thailand (*1*,*11*). Most cases occurred during or shortly after the rainy season (May–November); diabetes mellitus was the most relevant risk factor, which is consistent with findings from other regions where melioidosis is endemic (*1*,*11*,*12*). Diabetes is quickly emerging in Cambodia and remains a difficult-to-treat chronic disease in poor rural settings (*13*).

In this study, nearly two thirds of patients had BSI and half had pneumonia. These data are consistent with studies from Thailand and Australia, where BSI and pneumonia accounted for 46%–60% and 50%–60% of manifestations, respectively (*11*,*12*). Soft tissue and deep organ abscesses were also frequent. The finding of a spleen abscess in a melioidosis-endemic area should trigger suspicion of melioidosis.

In our study, distinguishing primary infection or reinfection from relapse was not possible, but the seasonal link suggests recent infections. The 2 recurrences in our study were probably relapses caused by insufficient

Table 1. MICs for 54 *Burkholderia pseudomallei* isolates, Phnom Penh, Cambodia, July 1, 2007–January 31, 2010*

	MIC, μg/mL															Breakpoints, μg/mL	
Antimicrobial drug	0.38	0.5	0.75	1	1.5	2	3	4	6	8				MIC_{50}	MIC_{90}	S	R
Meropenem	3	29	16	1	3	2	–	–	–	–				0.5	1.5	≤4	≥16
Doxycycline†	–	13	19	18	3	1	–	–	–	–				0.75	1	≤4	≥16
Ceftazidime†	–	2	0	18	25	7	2	–	–	–				1.5	2	≤8	≥32
Amoxicillin/ clavulanic acid	–	1	0	13	30	7	2	1	–	–				1.5	2	≤8	≥32
Chloramphenicol	–	–	–	–	1	1	0	17	16	12				6	8	≤8	≥32
	0.032	0.038	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.75	1	1.5	3				
Sulfamethoxazole/ trimethoprim	3	1	7	12	5	9	4	7	1	1	3	1	0	0.125	0.75	≤2	≥4

*MICs determined by Etest (AB Biodisk, Solna, Sweden). MIC_{50}, MIC for 50% of isolates; MIC_{90}, MIC for 90% of isolates; S, susceptible; R, resistant; –, no isolates with this MIC.
†53 isolates included.

Table 2. Predictors of death for 55 patients with melioidosis, Phnom Penh, Cambodia, July 1, 2007–January 31, 2010*

Risk factor	Presence of risk factor	No. patients	No. patients who died	Relative risk (95% CI)	p value
Age >55 y	Y	24	14	1.13 (0.70–1.83)	0.786
	N	31	16		
Male sex	Y	31	18	1.16 (0.70–1.91)	0.595
	N	24	12		
Rainy season	Y	36	23	1.73 (0.92–3.28)	0.087
	N	19	7		
Diabetes	Y	32	14	0.70 (0.41–1.21)	0.359
	N	16	10		
Alcoholism	Y	7	6	0.97 (1.19–3.22)	0.092
	N	32	14		
Clinical sign					
Duration of symptoms <2 mo	Y	12	3	2.26 (0.80–6.42)	0.152
	N	23	13		
Bloodstream infection	Y	37	28	6.81 (1.82–25.50)	**<0.001**
	N	18	2		
Pneumonia	Y	28	18	1.52 (0.90–2.57)	0.172
	N	26	11		
Deep abscesses	Y	15	6	0.80 (0.38–1.67)	0.742
	N	24	12		
Bone/joint infection	Y	8	4	1.04 (0.47–2.28)	1.000
	N	29	14		
Urogenital infection	Y	5	1	0.38 (0.64–2.25)	0.345
	N	38	20		
Skin and soft tissue infection	Y	19	6	0.48 (0.24–0.97)	**0.023**
	N	35	23		
Shock or multiorgan failure	Y	17	13	4.59 (1.60–13.32)	**<0.001**
	N	18	3		
Therapy					
Inappropriate empiric therapy	Y	18	18	3.50 (2.07–5.90)	**<0.001**
	N	35	10		

*Not all information about outcome predictors was available from all patients. Fisher exact test was used for categorical variables and Student *t* test for continuous variables. CI, confidence interval. Statistically significant associations (p<0.05) are shown in **boldface**.

treatment of the first (unrecognized) episode, as has been described in patients in Thailand and Australia (*10*). The possibility of relapse emphasizes the need for intense follow-up during and after the treatment course.

We noted a high case-fatality rate, especially among patients with BSI or pneumonia, who were in shock or had multiorgan failure, or who were receiving inappropriate empirical therapy. Potential interventions to decrease risk factors for death caused by melioidosis include improved sepsis care and ensured availability of effective drugs such as ceftazidime, carbapenems, and amoxicillin/clavulanic acid. Although we did not demonstrate resistance to any of these antimicrobial drugs, resistance can occur during therapy; follow-up blood cultures during treatment is essential (*14*).

During the 19-month study, we observed a learning curve on melioidosis at several levels in the hospital. Even though melioidosis is well known in the Southeast Asian region (*2*), it was unfamiliar to most clinicians and laboratory staff at the start of the study. Our findings may also have an effect at the national level, especially regarding early diagnosis and treatment. Awareness must be raised among health care workers and high-risk patient groups (e.g., diabetes patients). Development of quality-assured and affordable microbiological capacity throughout the country is also crucial in the broader picture of surveillance and containment of antimicrobial drug resistance. Careful adaption of local treatment guidelines is essential and has been successful in other settings, e.g., Northern Territory, Australia (*15*). Because melioidosis appears endemic to Cambodia, the public health impact of this disease warrants further research and action.

The Bloodstream Infection Surveillance Project was supported by Project 2.08 of the third framework agreement between the Belgian Directorate General of Development Cooperation and the Institute of Tropical Medicine, Antwerp, Belgium.

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Viability of *Baylisascaris procyonis* Eggs

Shira C. Shafir, Frank J. Sorvillo, Teresa Sorvillo, and Mark L. Eberhard

Infection with *Baylisascaris procyonis* roundworms is rare but often fatal and typically affects children. We attempted to determine parameters of viability and methods of inactivating the eggs of these roundworms. Loss of viability resulted when eggs were heated to 62°C or desiccated for 7 months but not when frozen at –15°C for 6 months.

Baylisascaris procyonis, the common intestinal roundworm of raccoons, has increasingly been recognized as a source of severe, often fatal, neurologic disease in humans, particularly children (*1*,*2*). Although this devastating disease is rare, lack of effective treatment and the widespread distribution of raccoons in close association with humans make baylisascariasis a disease that seriously affects public health (*3*). Raccoons infected with *B. procyonis* roundworms can shed millions of eggs in their feces daily (*4*). Given the habit of raccoons to defecate in and around houses, information about optimal methods to inactivate *B. procyonis* eggs are critical for the control of this disease. However little information is available about survival of eggs and effective disinfection techniques. This study expands on our previous work by providing additional data on thermal death point and determining the impact of desiccation and freezing on the viability of *B. procyonis* eggs to provide additional information for risk assessments of contamination and guide attempts at environmental decontamination (*5*).

The Study

Worms were harvested from the intestines of infected raccoons from Goleta, California, USA. Eggs were removed from adult female worms and allowed to embryonate in sterile saline at room temperature for 3 weeks.

To determine the thermal death point, 150 µL each of embryonated eggs, at a concentration of 100 eggs per µL were added to six 1-mL polypropylene tubes of sterile water. The 6 tubes were then added to a water bath at 35°C and allowed to sit for 10 min to equilibrate. Temperature of the water bath was increased at a rate of ≈5°C every 7 min, and 1 tube was removed at 5° increments from 37°C to 62°C. Eggs were then examined by light microscopy to determine viability as judged by larval motility. A minimum of 50 eggs were examined per assay, and larval motility was judged as +/–. The experiment was repeated using a more objective assessment of viability through examination of hatched larvae. Inactivation was measured by using a viability dye (methylene blue) exclusion method where uptake of dye indicates cell death and inactivation. After the eggs were removed from the heat, the mammilated layer was removed through exposure to undiluted household bleach (6% sodium hypochlorite) to promote subsequent emerging of larvae, then washed 5 times in 0.85% saline for 1 min at 600 × *g*. Hatching was achieved by the glass bead method (*6*,*7*). Hatched larvae were then removed and mixed 1:1 with a 1:10,000 dilution of methylene blue. Viable larvae remained motile and remained unstained (Figure 1), whereas nonviable larvae absorbed the methylene blue stain (Figure 2) (*8*).

The experiment was repeated by adding the heated water, in 5° increments from 37°C to 62°C, directly into the tube containing the eggs. Eggs were exposed to the water for <1 min. Eggs were then processed as described and examined by light microscopy. All experiments were replicated.

To determine the ability of eggs to withstand freezing temperatures, 150 µL each of embryonated eggs at a concentration of 100 eggs per µL were added to ten 1-mL polypropylene tubes of sterile water. The tubes of eggs were exposed to an environment of –15°C. Every 30 days, 1 tube was removed and thawed at room temperature. Viability was evaluated by using both motility and methylene dye

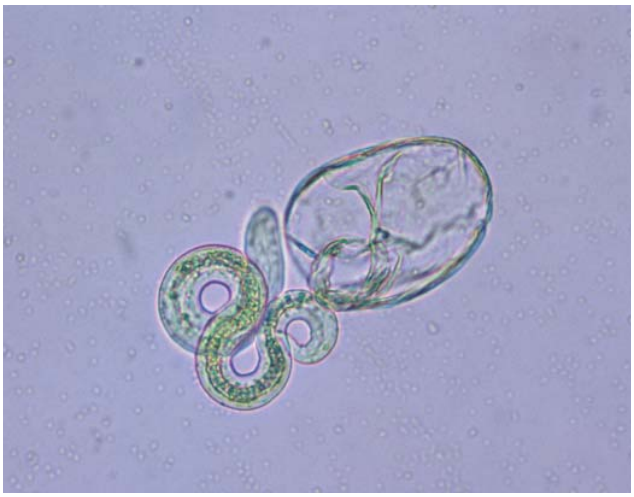

Figure 1. Viable *Baylisacaris procyonis* larvae demonstrating intact membrane and impermeability to methylene blue. Original magnification ×40.

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DOI: 10.3201/eid1707.101774

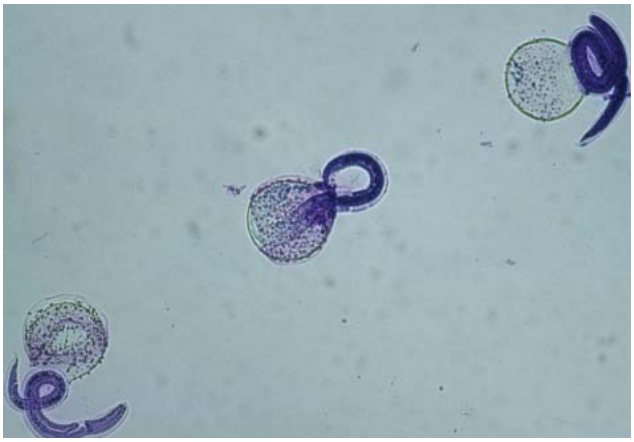

Figure 2. Hatched nonviable *Baylisascaris procyonis* larvae demonstrating uptake of methylene blue. Original magnification ×40.

exclusion as described above. After examination, tubes were refrozen. Each month, in addition to the evaluation of a fresh tube, tubes from the previous months were reevaluated to examine the effects of freeze–thaw. A total of 5 freeze–thaw cycles were evaluated.

To assess survival of eggs to total desiccation, 150 μL each of embryonated eggs, at a concentration of 100 eggs per μL, were added to ten 1-mL wells of sterile water in a microtiter plate. The plates were stored at room temperature and allowed to dehydrate to complete desiccation over a 1-week period. Monthly, 1 mL of sterile water was added and evaluated for eggs and assessed for viability. Assessments were made until complete inactivation occurred.

All larvae remained viable to 47°C; >90% of the larvae were viable at 52°C, and 50% were viable at 57°C. Larve were completely inactivated at 62°C (Table). When the heated water was added directly to the eggs, all larvae remained viable through 42°C, and most larvae were viable at 47°C and 52°C. Complete inactivation occurred at 57°C (Table).

Eggs survived complete desiccation for at least 6 months at room temperature. Total loss of viability was observed after 7 months of desiccation. Eggs frozen at –15°C for 6 months demonstrated no reduction in viability. Freeze–thaw, including exposure to 5 cycles, did not affect survival. Although we did not specifically design experiments to assess the effect of chlorine on inactivation of *B. procyonis* eggs, exposure to undiluted household bleach for 90 min to remove the mammillated layer did not affect viability.

Conclusions

These findings indicate that *B. procyonis* eggs have a low thermal death point at ≤62°C, similar to that reported for other ascarids (*5*,*9*). Moreover, inactivation was achieved at relatively short exposure times. However, the eggs are highly resistant to desiccation. Additionally, extended freezing or freeze–thaw exposure is unlikely to have affect viability, similar to *Parascaris equorum* (*10*). Exposure to undiluted bleach had no observable effect on survival.

In North America, the prevalence of *B. procyonis* is high, and infection is possible wherever raccoons are found (*11–14*). Given the close association of raccoons with human populations and the serious nature of infection, identification of the parameters of viability of *B. procyonis* larvae has public health implications (*15*). Potential for human infection can be mitigated by decontamination of areas known to be contaminated with raccoon feces. Such contamination of yards, pools, and homes is commonly reported. Hyperchlorination of contaminated swimming pool water is unlikely to be effective. However, current recommendations are to completely drain water and refill pools or to allow complete filtration and turnover of water and to discard and replace the filter material. Given the low thermal death point of *B. procyonis* eggs, the use of readily available steam-producing devices may be of value in disinfecting contaminated areas. Moreover, because freezing and freeze–thaw do not affect viability, contamination in areas that have cold winters represents potential risk. Because eggs can survive periods of prolonged desiccation, areas contaminated with raccoon feces that have dried cannot be considered safe. However, the recognition of complete inactivation of eggs at relatively low temperatures provides guidance in circumstances where contamination with *B. procyonis* eggs requires disinfection efforts and indicates that approaches well short of incineration or boiling will be effective. Furthermore, these results suggest that temperatures achievable in point-of-use hot water heaters (household units) can deactivate infectious *B. procyonis* eggs and provide an option for maintaining safe drinking water during a possible bioterrorism event or a boil water advisory. Further studies are needed to determine the effectiveness of heat and other possible disinfection methods on inactivation of eggs in natural circumstances, such as contaminated play areas and soil.

Table. Viability of *Baylisacaris procyonis* eggs in sterile water when exposed to 2 different heating conditions

Temperature water slowly heated to, °C	% Viable	Temperature of hot water added, °C	% Viable
37°C	100	37	100
42°C	100	42	100
47°C	100	47	98
52°C	94	52	92
57°C	50	57	0
62°C	0	62	0

Acknowledgment

We thank Lawrence Ash for reviewing the manuscript and critiquing the experimental design of the study.

This study was supported by grant S3049 from the Associated Schools of Public Health and the Centers for Disease Control and Prevention and by the UCLA Weisman Fund.

Dr Shafir recently completed her PhD in infectious disease epidemiology at the UCLA School of Public Health and is working there as an assistant professor in the Department of Epidemiology. Her primary research interest is parasitic diseases.

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Melioidosis Acquired by Traveler to Nigeria

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We describe melioidosis associated with travel to Nigeria in a woman with diabetes, a major predisposing factor for this infection. With the prevalence of diabetes projected to increase dramatically in many developing countries, the global reach of melioidosis may expand.

The Patient

A 46-year-old woman sought treatment in the emergency department of St Thomas' Hospital, London, UK. She described a 2-day history of frontal headache, fever, and painful swelling in the right postauricular region. She had received a diagnosis of type 2 diabetes mellitus 5 months before admission, for which she took metformin (500 mg 2×/d). She did not smoke or drink alcohol. She was born in Ogun State, Nigeria, and had moved to the United Kingdom at the age of 31 years. She recently had visited relatives in Sagamu City, Ogun State, and returned to the United Kingdom 6 weeks before onset of her illness. She had no history of travel outside the United Kingdom or Nigeria.

Examination at admission indicated a temperature of 39.5°C, a heart rate of 108 beats per minute, and a blood pressure level of 132/84 mm Hg. Other examination findings were unremarkable, apart from a tender, firm, erythematous, and hot swollen area (4 cm × 3 cm) in the right postauricular region. Initial investigations found a leukocyte count of 6.1×10^9 cells/L (neutrophils 4.4×10^9 cells/L) and a C-reactive protein level of 406 mg/L; renal and liver profiles were normal. Results of a hemoglobin A1c blood test for diabetes were 13%, which is consistent with poorly controlled diabetes, and results of an HIV test and malaria screen were both negative. Ultrasonography of the swollen area showed localized, superficial, enlarged lymph nodes, posterior and inferior to the right pinna. Blood was drawn for culturing, and a course of intravenous flucloxacillin treatment was begun. Subsequently, the aerobic bottles of 2 sets of blood cultures were positive for gram-negative bacilli, and subculturing yielded an oxidase-positive, gram-negative bacillus that grew rapidly on blood agar as a gray colony with a metallic sheen. The organism was subsequently identified as *Burkholderia pseudomallei* (see characterization of blood culture isolate). The antimicrobial drug therapy was changed to intravenous co-amoxiclav (1.2 g 3×/d), and 3 days later, when the identification of the organism was confirmed, the patient's treatment was switched to intravenous meropenem (2 g 3×/d). Results of a computed tomography scan of the chest and abdomen were normal. The patient's fever and lymphadenopathy subsequently resolved, and after 9 days of receiving meropenem, she was discharged with a 12-week course of oral co-trimoxazole (1,920 mg daily). The patient did not attend her scheduled outpatient appointment.

Two microbiology laboratory workers were judged to have had low-risk exposure to the organism before its identification (*1*). They were counseled, and both chose not to receive antimicrobial drug prophylaxis. Serologic follow-up at 2 and 6 weeks postexposure for 1 worker showed no evidence of seroconversion to *B. pseudomallei*. The other did not attend her scheduled occupational health outpatient appointment.

Because the isolate could not be definitively identified by using API20NE (bioMérieux, Marcy l'Etoile, France), it was sent to the UK Health Protection Agency reference laboratory for further analysis. The appearance of the organism in colonies on Ashdown agar was typical of *B. pseudomallei*. Moreover, the same identification was reached through fatty acid methyl ester analysis by gas chromatography with the rapid bioterrorism database (MIDI Sherlock, Newark, NJ, USA). PCR amplification, using primers that specifically detect the type III secretion system gene cluster of *B. pseudomallei* (*2*), produced the predicted 115-bp band for our isolate and the positive control, *B. pseudomallei* strain 204 (*3*). Multilocus sequence typing, performed by using PCR amplification conditions and primers as previously described (*3*), generated an allelic profile (1, 1, 10, 2, 5, 2, 1) that was compared with those of strains of *B. pseudomallei*, *B. mallei*, and *B. thailandensis* stored in the database (http://bpseudomallei.mlst.net). This profile was unique among strains in the multilocus sequence typing database and therefore represents a novel sequence type (ST), ST707. An evolutionary analysis of ST707 with eBURST version 3 (http://eburst.mlst.net) suggested that it was most closely related to other strains of *B. pseudomallei,* including ST26 from Niger, ST82 from France, and ST7 from Vietnam (Figure).

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DOI: 10.3201/eid1707.110502

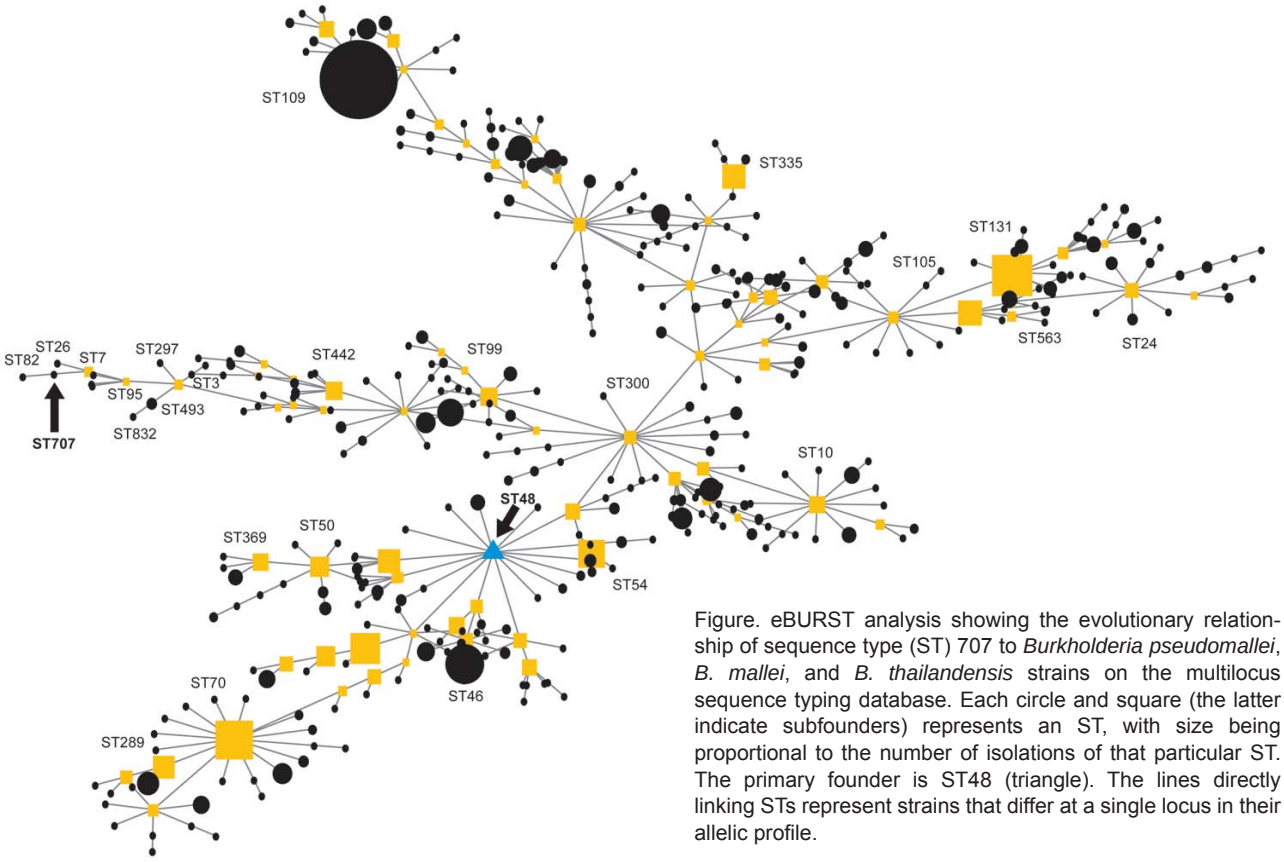

Figure. eBURST analysis showing the evolutionary relationship of sequence type (ST) 707 to *Burkholderia pseudomallei*, *B. mallei*, and *B. thailandensis* strains on the multilocus sequence typing database. Each circle and square (the latter indicate subfounders) represents an ST, with size being proportional to the number of isolations of that particular ST. The primary founder is ST48 (triangle). The lines directly linking STs represent strains that differ at a single locus in their allelic profile.

B. pseudomallei is the causative agent of melioidosis, a severe illness usually characterized by fever, lymphadenopathy, and suppurative lesions in skin, liver, spleen or the lung. The organism is present in the soil to disease-endemic areas, and infection is thought to be acquired through breaks in the skin. The disease is endemic in many parts of Southeast Asia, with most cases being reported from Thailand (*4*). The infection is also a well-recognized pathogen in the Northern Territory of Australia (*5*). In addition, sporadic cases have been described in Central and South America and the Pacific islands. By contrast, strikingly few human cases of melioidosis have previously been reported in Africa. Wall et al. reported a case in The Gambia in a patient originally from Sierra Leone (*6*), and Bremmelgaard et al. noted a case in a Danish patient who had probably acquired the infection in Kenya (*7*). Despite the paucity of clinical cases reported from Africa, serologic surveys suggest that the organism is present in several countries, including Burkina Faso (*8*) and Uganda (*9*). Moreover, *B. pseudomallei* has been isolated from the soil and from animals in several African countries (*10*,*11*).

Conclusions

Although we cannot be certain, the patient reported here likely acquired her infection in Nigeria, the most populous country on the African continent. To our knowledge, no documented cases of melioidosis acquired in Nigeria have been reported. Most cases imported into the United Kingdom originate in Southeast Asia and the Indian subcontinent (*12*). Given the frequency of travel between the United Kingdom and Nigeria, the absence of imported cases from that country until now is striking. However, the patient's strain appears to be related to a strain that was isolated from neighboring Niger ≈40 years earlier. Although the case we report here may represent a recent introduction of the organism into Nigeria, the disease may also be underdiagnosed because of the difficulty of identifying the organism in a resource-limited setting.

Although melioidosis may affect healthy persons, infection is strongly associated with underlying diseases and with diabetes in particular. In a case–control study in Thailand, diabetes was associated with an adjusted odds ratio of 12.9 (*13*). With the prevalence of diabetes projected to increase dramatically over the next few decades in the developing world (*14*), melioidosis may become more

common in countries such as Nigeria. Finally, from a clinical standpoint, the patient reported here highlights the need to consider the diagnosis of melioidosis, even in the absence of travel to traditional disease-endemic countries.

Acknowledgment

We thank Peter Staves for his help with the eBURST analysis.

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Implications of the Introduction of Cholera to Haiti

Scott F. Dowell and Christopher R. Braden

With more than 250,000 cases and 4,000 deaths in the first 6 months, the cholera epidemic in Haiti has been one of the most explosive and deadly in recent history. It is also one of the best documented, with detailed surveillance information available from the beginning of the epidemic, which allowed its spread to all parts of the country to be traced. Piarroux et al. make good use of this information, along with their own careful field investigations, to trace the epidemic to its beginning and propose an explanation for its origins (*1*).

Multiple lines of evidence indicate that *Vibrio cholerae* was newly introduced into Haiti. Cholera had not been documented in Haiti for at least several generations. Although there was a reference to a small number of "cholera" cases during the American occupation in 1928 (*2*), there was no culture confirmation, and the likelihood is these represented cases of severe diarrhea caused by other pathogens. In the current situation, 14 *V. cholerae* isolates from the Artibonite Department early in the epidemic were indistinguishable by multiple phenotypic and molecular characterization methods, which indicated the infections were due to a single clone of *V. cholerae* introduced into Haiti in 1 event (*3*). Piarroux et al. present strong evidence that the earliest cases of cholera occurred in the upper Artibonite valley, near the town of Mirebalais (*1*). Within days of the Mirebalais cases, hundreds of additional cases were reported in the lower Artibonite valley, more than 50 km away, and over the next 2 days in neighboring departments outside the Artibonite valley. This rapid spread is characteristic of cholera transmission in a mobile, immunologically naive population with widespread exposure to sewage-contaminated drinking water. The mobility of the population ensured transmission of the pathogen to all 10 departments of Haiti within weeks, and the uniformly poor water and sanitation infrastructure ensured that where the pathogen was introduced local transmission would follow. Indeed, residents of the camps for internally displaced persons, where chlorinated water and sanitation was in many instances provided by outside organizations, experienced relatively low numbers of cholera infections (*1*).

Piarroux et al. provide circumstantial evidence that fecal contamination of a local stream draining into the Artibonite River initiated the epidemic, that further spread then occurred to more heavily populated towns downstream in the river valley, and that a foreign peacekeeping battalion may have been the source of *V. cholerae* introduction into Haiti. The origin of cholera in Haiti is the subject of study by an independent panel appointed by the Secretary General of the United Nations. Certainly the spread within days to remote departments outside the valley indicates an important role for travel of infected persons along land routes in the subsequent if not the initial spread. However it occurred, there is little doubt that the organism was introduced to Haiti by a traveler from abroad, and this fact raises important public health considerations.

Introduction into an immunologically naive population was necessary but not sufficient for such explosive epidemic spread. During the course of this epidemic, there were multiple introductions into camps for internally displaced persons in Haiti, with limited spread; into communities in the Dominican Republic, resulting in local but not widespread transmission; and into Florida and other states in the United States, with no secondary spread and no epidemics (*4*). These populations were also immunologically naive to cholera but were protected from exposure by physical and chemical barriers—the infrastructure for water treatment and distribution and for the collection and treatment of fecal waste—that effectively prevent contamination of food and drinking water by enteric pathogens.

International travelers, including those going to serve vulnerable populations, are potential carriers of epidemic-prone disease. These travelers and their service organizations should take appropriate precautions (such as vaccination and chemoprophylaxis) to protect themselves and to forestall introducing such pathogens to local populations (*5*).

After travelers' arrival in a country, the spread of cholera and other enteric infections should be prevented by ensuring adequate sanitation such that pathogens excreted

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DOI: 10.3201/eid1707.110625

by either symptomatic or asymptomatic persons cannot be introduced into local food or water supplies. Prompt diagnosis and treatment of acute illnesses that may arise will also limit the opportunities for further spread. Realistically, however, it is impossible to completely guard against the introduction of infectious diseases, including cholera, into new populations and places, given the mobility of vast numbers of persons, animals, and products around the globe.

For Haiti, the future course of the cholera epidemic is difficult to predict, especially given the chronic degradation of water and sanitation infrastructure over many years and the acute disruption from the earthquake in Haiti in January 2010 (*6*). Improving water and sanitation infrastructure is clearly the most effective and lasting approach to prevent the spread of cholera in countries where it is endemic as well as in those that are currently cholera-free. In the United States, Europe, and worldwide, disinfection of municipal water supplies and improvements in sanitation have dramatically reduced the incidence of cholera, typhoid, and overall mortality (*7*). In Haiti, the short-term public health response has included real-time surveillance, laboratory confirmation of infections, training of health workers on case management, and public education for basic cholera prevention. Enhanced access to medical services were scaled up quickly among many partner organizations by using the existing clinic infrastructure supported by the US President's Emergency Plan for AIDS Relief. These efforts helped to reduce mortality to <1% within 4 months and bring the epidemic temporarily under control. Even as we continue to learn more about the intercontinental spread of this ancient human scourge, we are reminded of the continued effectiveness of traditional public health control measures.

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Trichostrongylus colubriformis Nematode Infections in Humans, France

To the Editor: In April 2009, a 47-year-old woman in Saint-Jeannet in southern France reported stomach aches, abdominal bloating, and occasional diarrhea. Blood analyses found an increased eosinophil level (8,800 cells/mm^3), which represented 52% of 16,900 leukocytes/mm^3.

Parasitologic examinations for helminths were conducted with 6 fecal specimens obtained during June 9–July 2, 2009. Analyses included direct wet mount microscopic examination, Merthiolate–iodine–formaldehyde concentration, formalin–ethyl acetate concentration, and Baermann larval extraction.

Results of direct examination and the Baermann technique were negative for all samples. The formalin–ethyl acetate concentration technique detected a parasite egg (Figure, panel A) and first-stage larvae. Fecal cultures grew mature third-stage larvae (length 700–800 µm, 16 intestinal cells, length of the sheath <40 µm), belonging to the genus *Trichostrongylus* (Figure, panel B). Because of the ambiguous morphologic features of this genus, a molecular approach was necessary for specific identification (*1,2*).

Identical symptoms developed in 2 children of the patient and in 2 friends. The mother of the patient had additional symptoms (weight loss 5 kg in 1 month and 35,000 eosinophils/mm^3, which represented 85% of 43,200 leukocytes/mm^3). However, results of fecal examinations were negative for these 5 persons.

DNA was extracted separately from 2 third-stage larvae (Figure, panel B) by using the DNA Tissue Mini Kit (QIAGEN, Hilden, Germany). To amplify internal transcribed spacer 2 (ITS2) sequences, we used primers NC1: 5′-ACGTCTGGTTCAGGGTTGTT-3′ (forward) and NC2: 5′-TTAGTTTCTTTTCCTCCGCT-3′ (reverse) (*3,4*), which were used by Hoste et al. for *Trichostrongylus* spp. typing (*3*). ITS2 rDNA was sequenced, and third-stage larvae sequences were registered in GenBank (accession nos. HQ174256 and HQ174257).

Complete (100%) homology was obtained with known sequences (*3,4*) for adult *Trichostrongylus colubriformis* nematodes from sheep (GenBank accession nos. S69220, X78063, and EF427624). Parasite sequences also showed 100% homology with the main haplotype observed in humans in Laos (*2*). If one considers the absence of intraspecific variability within *T. colubriformis* nematodes (*3,4*), the specimens isolated from the patient and most likely from the other 5 persons presumed to be affected in this outbreak belong to this species.

The 6 symptomatic patients were treated according to published recommendations (*5*) with albendazole, 400 mg/day for 10 days. Clinical remission was obtained in <3 days, and eosinophil counts returned to reference levels 3 months later.

Specific questioning of the 6 persons indicated that the source of infection most likely was a meal eaten in April 2009, which included strawberries picked in the vegetable garden of the patient's mother. The patient's father and brother did not eat any strawberries and did not have any symptoms. The garden was fertilized yearly with dried manure from a local sheep farm. Lack of dried manure in 2009 led to use of fresh sheep manure from the same farm. Sheep manure from breeding stock on the farm was examined. *Trichostrongylus* spp. third-stage larvae were found despite prophylactic treatment of sheep on the farm against helminths.

T. colubriformis nematodes are mainly parasites of herbivorous mammals and have a worldwide distribution. Human infections are found predominantly in warm areas. They are usually asymptomatic or as described in the present case. *T. colubriformis* adults live in the intestines of the host (*6*). The female lays eggs, which are excreted in feces. Eggs then hatch and mature into infectious larvae. Humans become

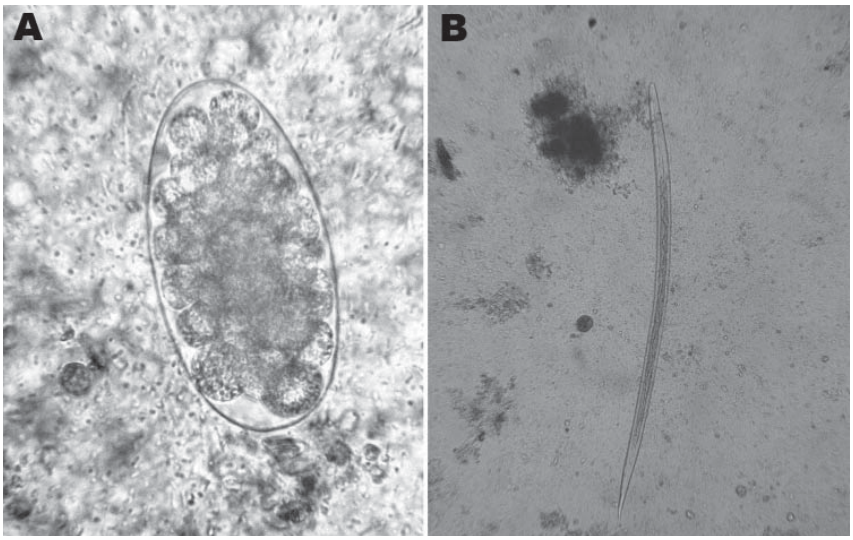

Figure. *Trichostrongylus colubriformis* nematode isolated from feces of a 47-year-old woman, France. A) Egg (length 89 µm) isolated by using direct examination (original magnification ×200). B) Third-stage larvae (length 740 µm, 16 intestinal cells, length of distal part of the sheath <40 µm) isolated by using fecal culture (original magnification ×50). A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1301-F.htm).

infected by ingesting unwashed vegetables contaminated by animal feces containing strongyloid larvae. Larvae mature into adults in the intestines.

Sporadic cases of this infection in humans have been reported in many countries (*7*). In France, several autochthonous cases were suspected, but because of their rarity and difficulty in identification, they are not commonly reported (*8*). Eggs of *Trichostrongylus* spp. can be differentiated from those of *Necator* and *Ancylostoma* spp. because they are longer, narrower, and elongated. After 6 days of culture, *T. colubriformis* nematodes can be distinguished from similar stages in *Strongyloides* and *Ancylostoma* spp. by the bead-like swelling at the tip of the tail. Except for isolation of adult worms, which are rarely found in feces, sequencing of the ITS2 region is the most accurate method for specific identification of *Trichostrongylus* spp. isolated from humans.

This familial outbreak highlights increased risk for animal parasitosis in humans in an industrialized country, which may have been caused by an increasing trend of persons using ecologic and organic farming methods. These cases confirm that hygienic recommendations for use of organic fertilizer must be disseminated on a large scale. It is also mandatory that fresh vegetables be washed carefully and thoroughly before ingestion, and only dried manure should be used as an organic fertilizer.

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DOI: 10.3201/eid1707.101519

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Adult *Opisthorchis viverrini* Flukes in Humans, Takeo, Cambodia

To the Editor: *Opisthorchis viverrini* and *Clonorchis sinensis,* the 2 major species of small liver flukes (family Opisthorchiidae), cause chronic inflammation in the bile duct, which leads to cholangitis and cirrhosis of the liver, and are a predisposing factor for cholangiocarcinoma (*1*). Human infections with *O. viverrini* flukes are found along riverside areas of Indochina (Thailand, Lao People's Democratic Republic [PDR], and Vietnam) (*2*).

Small trematode eggs (length 20–32 μm) have been found in human fecal samples in Cambodia (*1,3,4*). During 1981–1982, two of 102 Cambodian refugees in the United States were found to be positive for *C. sinensis* (likely *O. viverrini*) eggs (*3*). Egg-positive cases were later detected in several provinces of Cambodia (*4,5*). Presence of *O. viverrini* flukes in Cambodia was verified by detection of metacercariae in freshwater fish in a lake on the border between Takeo and Kandal Provinces and by isolation of adult flukes in experimentally infected hamsters (*6*).

In May 2010, we analyzed fecal samples from 1,993 persons in 3 villages (Ang Svay Chek, Kaw Poang, and Trartang Ang) in the Prey Kabas District, Takeo Province, Cambodia, ≈45 km south of Phnom Penh, to confirm the presence of *O. viverrini* flukes among humans. We found an egg-positive rate of 32.4% for small trematode eggs. Because these eggs may be those of *Haplorchis* spp. flukes (*H. taichui*, *H. pumilio*, and *H. yokogawai*) and lecithodendriid flukes (*Prosthodendrium molenkampi* and *Phaneropsolus bonnei*) (*1*), we attempted to detect adult flukes that are responsible for these eggs.

Six of the small trematode egg–positive villagers, 1 man and 5 women (age range 16–72 years), who had occasional epigastric discomfort were selected for anthelmintic treatment, purgation, and recovery of adult worms. Fecal examination and anthelmintic treatment of villagers were approved by the Ministry of Health, Cambodia, under the agreement with the Korea–Cambodia International Collaboration on Intestinal Parasite Control in Cambodia (2006–2011). After obtaining informed consent, the villagers were treated with a single oral dose of praziquantel, 40 mg/kg (Shinpoong Pharmaceutical Co., Seoul, South Korea), and given a purgament (solution containing 30–40 g $MgSO_4$). Feces was obtained 3 or 4 times in a 2–3-hour period after purgation, pooled individually, and processed as described (*7*). Worms obtained were fixed with 10% formalin, stained with acetocarmine, and identified by morphologic features.

A total of 34 *O. viverrini* adult worms were obtained from the 6 villagers (14, 9, 5, 3, 2, and 1 from each villager, respectively). No other species of trematodes were obtained. Five worms were lanceolate and had a mean length of 9.5 mm (range 6.5–12.0 mm), a mean width of 1.5 mm (range 1.2–1.7 mm), and 2 characteristic 4–5-lobulated testes (Figure, panel A). Ten eggs in uteri were 27 μm long (range 25–29 μm) and 15 μm wide (range 13–16 μm).

To detect the source of infection, 2 freshwater fish species, *Puntioplites proctozysron* (n = 5) and *Cyclocheilichthys apagon* (n = 10), were caught in nearby Ang Svay Chek village and examined for *O. viverrini* metacercariae by using a digestion technique (*8*). A total of 50 metacercariae (Figure, panel B) were obtained from 5 *P. proctozysron* fish and fed to a hamster. Six weeks later, 42 young *O. viverrini* flukes (Figure, panel C) were isolated from the biliary tract of the hamster.

Our study identified only *O. viverrini* infections in humans in Cambodia. However, eggs of other hepatic and intestinal flukes also can be found in humans (*1*). In Thailand, Vietnam, and Lao PDR, opisthorchiids (*O. viverrini* and *C. sinensis*), heterophyids (*Haplorchis* spp., *Centrocestus formosanus*, and *Stellantchasmus falcatus*), and lecithodendriids have been found in humans (*1,7,9*). In several provinces in Lao PDR, mixed infections with *O. viverrini* and heterophyids or lecithodendriids were common (*7,9*), and the relative prevalence of each fluke species varied by locality. In Vientiane, Lao PDR, *O. viverrini* was the predominant species, whereas in Saravane Province, *H. taichui* predominated (*7*). In a mountainous area of Phongsaly Province, *H. taichui* and *H. yokogawai* worms were obtained from 10 villagers; however, no *O. viverrini* worms were detected (*10*). Thus, in Cambodia, the presence of human infections with intestinal flukes, including *Haplorchis* spp. and lecithodendriids, cannot be ruled out.

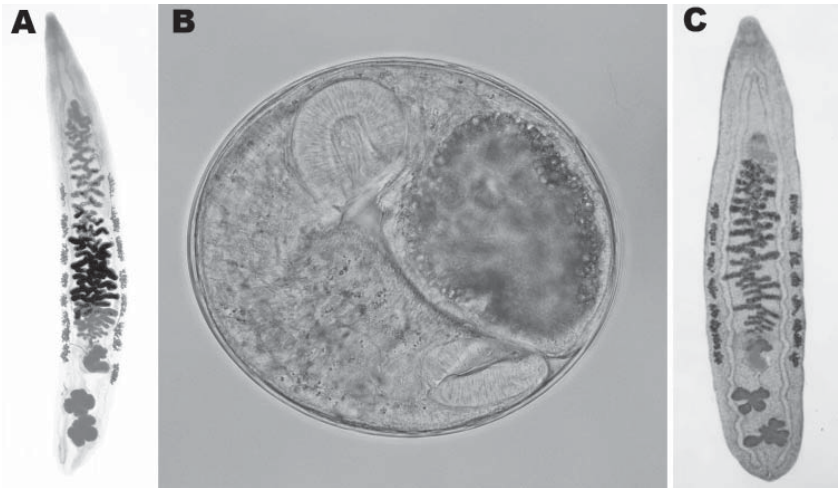

Figure. A) Adult *Opisthorchis viverrini* liver fluke (length 12.0 mm) isolated from a human after chemotherapy and purgation in Takeo Province, Cambodia, showing the characteristic morphology of the two 4–5-lobulated testes. B) Metacercaria of *O. viverrini* fluke (diameter 0.22 mm) detected in a freshwater fish (*Puntioplites proctozysron*). C) Young adult *O. viverrini* fluke (length 5.5 mm) isolated 6 weeks after experimental infection of a hamster with metacercariae from *P. proctozysron* fish. Original magnification levels ×8 (A), ×120 (B), ×9 (C). A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1302-F.htm).

Acknowledgment

We thank the staff of the Centre for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia, and Dong-Min Lee, Yoon-Hee Lee, Ji-Hae Kang, Hyun-Ju Woo, and Jun-Yong Hong for assistance with Kato-Katz fecal examinations.

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DOI: 10.3201/eid1707.102071

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Easy Test for Visceral Leishmaniasis and Post–Kala-azar Dermal Leishmaniasis

To the Editor: Diagnosis of visceral leishmaniasis (VL), fatal if untreated, is complex because the symptoms are the same for many fever-associated ailments. Despite limitations, diagnosis remains based on finding *Leishmania* amastigotes in spleen and/or bone marrow aspirates (*1*). Sophisticated laboratory methods, although sensitive, are costly. The immunochromatographic strip test that uses recombinant K39 antigen (rK39), although satisfactory in India, is less sensitive in Africa, Latin America, and Mediterranean regions (*2*). Post–kala-azar dermal leishmaniasis (PKDL), a sequel to VL in India and Africa, is often confused with other skin diseases (*3,4*). Diagnosis of VL in dogs in Latin America and Mediterranean countries remains confusing because of rampant asymptomatic infections and elevated antibodies against *Leishmania* spp. (*5*).

Earlier we reported the diagnostic potential of *L. donovani* (MHOM/IN/83/AG83) promastigote membrane antigens (LAg) (*3,6*). Here we report applicability of LAg-based ELISA and dipstick systems even at primary health centers. Using randomized sampling, we tested samples from 122 kala-azar patients from India, 20 PKDL patients from India, and 40 VL patients from Brazil. VL was confirmed by finding parasites in aspirates. Serum samples were collected before chemotherapy was given. PKDL was diagnosed as described (*3*). Control samples were collected from 24 healthy persons from non–disease-endemic areas in India; 15 healthy persons from disease-endemic areas in India; 20 healthy persons from disease-endemic areas in Brazil; and 21 persons with Hansen disease, 7 with filariasis, 4 with tuberculosis, 1 with lymphoma, 1 with leukemia, 2 with virus-induced fever, and 5 with malaria. Consent was obtained from all human donors. This study was approved by Ethical Committee on Human Subjects at Indian Institute of Chemical Biology and the Ethical Board for Human Subjects and Animal Experimentation of the Federal University of Piauí.

We developed a diagnostic ELISA with modifications of our previous method (*6*). Microtiter plates were coated with 2.5 μg LAg at pH 7.5 (100 μL/well) and kept at 4°C overnight, after which they were blocked with 1% bovine serum albumin, dried, and stored at 4°C as precoated plates. The assay performed at room temperature took ≈2.5 h. Test and control serum samples (1:1,000 dilution, 100 μL/well) were applied to the plates for 45 min and shaken occasionally. Horseradish peroxidase–conjugated goat anti-human immunoglobulin (Ig) G (Genei, Bangalore, India) was applied at 1:5,000 (100 μL/well) for 45 min. Color development with orthophenylenediamine (Sigma-Aldrich, St. Louis, MO, USA) was allowed for 5–10 min. Positive results were determined by comparing colors with those on a card previously prepared for positive and negative wells. ELISA, performed for the VL and PKDL patients from India, was 100% sensitive (percentage of patients with confirmed disease and positive

test results) and 96.3% specific (percentage of negative controls with negative test results) (Figure, panel A); sensitivity and specificity were higher than that reported earlier (*6*) and by other studies that used crude leishmanial antigens (*2*). One sample from each of filariasis, lymphoma, and disease-endemic area controls was marginally false positive. Seropositivity was diagnosed for 1 patient who had a negative spleen aspirate but clinical signs of VL and for 1 patient who refused spleen or bone marrow aspiration.

To avoid any visible cross-reaction in the dipstick assay, we optimized LAg concentration, test serum dilution, and control serum dilution. Optimum concentration for human studies is 500 μg/mL LAg, 1:2,000 serum dilutions, 1:2,000 horseradish peroxidase–conjugated goat anti-human IgG, and 15 mg 3,3′-diaminobenzidine (Sigma-Aldrich) as substrate in 30 mL Tris-buffered saline. LAg was bound to a long nitrocellulose piece at the test line (line on which LAg is coated). Goat anti-human IgG (Genei) at 1:25 was coated as an internal control line. Free sites were blocked with 2% bovine serum albumin containing 0.01% NaN_3 and were air dried. LAg-coated membranes were affixed to the end of a plastic support (with a free end as handle) with double-adhesive tape, cut into 4-mm–wide sticks, and stored at room temperature. During the testing process at room temperature, dipsticks were incubated in diluted serum for 30 min, washed 2×, incubated for 30 min with secondary antibody, washed 3×, and incubated in substrate solution for 1 min. Finally, dipsticks were washed in water, dried on tissue paper, and examined for specific reaction. When stored at room temperature without desiccation, dipsticks performed consistently for 12 months. Dipsticks appeared equally sensitive and specific (100%) for VL from India and Brazil and for PKDL. Because internal control lines remained positive, analyses were considered valid (Figure, panel B).

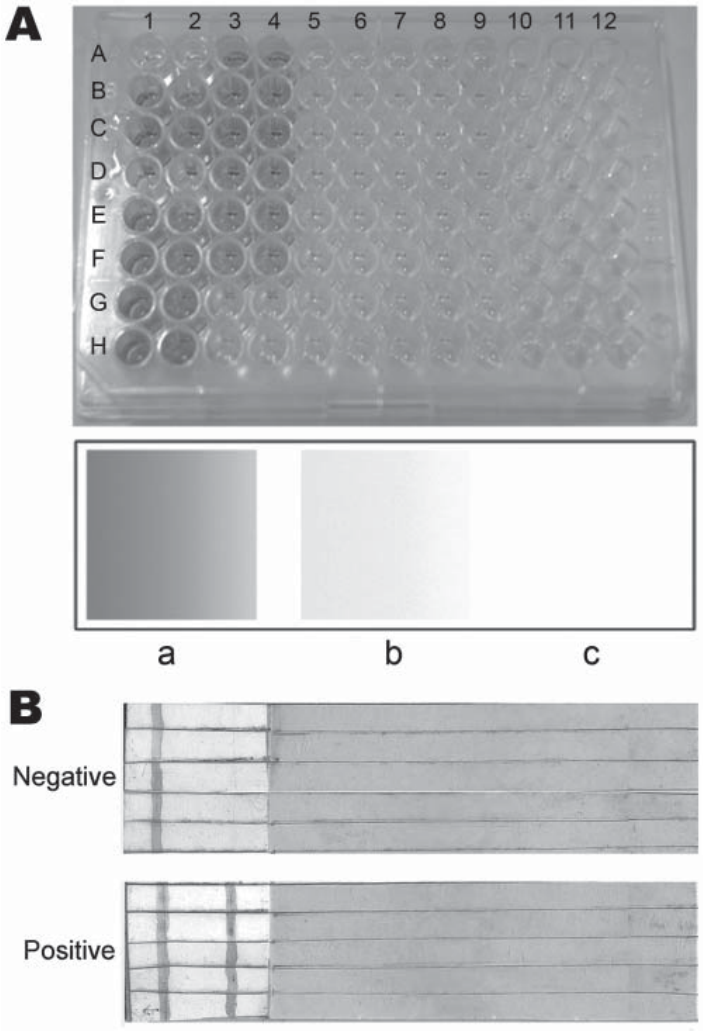

Figure. Representative results of ELISA and dipstick testing. A) Samples underwent ELISA in duplicate. Upper panel, positive samples in duplicate (1–2 and 3–4) in wells A–H, except A1–A2 (blank), and G3–G4 and H3–H4 (negative controls). Wells in columns 5–10 represent different negative controls in duplicate (5–6, 7–8, and 9–10), except F9–F10, G9–G10, H9–H10, and all wells in columns 11–12 (unused wells). Lower panel, the reference color card: a, positive; b, negative; c, blank. B) Dipstick test results. The left band is the internal control line; the right band is the test line. A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1304-F.htm).

LAg dipsticks are more sensitive for diagnosing VL in Brazil than rK39 (*7*) and cost ≈70× less (*8*). Although further validation with a larger sample size and healthy controls from disease-endemic areas and controls for other diseases is warranted, these easy, simple, and low-cost methods could emerge as efficient tools for diagnosis of VL and PKDL.

Acknowledgments

We thank all patients and donors for participating in this study and Pradyot Bhattacharya for assistance with sample collection.

The Council of Scientific and Industrial Research and University Grants Commission, Government of India, provided financial support.

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DOI: 10.3201/eid1707.100801

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Ameba-associated Keratitis, France

To the Editor: Amebic keratitis is an aggressive infection usually associated with soft contact lenses, and its poor outcome can lead to a corneal graft (*1*). Ameba can host ameba-resistant bacteria (*2*) and serve as a source for numerous organisms to exchange DNA, adapt to changing environments, and become pathogenic to the host (*3*). However, mixed keratitis caused by amebae in association with ameba-resistant pathogens is rarely seen.

A 17-year-old woman who was myopic and had worn soft contact lenses for 3 years consulted our ophthalmology department for pain and redness of the left eye that had persisted for 2 weeks. No visual loss was reported, and a slit-lamp examination showed a millimetric epithelial defect associated with a round stromal infiltrate (Figure). No intraocular reaction was observed, and bacterial keratitis was diagnosed. Her condition improved after a 7-day topical fluoroquinolone treatment, and follow-up at 13 months showed only a slight superficial stromal scar. The patient reported that she had inappropriately worn monthly contact lenses for 3 months, cleaned and rinsed lenses with a commercial cleaning solution but diluted the solution with tap water, and washed her hands with tap water but did not dry them before handling the lenses.

Results of microbiologic analysis of a corneal scraping, which included molecular detection of ameba (18S rDNA), bacteria (16S rDNA), and herpesvirus (DNA polymerase), were negative. However, culture of contact lens storage case liquid in Page-modified Neff ameba saline enriched with heat-killed *Enterobacter aerogenes* (*2*) identified *Pseudomonas fluorescens* and *Stenotrophomonas maltophilia*, both of which were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (*4*); *Mycobacterium chelonae*, which was identified by *rpo*B gene sequencing; and *Acanthamoeba polyphaga*, which was identified by partial 18S rDNA sequencing.

Culturing this ameba in sterile peptone–yeast extract–glucose broth showed that it hosted 4 organisms. The first organism was a new intracellular Deltaproteobacterium provisionally named *Candidatus* Babela massiliensis (100% 16S rDNA sequence similarity with a reference strain [GenBank accession no. GQ495224], which was susceptible to 200 μg/mL rifampin and resistant to 20 μg/mL ciprofloxacin). The second organism was a new, unnamed, gram-negative, ciprofloxacin-susceptible (20 μg/mL) *Alphaproteobacterium* bacillus with 99% 16S rDNA sequence similarity (GenBank accession no. HM138368) with *Candidatus* Odyssella sp. and *Acanthamoeba* endosymbiont KA/E9 (*5*). The third organism was a new giant virus related to an *A. polyphaga* mimivirus (*6*) strain Lentille. The

fourth organism was a new virophage referred to as Sputnik 2 (*7*).

Acanthamoeba spp. are ubiquitous in tap water (*2,5*), including that used by contact lens wearers to wash hands before manipulating the lens (*8*). Tap water hosts many organisms, including bacteria (*2,5*); large DNA viruses (*9*); and the recently described Sputnik virophage, a virus that infects ameba-resistant large DNA viruses (*10*). We found 5 bacterial species, a giant virus, and a virophage in the contact lens storage case liquid for this patient.

Although *Acanthamoeba* spp. and *M. chelonae* are well-described agents of keratitis, other ameba-resistant organisms have not been associated with keratitis. However, 1 of the 2 new ameba-resistant bacteria isolated from the contact lens storage case liquid had a 16S rDNA sequence similar to that of the endosymbiont of an *Acanthamoeba* species isolated from the corneal sampling of a patient with keratitis in South Korea (*5*).

These data indicate the ubiquity of these emerging organisms and raise questions about roles of the ameba host and the symbiont as causative agents of keratitis. We therefore estimate that any of the organisms reported or any combination of these agents could have been involved in the keratitis that developed in the patient. Because the minute quantity of corneal scraping material prevented molecular detection, the relative contribution of each organism could not be specified.

This case illustrates that culturing ameba from ocular specimens and contact lens storage case liquid is mandatory for determining the diversity of pathogens potentially responsible for ameba-associated infections, such as keratitis, in patients who wear contact lenses. Ameba-resistant organisms have complex reciprocal interactions with the host, reminiscent of the mafia behavior.

The patient's practice of not drying her hands after cleansing them with tap water and diluting the contact lens cleansing liquid with tap water may have provided a route for contamination of the contact lens fluid with ameba-resistant, tap water–borne organisms. Patients should be informed of the risk for keratitis caused by water-borne, amebal-resistant pathogens. They should also be educated to avoid contact with tap water when manipulating contact lenses, to dry hands after washing them with water and soap, or to use antiseptics containing ≥70% alcohol for hand disinfection before contact lens manipulation.

This study was supported by the Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Unite Mixte de Recherche, Centre National de la Recherche Scientifique 6236.

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DOI: 10.3201/eid1707.100826

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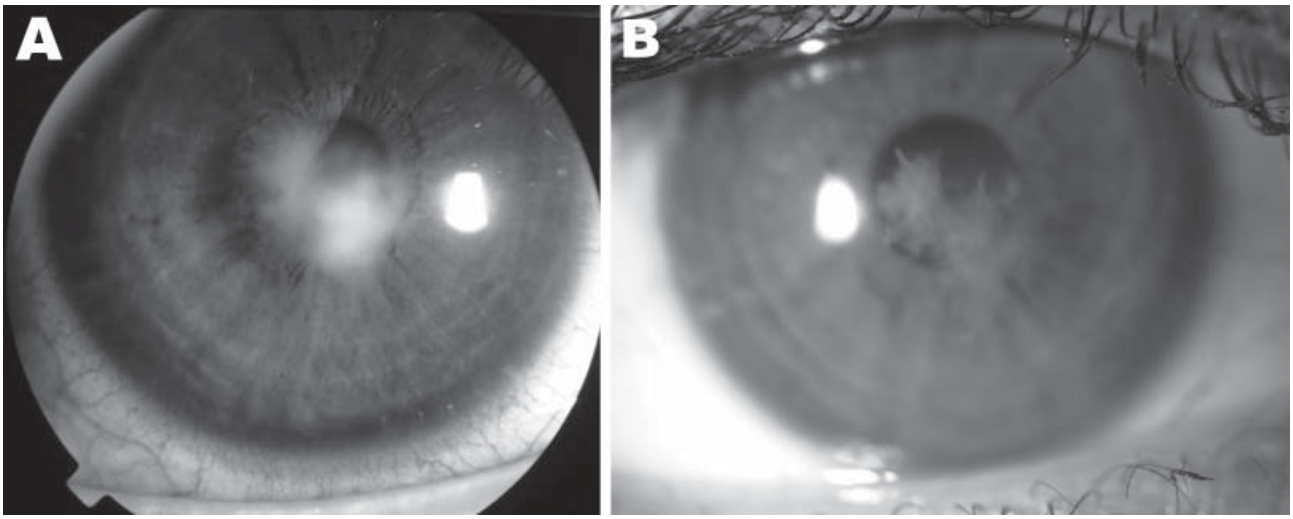

Figure. Ameba-associated keratitis in a 17-year-old woman (contact lens wearer), France, showing a paracentral corneal scar (A) and recovery at 13-month follow-up (B). Original magnification ×10. A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1306-F.htm).

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Human Herpesvirus 1 in Wild Marmosets, Brazil, 2008

To the Editor: Human herpesvirus 1 (HHV-1) infections in New World monkey species, especially in the Callithrichid family, have been described (*1–6*), but most reports have discussed experimental infections or isolated spontaneous infections in pet, zoo, or research animals. We report an outbreak of HHV-1 in wild marmosets (*Callithrix* spp.) in the city of Rio de Janeiro, Brazil.

In October 2008, the Empresa de Pesquisa Agropecuária received 5 marmosets (*Callithrix* spp.) from the Campo Grande district of Rio de Janeiro for necropsy. These animals were usually fed by residents of a condominium complex and were having neurologic signs and severe prostration, physiologic changes suggestive of herpesvirus infections. Euthanasia, followed by necropsy and histopathologic examinations to determine the cause of illness, were recommended.

The primary changes observed during necropsy were vesicular and necrotic plaques on tongues (online Appendix Figure, panel A, www.cdc.gov/EID/content/17/7/1308-appF.htm) and ulcerations in oral mucosa of all examined animals, as well as large lymph nodes of the cervical region, mainly retropharyngeal. Three animals showed marked brain congestion (online Appendix Figure, panel B). Other alterations were splenomegaly, lung congestion, and adrenomegaly.

Histopathogic examinations found superficial ulcerations of the tongue, variable in dimension, that showed fibrinopurulent exudates, mononuclear cell infiltrates on lamina propria, and balloon degeneration of epithelial cells. The brains had multifocal nonsuppurative meningoencephalitis with perivascular and vascular infiltrates of mononuclear cells and gliose foci (Figure, panels A, B). Adrenal glands had hyperemia, hemorrhage, perivascular infiltrates of mononuclear cells, and focal necrosis. Mild hyperemia and alveolar emphysema had occurred in lungs. The livers showed hyperemia and mild to moderate periportal infiltrates of mononuclear cells. Lymph nodes showed hemorrhages, lymphoid hyperplasia, and small foci of subcapsular necrosis. Hyperemia and decreased lymphoid cells population were present in the spleens. In addition, intranuclear inclusion bodies in cells of brains, peripherical nerves, tongues, and adrenal glands were observed. These changes were found in all animals. All changes were consistent with HHV-1 in nonhuman primates (*2–6,7,8*).

To confirm the diagnosis, immunohistochemical examination was done by using polyclonal antibody directed against HHV-1. We used the avidin–biotin–peroxidase complex method with Harris hematoxylin counterstain. Sections taken of the ulcerated oral lesions had intranuclear inclusion areas strongly marked by immunoperoxidase (Figure, panels C, D). HHV-1 infection was confirmed in the 5 marmosets.

Many reports have described human herpesvirus in New World monkeys. Most of the reports were of experimental or isolated spontaneous infections in pets (*1*,*2*), zoo (*3*), research (*4*,*5*) or wild animals (*6*). This is the second report of a naturally occurring infection in wild marmosets. Both infections occurred in the Grande Rio region, where *Callithrix* spp. imported from other Brazilian states were accidentally introduced. These species came to occupy a niche that once belonged to the golden lion tamarin (*Leontopitecus rosalia*) (*9*,*10*).

Humans are the reservoir and the natural host of human herpesvirus (*3–6*), which can be disseminated by direct contact, through sexual

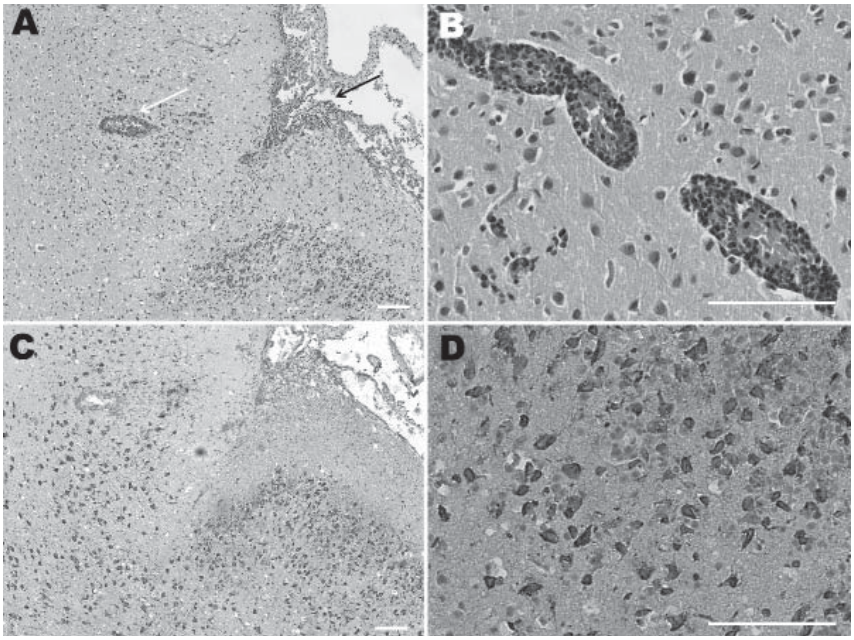

Figure. Microscopic lesions of brain caused by human herpesvirus 1 infection in marmosets. A) Histopathogic sample stained with hematoxylin and eosin showing nonsuppurative meningoencephalitis with perivascular infiltrates (black arrow) and infiltrates in piamater (white arrow). B) Histopathologic sample stained with hematoxylin and eosin showing perivascular and vascular infiltrates of mononuclear cells. C, D) Immunohistochemical examination by using polyclonal antibody directed against human herpesvirus 1 and the avidin–biotin–peroxidase complex method, Harris hematoxylin counterstain. Neural cells strongly marked by immunoperoxidase, indicating a positive finding. Scale bars = 100 μm. A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1308-F.htm).

activity (*5*) and, in a brief period after contamination, through domestic tools and food remains (*6*). Once brought to the colony, the disease spreads quickly with high rates of illness and death (*4*,*5*). In general, the herpesviruses produce asymptomatic and latent infections in their natural hosts but cause severe disease when transmitted to other species (*5*,*7*,*8*).

In Old World primates, benign and localized human herpesvirus infections have been described. Although systemic infections with fatal outcome occur, infection usually remain confined to the skin, oral cavity, external genitalia, and conjunctiva (*1–3*,*5*,*6*) rather than affecting the nervous system.

New World primates are highly susceptible to infection and severe disease, with spontaneous infections more commonly reported in *Callithrix* spp. The clinical course is severe, resulting in death in most reported cases (*2*,*4*,*5*). In marmosets, human herpesvirus produces an epizootic disease with substantial illness and death (*7*). This viral infection has already been described in 3 species of marmosets (*C. jacchus, C. penicillata* and *C. geoffroyi*) and in owl monkeys (*Aotus trivirgatus*) and cotton-head tamarins (*Saguinus oedipus*) (*1–3*,*5*).

There is only 1 report of spontaneous infection in free-living black tufted-ear marmosets (*C. penicillata*), which occurred at the State Park of Serra da Tiririca, Niterói, Brazil (*6*). In this report, the infection is thought to have been related to the proximity between local human residents and wildlife; the disease also reportedly developed with substantial illness and death in the marmoset population (*6*). Similarly, the cases presented here presumably were acquired from close contact with humans because the animals were fed regularly at a residential condominium, and the virus can be transmitted through contact with contaminated saliva, aerosols, and fomites, such as tools. The high susceptibility and mortality rates for New World monkeys that contract this infection argues strongly for prophylactic strategies, considering that the infection occurs even in conservation parks and could seriously affect the local primatologic fauna and thus species conservation.

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DOI: 10.3201/eid1707.100333

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Melioidosis in Birds and *Burkholderia pseudomallei* Dispersal, Australia

To the Editor: Melioidosis is an emerging infectious disease of humans and animals caused by the gram-negative bacterium *Burkholderia pseudomallei*, which inhabits soil and surface water in the disease-endemic regions of Southeast Asia and northern Australia (*1*). The aim of this study was to assess the potential for birds to spread *B. pseudomallei*. Birds are known carriers of various human pathogens, including influenza viruses, West Nile virus, *Campylobacter jejuni*, and antimicrobial drug–resistant *Escherichia coli* (*2*).

During February–August 2007, we conducted a survey to determine *B. pseudomallei* carriage in 110 wild native finches and doves from the melioidosis-endemic Darwin region, Northern Territory, Australia. Swab specimens from the beaks, feet, cloacae, and feces were cultured for *B. pseudomallei* as described (*3*). One healthy (normal physical appearance, weight, and hematocrit) native peaceful dove (*Geopelia placida*) at a coastal nature reserve was found to carry *B. pseudomallei* in its beak. The peaceful dove is a common, sedentary, ground-foraging species in the Darwin region. *B. pseudomallei* was not detected in environmental samples from the capture site, but *B. pseudomallei* is known to occur within 3 km of the capture site (*4*), the typical movement range for this bird species. On multilocus sequence typing (MLST) (*5*), the *B. pseudomallei* isolate was identified as sequence type (ST) 144, which we have previously found in humans and soil within 30 km of the site.

Numerous cases of melioidosis in birds have been documented (online Technical Appendix, www.cdc.gov/EID/content/17/7/1310-Techapp.pdf). However, these are mostly birds in captivity and often exotic to the location, suggesting potential reduced immunity. Little is known about melioidosis in wild birds. In Sabah, Malaysia, only 1 of 440 wild birds admitted to a research center over 9 years was found to have melioidosis (*6*).

Although birds are endotherms, with high metabolic rates and body temperature (40°C–43°C) protecting them from many diseases, some birds appear more susceptible to melioidosis. Indeed, high body temperature would not deter *B. pseudomallei*, which is routinely cultured at 42°C and at this temperature shows increased expression of a signal transduction system, which is involved in pathogenesis (*7*).

Examples of birds with fatal melioidosis in our studies in the Darwin region include a domesticated emu in 2009 with *B. pseudomallei* cultured from brain tissue and a chicken in 2007 with *B. pseudomallei* cultured from facial abscesses. In 2007, an outbreak of melioidosis occurred in an aviary; 4 imported exotic yellow-bibbed lorikeets (*Lorius chlorocercus)* died within months of arriving from a breeder in South Australia. On necropsy, the birds showed nodules throughout the liver and spleen (Figure). *B. pseudomallei* was cultured from the liver, spleen, crop, beak, and rectum. At the aviary, *B. pseudomallei* was also found in water from sprinklers, the water bore head, soil next to the bore, and the drain of the aviary. The unchlorinated sprinkler system used to cool the aviary was identified as the likely source of infection. MLST and 4-locus multilocus variable-number tandem repeat analysis (*8*) suggested a point-source outbreak with an identical 4-locus multilocus variable-number tandem repeat analysis pattern and ST for all *B. pseudomallei* isolated from the diseased birds and the sprinkler system. The ST was novel (ST673),

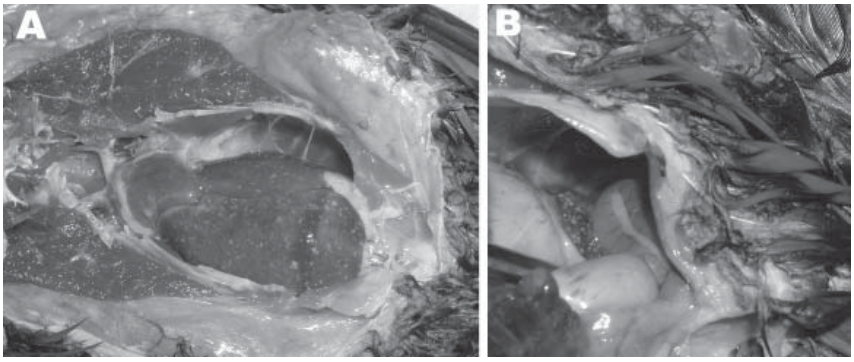

Figure. Images from necropsy of yellow-bibbed lorikeet that died of melioidosis, showing multiple diffuse nodular lesions in the liver (A) and spleen (B). Photographs by Jodie Low Choy. A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1310-F.htm).

with no single-locus variants in the global MLST dataset.

Although an infected exotic or captive bird is likely to quickly die from melioidosis, our survey suggests that native birds are not very susceptible to infection with *B. pseudomallei* and resulting disease. Further studies are required to quantify the carriage of *B. pseudomallei* in wild native birds in melioidosis-endemic locations. Nevertheless, although no direct proof exists for spread of *B. pseudomallei* by birds, our finding of an asymptomatic native bird with *B. pseudomallei* in its beak supports the hypothesis of potential dispersal of these bacteria by birds from melioidosis-endemic regions to previously uncontaminated areas. For instance, carriage by birds could explain the introduction of *B. pseudomallei* to New Caledonia in the Pacific, 2,000 km east of Australia. *B. pseudomallei* strains from New Caledonia are related by MLST to Australian strains; 1 strain is a single-locus variant of a strain from Australia's east coast (*9*). Vagrant water birds are known to irregularly disperse from eastern tropical Australia to the southwestern Pacific, presumably driven by drought and offshore winds (G. Dutson, pers. comm.). Thus, *B. pseudomallei* could have been introduced to New Caledonia by an infected bird that flew there from northeastern Australia.

In summary, melioidosis is uncommon in wild birds but occurs in captive or exotic birds brought to melioidosis-endemic locations. Asymptomatic carriage of *B. pseudomallei* can occur in wild birds but appears to be unusual. We believe the risk for spread of *B. pseudomallei* by birds is low, but such occurrence does provide a possible explanation for the spread of melioidosis from Australia to offshore islands.

Acknowledgments

We are grateful to Cathy Shilton for advice and to the owners of the lorikeet aviary for their enthusiastic cooperation and help. We also thank the Charles Darwin University Animal Ethics Committee and Northern Territory Parks and Wildlife Commission for permission to trap wild birds, collect swab specimens, and release the wild birds.

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DOI: 10.3201/eid1707.100707

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Rare Case of Trichomonal Peritonitis

To the Editor: Trichomonads are flagellated protozoa with several species capable of infecting or colonizing humans. The most common, *Trichomonas vaginalis*, causes genitourinary infection in adults and, rarely, respiratory infections in premature neonates of infected mothers. *Pentatrichomonas hominis* has been isolated from the human gastrointestinal tract, and *Trichomonas tenax*, a human oropharynx commensal, has caused empyema in immunocompomised persons. Rare cases of human peritonitis caused by trichomonads have been reported.

Some trichomonads, including *Tritrichomonas foetus* and *Tritrichomonas suis*, primarily infect and colonize animals. Although they were previously thought to be different species, current molecular and biologic evidence suggests they are indistinguishable (*1*). *T. foetus* (synonym *T. suis*) causes genitourinary infection in cattle and diarrhea in cats and colonizes the gastrointestinal tract of swine.

We report *T. foetus* peritonitis in a 52-year-old man with common variable immunodeficiency, rheumatoid arthritis, splenectomy, and cryptogenic cirrhosis. In June 2007, he was admitted with peritonitis to a community hospital in Pennsylvania, United States. He lived on a farm that had swine, horses, and cats. Exposure to cattle was unknown. He denied having a history of sexually transmitted infections or high-risk sexual behavior.

Initial examination showed paracentesis fluid with numerous motile, flagellated organisms consistent with trichomonads. Bacterial fluid cultures had no growth. Despite receiving antimicrobial drugs (including metronidazole 500 mg intravenously every 6 hours), he became increasingly ill over the following 72 hours with hypotension, acute renal failure, and metabolic acidosis, which required transfer to Penn State Milton S. Hershey Medical Center (Hershey, PA, USA) for further care.

Upon arrival, the man was afebrile but hypotensive and tachycardic. Abdominal examination showed ascites, decreased bowel sounds, and diffuse tenderness. Genitourinary examination results were normal. Repeat paracentesis demonstrated numerous motile trichomonads. Urinalysis and routine cultures of peritoneal fluid and blood were negative. Computed tomography of the abdomen and pelvis showed edematous bowel, ascites, and peritonitis. His condition deteriorated during the following 48 hours. Despite ongoing treatment with broad-spectrum antimicrobial drugs (including metronidazole 500 mg intravenously every 6 hours), he died of multiorgan failure.

Autopsy showed peritonitis with copious intraabdominal exudate and peripancreatic and perigastric abscesses. No intestinal perforation or genitourinary abnormalities were noted. No portal of entry for peritoneal infection was identified. Premortem abdominal fluid samples were sent to the Centers for Disease Control and Prevention (Atlanta, GA, USA) for analysis.

DNA was extracted from the trichomonad culture and peritoneal fluid by using the QIAamp DNA mini-kit (QIAGEN, Valencia, CA, USA). PCR testing for *T. vaginalis* was performed (*2*). PCR for *T. foetus* was performed by using primers TFR3 and TFR4 with thermocycling conditions outlined previously (*3*). PCR was performed in a 50-μL reaction volume with 1 μL of deoxynucleoside triphosphate mix (12.5 mmol/L each of dATP, dCTP, dGTP, and 5 mmol/L of dUTP; Applied Biosystems, Foster City, CA, USA), 5 μL of $MgCl_2$ (25 mmol/L; Applied Biosystems), 0.2 μM each primer, 2.5U of AmpliTaq Gold polymerase (Applied Biosystems), 5 μL of 10× PCR buffer (Applied Biosystems), and 5 μL of DNA. PCR products were analyzed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Amplicons were purified with the QIAquick PCR purification kit (QIAGEN) and directly sequenced with PCR primers on an ABI 3130-XL Genetic Analyzer (Applied Biosystems). Sequences were assembled and aligned with Lasergene software (DNASTAR, Inc., Madison, WI, USA) and deposited in GenBank (accession no. HQ849063).

Metronidazole sensitivity was tested with methods previously described (*4*). The patient's trichomonads had minimal lethal concentration (MLC) of 3.1 μg/mL for metronidazole, similar to MLCs of the known metronidazole–sensitive *T. vaginalis* isolate. *T. vaginalis* metronidazole MLCs >50 μg are associated with resistance (*5*).

PCR performed by using primers TFR3/4 produced a 348-bp amplicon with DNA extracted from peritoneal fluid and culture (Figure). Comparison of DNA sequence from the parasite to GenBank sequences showed 100% identity with cattle isolates of *T. foetus.*

Two human cases of *T. foetus* infection have been reported. *T. foetus* was identified by PCR in the respiratory tract of a patient with AIDS and pneumonia (*6*) and

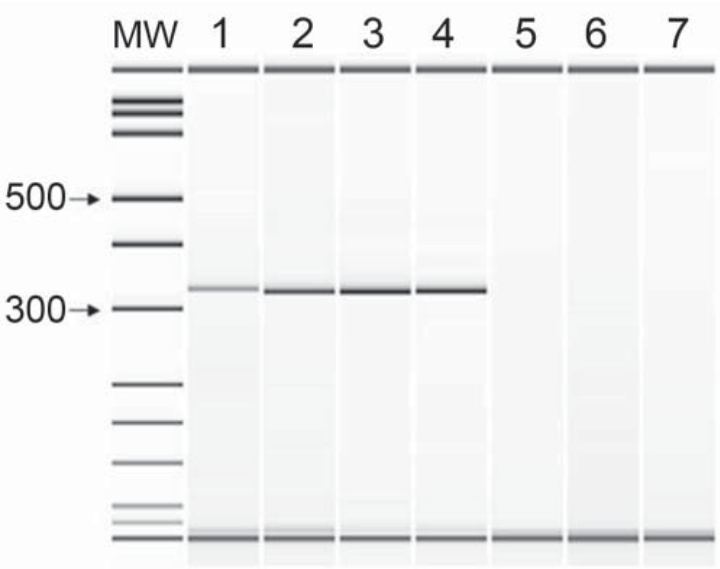

Figure. PCR amplification with primers TFR3 and TFR4. Lane 1, *Tritrichomonas foetus* ATCC 30231; lane 2, peritoneal fluid specimen; lane 3, culture of peritoneal fluid; lane 4, *T. suis* ATCC 30167; lane 5, *Pentatrichomonas hominis*; lane 6, *Trichomonas tenax*; lane 7, no template control. MW, molecular size standard. Values at left are bp.

by microscopy in cerebrospinal fluid from a hematopoetic stem-cell transplant recipient with fatal meningoencephalitis (*7*). The latter patient described by Okamoto et al. had a history of trichomonads in a urine sample before transplantation and clinical epididymitis when meningoencephalitis was diagnosed, leading to their conclusion that his infection was genitourinary in origin. The patient we report had no apparent signs of genitourinary infection.

Human trichomonal peritonitis has been reported (*8–10*). Straube et al. described a 54-year-old man with common variable immunodeficiency and cirrhosis. Peritoneal fluid contained numerous trichomonads, identified as *T. faecalis* (syn *T. equi*), an intestinal commensal in horses. This patient died shortly after diagnosis. These authors did not describe animal exposures. The patient we report had animal contact. We found no reported cases of *T. foetus* peritonitis.

Two reported patients with trichomonal peritonitis recovered after treatment with metronidazole. Thus, we propose initial treatment with metronidazole in patients with trichomonal peritonitis but confirmation of species and sensitivity to antimicrobial drugs is essential.

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DOI: 10.3201/eid1707.100892

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Plasmodium knowlesi Reinfection in Human

To the Editor: In 2004, a large number of patients infected with *Plasmodium knowlesi* (simian malarial species) were reported in Sarawak, Malaysia (*1*). *P. knowlesi* infection was also reported in Peninsular Malaysia (*2*).

Here we report a case of human *P. knowlesi* reinfection. Phylogenetic sequence analysis shows that the first and second infections were caused by different strains of *P. knowlesi*.

The patient was a 41-year-old businessman from Peninsular Malaysia. He was first admitted to the hospital in October 2009 with a 4-day history of fever, chills, and headache. His symptoms started ≈2 weeks after a 4-wheel-drive expedition with overnight camping in a jungle in Raub in the state of Pahang. Initial examination showed thrombocytopenia and hepatitis, and *P. knowlesi* malaria was subsequently confirmed with nested PCR by using diagnostic primers for *Plasmodium* small subunit (SSU) rRNA as described (*3*). He recovered fully after a treatment course of oral quinine plus doxycycline.

The patient was readmitted to the hospital on June 11, 2010, with a 5-day history of fever and chills and rigors, followed by epigastric pain, nausea, and vomiting. His symptoms began 15 days after another 4-wheel-drive expedition with overnight camping in a jungle in Tanjung Malim in the state of Perak. Laboratory investigations showed severe thrombocytopenia. Falciparum malaria was diagnosed initially on the basis of blood film examination with 1% parasitemia. The patient was administered oral mefloquine (750 mg) followed by 500 mg and 250 mg at 6 hours and 12 hours, respectively. His parasitemia level increased from 1.0% to 2.5% despite treatment with mefloquine. Oral quinine and doxycycline were initiated. However, renal function deteriorated further, and acute hemolysis was evident. Oral quinine was changed to intravenous quinine infusion, and oral combination of artemether and lumefantrine was added. Intermittent hemodialysis was initiated, and 1 unit each of packed erythrocyte cells and whole blood were transfused. Parasitemia eventually cleared on June 16, 2010. PCR confirmed *P. knowlesi* in the patient's blood sample.

P. knowlesi has a 24-hour asexual life cycle, resulting in daily schizont rupture, which leads to high parasitemia levels. Delay in appropriate treatment, as seen in the second infection of the patient in our study, can cause severe conditions, such as thrombocytopenia, acute renal failure, and hemolysis (*4*).

To confirm the reinfection, blood samples collected from the patient at the first and second infections were reexamined. Giemsa-stained thin and thick blood films showed 2.0% and 2.5% parasitemia for the first and second infections, respectively. Some parasites showed morphologic features resembling those of *P. falciparum* ring forms and *P. malariae* trophozoite band forms.

We confirmed the *P. knowlesi* in the first and second infections by PCR, sequencing and analysis of the highly variable *csp* gene (*5*), and SSU rRNA. The nucleotide sequences of the gene were aligned by using ClustalW and analyzed by using MEGA4 software (*6*). The *csp* gene of the isolate from the first infection (denoted as Pkpah-1) was 1,217 nt, whereas the gene of the isolate from the second infection (denoted as Pkprk-1) contained 1,277 nt. This difference was due to the absence of 2 repetitive sequences

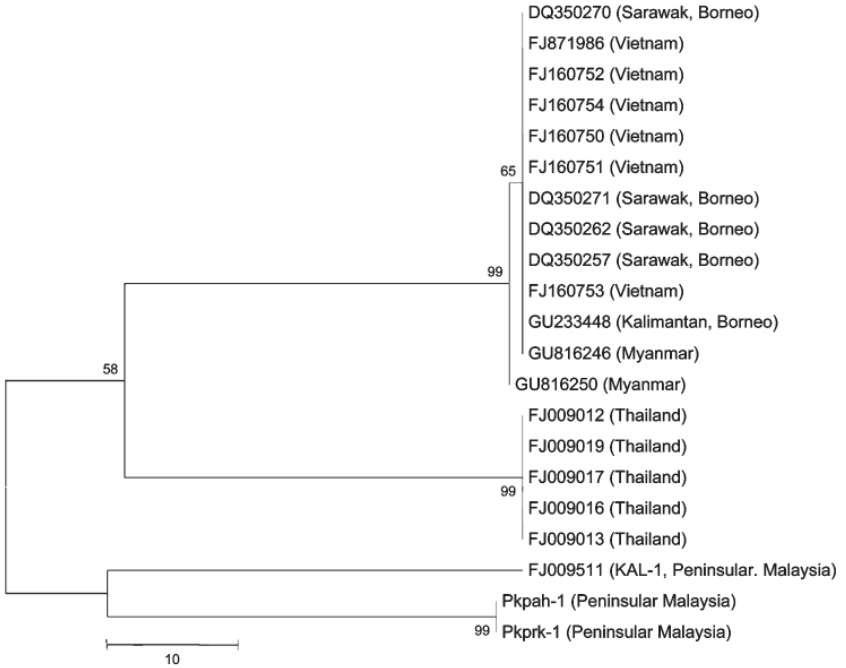

Figure. Phylogenetic tree based on nucleotide sequences of small subunit rRNA of *Plasmodium knowlesi* isolates from Peninsular Malaysia (Pkpah-1, Pkprk-1, KAL-1) and surrounding regions (denoted by GenBank accession nos.). The tree was constructed by using the maximum-parsimony method. The percentage of replicate trees in which the associated isolates cluster together in the bootstrap test (10,000 and 1,000 replicates, no differences were observed) is shown next to the branches. Phylogenetic analysis was conducted by using MEGA4 (*6*). Scale bar indicates nucleotide substitutions per site.

(5′-GTGGAGCAAATGCAGGACA ACCGAATGCAG-3′;5′-AGCAA ATGCAGGACAACCGAAT GCAGAAGG-3′) in the *csp* gene of Pkpah-1. Furthermore, the alignment showed 21 variable sites between the genes. Phylogenetic analysis based on SSU rRNA sequences indicated that Pkpah-1 and Pkprk-1 formed a cluster, which was more related to *P. knowlesi* isolates from Thailand than to isolates from Sarawak, a Malaysian state in Borneo Island (Figure). Another isolate from Peninsular Malaysia, KAL-1, was also grouped with Pkpah-1 and Pkprk-1. The KAL-1 isolate was detected in a traveler from Finland returning from Peninsular Malaysia in 2007 (*7*).

P. knowlesi infection does not relapse because the parasite has no liver hypnozoite stage (*8*). Reinfection and recrudescence are not uncommon in malaria. The finding in the patient reported here indicates reinfection rather than recrudescence as confirmed by PCR genotyping. He acquired his infection while jungle trekking in Raub in October 2009 and was reinfected in Tanjung Malim in June 2010 (the great-circle distance between the 2 locations is 38.61 km). This case shows that immunity toward *P. knowlesi* infection is strain specific as has been observed in other malaria species (*9*). *P. knowlesi* is a nonhuman primate malaria species, and humans are accidental hosts when they go into areas where macaques dwell. *P. knowlesi* can cause reinfection and can potentially be severe in areas where it is endemic. Travelers to forested areas of endemicity should be advised to take strict antimosquito measures and prophylaxis. Physicians should be aware that *P. knowlesi* infection is a vital differential diagnosis in febrile travelers with a recent travel history to forested areas in Southeast Asia.

This study was supported by High Impact Research grant no. J-20011-73592 from the University of Malaya.

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DOI: 10.3201/eid1707.101295

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Antibody to Arenaviruses in Rodents, Caribbean Colombia

To the Editor: The ≈20 recognized arenaviruses in the Americas are hosted by rodents of the family *Cricetidae*; 1 exception may be hosted by a bat (genus *Artibeus*, family *Phyllostomidae*) (*1*). Pichindé virus, hosted by O*ryzomys albigularis*, was described from animals in the Pichindé Valley near Cali, Colombia (*2*), and antibody reactive to Pichindé virus was found in 2 of 82 serum samples from humans in the same area. No studies of arenavirus infection in rodents or humans have been conducted in Colombia since 1971. Although Pichindé virus is not associated with human disease, Guanarito virus, which is hosted by *Zygodontomys brevicauda*, the short-tailed cane mouse (*3,4*), causes Venezuelan hemorrhagic fever in the Venezuelan state of Portuguesa (*5*). This state borders on Colombia, and *Z. brevicauda* is a common species in Caribbean Colombia. Our aim was to determine the prevalence of antibody to arenaviruses among wild rodents in this region.

During November 1, 2008–June 10, 2009, we trapped 322 rodents in 3 rural localities in the Department of Córdoba, Colombia (Monteria, Vereda El Escondido, 8°34.183′N,

75°42.776′W; Sahagun, Vereda Las Llanadas, 8°56.533′N, 75°20.909′W; and Lorica, Colegio Instituto Técnico Agrícola, 9°24.067′N, 075°75.707′W). The landscape at the study sites is dominated by tropical savanna, small patches of forest, and cultivated land. Live-capture traps were set in a variety of habitats at each locality, and captured rodents were processed according to methods by Mills et al. (*6*). Rodents were anesthetized, and blood (by cardiac puncture) and tissue (liver, lung, spleen, hearty, kidney) samples were collected into individual cryovials, placed in liquid nitrogen in the field, and transferred to freezers and stored at –80°C at the Instituto de Investigaciones Biológicas del Trópico, Universidad de Córdoba, Montería, Colombia. Rodent species were identified on the basis of morphologic analyses of formalin-fixed carcasses; chromosomal data and mitochondrial DNA sequencing of the cytochrome b gene were used to confirm identification of most antibody-positive animals. Alcohol-preserved voucher specimens are archived at the Museum of Texas Tech University (Lubbock, TX, USA).

Blood samples were tested for arenavirus immunoglobulin (Ig) G by indirect immunofluorescent antibody assays. Guanarito and Pichindé virus–infected Vero E6 cells were used as antigens on spot slides. The secondary antibody was a fluorescein-conjugated goat, antimouse IgG. Serum samples were screened at a dilution of 1:10 and endpoint titers were measured by using serial 2-fold dilutions (1:10–1:320) (*7*). Attempts to amplify viral RNA in tissues by reverse transcription PCR were unsuccessful.

We collected 210 sigmodontine rodents of 3 species: 181 *Z. brevicauda*, 28 *Oligoryzomys fulvescens*, and 1 *Oecomys concolor*. Eleven serum samples, 10 from *Z. brevicauda* and 1 from *O. fulvescens* rodents, had detectable arenavirus antibody. Three *Z. brevicauda* rodent samples had antibody reactive to both Pichindé and Guanarito virus, and 7 more were positive for either Pichindé or Guanarito arenaviruses (Table).

We used only 2 viral antigens in our screening belonging to the 2 viruses that are either known to occur in Colombia (Pichindé virus) or known to be hosted by species that we captured (Guanarito virus). Among the 10 *Z. brevicauda* samples with detectable antibody, 5 reacted only to Pichindé virus antigen or their antibody titer to Pichindé virus was at least 4-fold higher than their titer to Guanarito virus (Table), suggesting those rodents were infected with Pichindé or a closely related virus. Additional studies, including isolation and sequencing are needed to definitively identify this virus.

Surprisingly, only 2 *Z. brevicauda* rodent (1.1%) had antibody only to Guanarito virus or had a 4-fold greater titer to Guanarito virus, much lower than the 15% antibody prevalence in the same species in the Venezuelan hemorrhagic fever–endemic area, Portuguesa State, Venezuela (*5*). Our testing protocols differed from the earlier study, and we have not definitively identified Guanarito virus in those 3 rodents; nevertheless, this low prevalence might help explain the absence of Venezuelan hemorrhagic fever in Colombia, although inadequate surveillance is a second possible explanation.

The single antibody-positive *O. fulvescens* rodent had a low antibody titer only to Pichindé virus. This apparent 4% antibody prevalence is based on only 28 mice. The significance of this finding is not clear but may represent spillover or an undescribed arenavirus specific to the species *O. fulvescens*. Again, additional studies are needed.

Our results demonstrate the presence of ≥1 arenaviruses circulating among common rodent hosts in Caribbean Colombia. We emphasize that many New World arenaviruses are likely cross-reactive to the antigens we used; recovery and sequencing of viral RNA will be essential to fully characterize these viruses. Hemorrhagic fever of arenaviral origin should be included in the differential diagnosis of tropical fevers, at least in our study region. As the human population of the rural Department of Córdoba and adjacent areas of

Table. Comparison of PICHV and GTOV antibody titers in rodents after serologic screening of 210 sigmodontine rodents, Córdoba, Colombia, November 1, 2008–June 10, 2009*

Specimen no.	Species	PICHV titer	GTOV titer	Rural locality
49	*Zygodontomys brevicauda*	**40**	<10	Monteria
70	*Z. brevicauda*	**80**	10	Monteria
105	*Z. brevicauda*	**40**	<10	Monteria
107	*Oligoryzomys fulvescens*	20	<10	Monteria
272	*Z. brevicauda*	**80**	10	Monteria
289	*Z. brevicauda*	20	<10	Monteria
317	*Z. brevicauda*	**40**	<10	Monteria
344	*Z. brevicauda*	20	<10	Monteria
345	*Z. brevicauda*	<10	20	Monteria
209	*Z. brevicauda*	10	**80**	Lorica
211	*Z. brevicauda*	<10	**40**	Lorica

*PICHV, Pichindé arenavirus; GTOV, Guanarito arenavirus. Titers in **boldface** are ≥4-fold higher than titers for the other antigen.

the Caribbean coast of Colombia continues to increase, the potential for arenavirus-related disease could become a public health concern.

This study was supported by grant CIUC FMV0406-1120222 from the Universidad de Cordoba.

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DOI: 10.3201/eid1707.101961

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High Incidence of Guillain-Barré Syndrome in Children, Bangladesh

To the Editor: Bangladesh has achieved remarkable success in its drive to eliminate poliomyelitis; no case has been reported from that country since 2000. Still, the nonpolio incidence rate of acute flaccid paralysis (AFP) in Bangladesh is 3.25 cases per 100,000 children <15 years of age (*1*). Guillain–Barré syndrome (GBS), an acute polyradiculoneuropathy, is the most frequent cause of AFP (*2*). GBS in Bangladesh is frequently preceded by an enteric infection caused by *Campylobacter jejuni* (*3*). Frequent exposure to enteric pathogens at an early age may increase the incidence of GBS. We hypothesized that most AFP cases in Bangladesh can be diagnosed as GBS. Our objective was to estimate the crude incidence rate of GBS among children <15 years of age in Bangladesh.

In collaboration with the World Health Organization (WHO), the Government of Bangladesh conducts active AFP surveillance. AFP is defined as acute onset of focal or general flaccid (hypotonic) weakness without other obvious cause (e.g., trauma) in children <15 years of age. Data on the number of reported AFP cases in Bangladesh during 2006 and 2007 were obtained. On the basis of clinical and other information routinely collected through the surveillance system, we defined a GBS case as presence of an acute flaccid (hypotonic) paralysis and symmetrical weakness (*4*) in the absence of injury or birth trauma.

Bangladesh is divided into 6 divisions (major administrative regions) comprising 64 districts. Crude incidence data for GBS were calculated per division and per district on the basis of the population <15 years of age reported by WHO and the Government of Bangladesh.

In 2006 and 2007, a total of 1,619 and 1,844 AFP cases, respectively, were reported in children <15 years of age, of which 608 (37%) and 855 (46%) cases, respectively, fulfilled the GBS case definition. The crude incidence rate for GBS in children <15 years of age varied from 1.5 to 1.7 cases per 100,000 population in the 3 northern divisions (Dhaka, Rajshahi, and Sylhet) and from 2.1 to 2.5 per 100,000 in the 3 southern divisions (Khulna, Barisal, and Chittagong) (online Appendix Figure, www.cdc.gov/EID/content/17/7/1317-appF.htm). Overall, the crude incidence rate of GBS in children <15 years of age varied from 1.5 to 2.5 cases per 100,000 population per year in the 6 divisions of Bangladesh. Incidence rates were high (>5.0/100,000) in the Meherpur and Barisal districts in southern Bangladesh. We found a seasonal fluctuation in the frequency of patients with GBS; the most cases occurred in May (n = 159) and the lowest in February (n = 84). GBS occurred predominantly among boys (59%).

Most incidence studies reported in the literature originate from Europe and North America (*5*). A recent review reported that the best estimate of the global incidence of GBS in children <15 years of age is

0.6 cases per 100,000 population per year. (*5*). Reports on incidence rates in developing countries are scarce. The crude incidence rate of GBS in Bangladesh in children <15 years of age reported here appears to be 2.5× to 4× higher than that reported in the literature. A statistically significant proportion of AFP cases in Bangladesh appear to be caused by GBS. We cannot explain the difference in incidence rates of GBS in the 3 northern divisions and the 3 coastal divisions in southern Bangladesh, in which rates are higher. However, differences in incidence rates between northern and southern Bangladesh may be explained by difference in climate or epidemiology. Further research is required to determine why GBS would occur more frequently in southern Bangladesh. Population-based data correlate well with a smaller scale hospital-based study in Khulna (*6*), in which GBS was diagnosed in 47% of hospitalized AFP case-patients. Our recent hospital-based study shows that 25% of GBS case-patients in Bangladesh are children <15 years of age (*3*). It is important to highlight that the present population-based analysis is based on a simplified case definition for GBS. All 25 children in the hospital study in whom GBS was diagnosed on the basis of National Institute of Neurological Disorders criteria (*7*) were also detected through population-based surveillance and fulfilled the simplified GBS case definition. Future studies, however, should include a validation of the case definition used here.

The possibility that GBS is related to pandemic (H1N1) 2009 and the affiliated vaccination campaign has resurfaced recently (*8*). For successful surveillance of excess GBS cases after pandemic (H1N1) 2009 and for postmarketing surveillance of the safety of new vaccines in general, data on the background incidence of GBS are critical. This report indicates that the effect of GBS in Bangladesh is substantial and suggests that data obtained through the ongoing global AFP surveillance program can be used to obtain crude incidence data on GBS worldwide.

Acknowledgments

We thank Tazul Islam and S.P. Luby for support and discussions with WHO and Alex van Belkum for critical review of the manuscript. We acknowledge with gratitude the commitment of the Government of Bangladesh to the research efforts of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).

This research was funded by ICDDR,B and the Government of Bangladesh through Improved Health for the Poor: Health Nutrition and Population Research Project. The following donors provide unrestricted support to ICDDR,B research efforts: Australian Agency for International Development, Government of the People's Republic of Bangladesh, Canadian International Development Agency, Embassy of the Kingdom of the Netherlands, Swedish International Development Cooperation Agency, Swiss Agency for Development and Cooperation, and Department for International Development, UK.

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DOI: 10.3201/eid1707.101999

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Rift Valley Fever in Ruminants, Republic of Comoros, 2009

To the Editor: Rift Valley fever (RVF) is caused by a *Phlebovirus* (family *Bunyaviridae*) transmitted by a wide range of mosquitoes (*1*). This zoonotic disease is present in Africa, the Middle East, and Madagascar. Infections by RVF virus (RVFV) in ruminants cause massive abortions in livestock and high death rates in young animals, which result in major economic consequences. Humans are infected by mosquito bites, contact, or inhalation of aerosols. RVF is frequently unapparent or mild for humans, inducing an influenza-like illness that occasionally leads to more serious complications such as hemorrhage, meningoencephalitis, retinopathy, or even death (*2*).

Cattle are socially important in Republic of Comoros because massive slaughtering occurs during traditional wedding ceremonies known as "Grands Mariages," especially on the main island, Grande Comore. Because of low meat production (only 20,000 head of local cattle), a large number of live ruminants enter Grande Comore from Anjouan and Mohéli, the other 2 islands of the Republic, from Madagascar and Tanzania without quarantine or any other preliminary veterinary control.

We report results from a serosurvey of the ruminant populations on the 3 islands of the Republic of Comoros during the 2009 dry season (April–August). A total of 488 blood samples were collected from randomly selected sheep, goats, and horned cattle and sent to laboratory facilities in Mayotte to be tested by an RVFV immunoglobulin (Ig) G competitive ELISA (*3*). Fifty IgG RVFV-negative and -positive serum samples were randomly selected for confirmation by a seroneutralization assay using the reference method described in the World Organisation for Animal Health manual (*4*,*5*).

Of the 488 serum samples tested, 160 were positive for RVFV, for a seroprevalence of 32.8% (95% confidence interval [CI] 28.6%–36.9%). The 3 species were positive for IgG, with prevalence of 30.6% (95% CI 24.2%–37.1%) for cattle, 33.5% (95% CI 27.6%–39.3%) for goats, and 39.0% (95% CI 24.1%–54.0%) for sheep. Using a χ^2 test, we found no statistically significant differences regarding species and gender, but more adults than young animals were seropositive for RVFV IgG ($p<0.001$). Significant differences in RVFV seroprevalence were found between islands ($p<0.005$), especially between Anjouan (26.6%; 95% CI 20.0%–33.3%) and Moheli (45.8%; 95% CI 31.7%–59.9%); $p = 0.011$). Of the 50 samples tested in seroneutralization, 31/31 (100%) of RVFV IgG ELISA-positive serum samples were confirmed as positive for RVFV (Table).

The serologic evidence of RVFV circulation in the ruminant population of the Republic of Comoros is in accordance with the epidemiologic situation described in other countries in the area. Actually, the serosurvey was implemented after the RVF outbreaks reported in several countries in eastern Africa in 2007 during El Niño rains (*6*). In August 2007, RVFV was detected in a young person from Comoros, and indigenous transmission of RVFV in Mayotte was confirmed in 2008 (*7*). RVF outbreaks were also reported in Madagascar during the 2008 and 2009 rainy seasons (January–May 2008 and November–March 2009) (*8*). To our knowledge, no circulation of RVFV in Republic of Comoros has been reported despite frequent legal and illegal movements of populations and goods between Republic of Comoros and eastern Africa, Mayotte, Madagascar, and the others islands of the area. With 1 of 3 ruminants having been in contact with RVFV, our results suggest that the human population in these islands have likely been widely exposed to this virus. However, several questions remain unanswered: Was RVFV recently introduced in the country? Has the virus settled down in a local reservoir for years without major clinical consequences before reemerging thanks to favorable conditions? Actually, no massive abortions in livestock or high death rate in young animals have been notified so far by the Comorian Sanitary Services. Therefore, the origin of infection is presently unknown because animals could have been infected on the island or in another country from where they have been imported.

Because live ruminants have been imported from neighboring countries for 20 years, the risk of introducing new diseases in the country is high. Despite efforts of the Comorian sanitary services, the Republic of Comoros is particularly vulnerable to

Table. Cross-sectional Rift Valley fever seroprevalence in sheep, goats, and horned cattle, Republic of Comoros, April–August 2009*

Animal	Positive IgG results by animal age, %	Positive IgG results by sex, %	No. animals positive by IgG/no. tested (%)				Animals with positive IgG results confirmed by SN
			Grande Comore	Anjouan	Mohéli	Total	
Sheep	Y, 25.0; A, 42.4	M, 12.5; F, 45.5	3/5 (60.0)	9/26 (34.6)	4/10 (40.0)	16/41 (39.0)	2/2
Goats	Y, 6.1; A, 43.2	M, 24.7; F, 37.4	55/139 (39.6)	20/97 (20.6)	9/15 (60.0)	84/251 (33.5)	17/17
Homed cattle	Y, 15.1; A, 38.5	M, 29.1; F, 31.2	35/127 (27.6)	16/46 (34.8)	9/23 (39.1)	60/196 (30.6)	12/12
Total	Y,11.4; A, 41.1	M, 25.7; F, 35.3	93/271 (34.3)	45/169 (26.6)	22/48 (45.8)	160/488 (32.8)	31/31

*Serum positivity was established when titers were >1:10. Ig, immunoglobulin; SN, seroneutralization; Y, young (before reproductive age); A, adult.

pathogens intrusion. Blackleg (1970, 1995) and the contagious ecthyma (1999) were probably introduced into the country by live ruminants imported from Madagascar (*9*). Since 2002, importation of live animals from Tanzania has been common, increasing the risk of introducing continental pathogens or vectors as illustrated with outbreaks of East Coast fever in 2003 and 2004 in Grande Comore (*10*). RVFV circulation presented in this study is another example of the exposure of the Republic of Comoros to emerging pathogens and potentially bears major consequences for the local economy and for public health. The improvement of the Comorian veterinary services and the setting up of surveillance programs are essential to limit the risk of introducing devastating diseases in the area.

Acknowledgments

We thank the vice president in charge of the Ministry of Agriculture, Fisheries, Environment, Industry, Energy, and Handicraft for providing logistical assistance and laboratory facilities and all herders and their representatives from the Comorian Farmers National Federation for their collaboration. We are also grateful to the Departmental Veterinary Laboratory at Mamoudzou, Mayotte, particularly Stéphanie Maeder and Abdou Achirafi for performing ELISA.

This work was supported with Fonds Européens de Développement Régional funds from the European Union within the Project "Programme de Coopération Scientifique sur les Maladies Animales Emergentes dans l'Océan Indien."

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DOI: 10.3201/eid1707.102031

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Yersinia pestis in Small Rodents, Mongolia

To the Editor: Plague is known to be endemic in several areas of Mongolia, but transmission to humans seems to play only a minor role because the number of recognized cases is relatively low (Figure) (*1*). The first human cases in Mongolia were reported to the World Health Organization in 1980, and ≤20 human cases have occurred each year since then (*2*). However, human plague was first reported in 1897 (*3*), such infections have been documented since the 1940s, and *Yersinia pestis* can be found in many provinces of Mongolia (Figure; T. Damindorj, pers. comm.) (*3*,*4*).

The most common source of

human plague in Mongolia is contact with and consumption of the marmot (*Marmota sibirica*) (*1*). Moreover, the great gerbil (*Rhombomys opimus*) and the Mongolian gerbil (*Meriones unguiculatus*) are suspected of being enzootic reservoirs. Although small rodents are also assumed to be reservoirs of *Y. pestis*, the interaction of individual mammals or fleas of particular species in the infectious cycle and the dynamics of an epizootic are not yet clear (*5*). In a retrospective study, we screened tissue samples from small rodents for *Y. pestis* DNA to investigate the prevalence of *Y. pestis* in a potential enzootic reservoir.

During the course of zoologic investigations in Mongolia during 2002, 2005, and 2006, 133 rodents (gerbils, jerboas, and squirrels) were trapped by standard methods (*5*), dissected, and cataloged (Figure). Documentation included species, sex, date and location of trapping, animal size (weight, length) and organ dimensions, as well as all pathologic findings. Although the trapped animals showed a high degree of parasitic infestation, signs of a severe infectious disease were not observed. After the dissection of animals, samples were conserved in 70% ethanol.

Subsequently, total DNA was extracted from alcohol-conserved spleen and liver tissue of 133 animals by using QIAamp DNA Mini Kit (QIAGEN, Hamburg, Germany), according to the manufacturer's instructions. Screening was performed by using a real-time PCR targeting the *pla* gene of *Y. pestis* pPCP1, including a PCR inhibition control, as described (*6*). As positive control, the *Y. pestis* vaccine strain EV76 was used. As negative controls, we included tissues of 53 laboratory rodents, which were processed analogs, beginning with DNA extraction.

In the real-time PCR targeting the *pla* gene, 7 (5.3%) of 133 spleen tissue samples were positive for *Y. pestis*. In contrast, all liver samples and samples of laboratory rodents tested negative. Identification of several host species was supported by partial sequencing of the cytochrome b gene (*7*). The animals tested positive for plague were gerbils (*Meriones* sp., 1; *M. unguiculatus*, 2; *Rhombomys opimus*, 2) and jerboas (*Allactaga sibirica*, 1; *Cardiocranius paradoxus*, 1).

The identity of the 230-bp *pla* PCR fragment was confirmed by DNA sequencing, showing 100% similarity to the *pla* gene sequences deposited in the European Molecular Biology Laboratory nucleotide database. Molecular subtyping of the 7 *pla*-positive DNA samples was attempted by clustered regularly interspaced short palindromic repeats analysis, targeting the 3 loci YPa, YPb, and YPc, respectively. Also included was DNA originating from the above-mentioned negative control tissues. However, only 1 sample from the spleen of a *M. unguiculatus* gerbil found the YPb locus, which then was sequenced, and resulted in the spacer signature *b1-b2-b3-b4-b5'*. This signature is known from a *Y. pestis* biovar, Orientalis, that has been isolated from *Rattus flavipectus* rats in the plague focus of the Yunnan–Guangdong–Fujian provinces in the People's Republic of China (*8*).

Detection of *Y. pestis*–specific DNA in wild rodents has been described. For instance, a wild rodent community in the eastern Sierra Nevada mountains in the United States was screened for plague by *pla*-specific real-time PCR; of 89 rodents, 1 chipmunk (1.1%) had positive results (*9*).

The permanent presence of *Y. pestis* in rodent communities in North America has led to smaller and more distant-living colonies of prairie dogs (*10*). Strikingly, in the present study, >5% of the screened rodents were found to carry *Y. pestis* DNA. This high number was unexpected for the investigated areas, which have had a low level of plague activity (Figure). To our knowledge, *Y. pestis* has also not yet been reported in Manlai Sum (district) in the Umnugovi Aimag (subdivision) (Figure) (*2–4*) nor has the presence of *Y. pestis* DNA in a *Cardiocranius paradoxus* jerboa.

Our findings emphasize that rodents play a role as zoonotic reservoirs of *Y. pestis* in Mongolia and

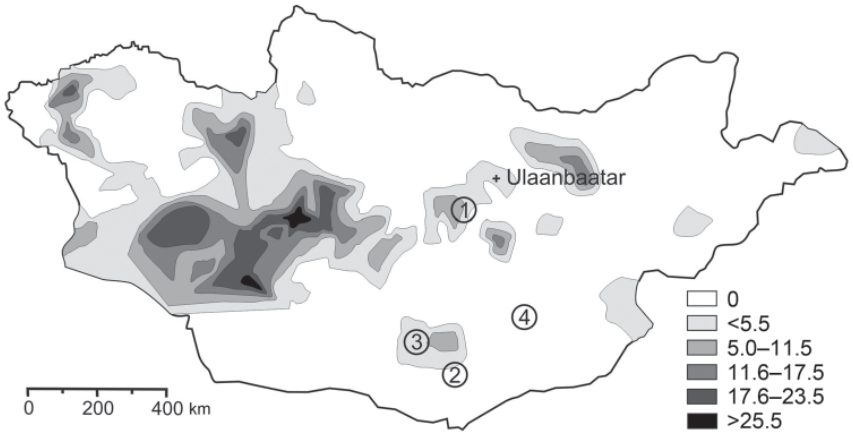

Figure. *Yersina pestis* in rodents in Mongolia. Shaded areas show the known distribution of enzootic plague in Mongolia during 1948–1999 (V. Batsaikhan, J. Myagmar, G. Bolormaa, National Center for Infectious Diseases with Natural Foci, Ulanbaatar, Mongolia; pers. comm.). The following 133 rodents were investigated: gerbils (*Meriones unguiculatus*, 61; *M. meridianus*, 25; *Rhombomys opimus*, 17); jerboas (*Allactaga sibirica*, 6; *Stylodipus telum*, 1; *Dipus sagitta*, 4; *Cardiocranius paradoxus*, 1), and squirrels (*Spermophilus alaschanicus*, 1; *Citellus dauricus*, 1). Plague-positive trapping loci were the following: 1, Tuv Aimag, Bayanunjuul Sum; 2–4, Umnugovi Aimag (2, Nomgon Sum; 3, Bayandalai Sum; 4, Manlai Sum). *Y. pestis* DNA was found in 7 rodents (gerbils and jerboas).

that the actual prevalence of plague seems to be underestimated. The low population density in Mongolia explains the low amount of illness in humans. Further investigations should include the screening of rodent populations near the plague-positive loci. In addition, fleas and other parasites (and also predators of small mammals) should be studied. Mongolia is a key area of plague genesis and therefore is an ideal location for more detailed study of the role of rodents as epizootic and enzootic reservoirs of *Y. pestis*.

Acknowledgments

We thank Gabriele Echle, Philipp Vette, and Astrid Thomas for excellent technical assistance and Gilles Vergnaud for providing access to the CRISPR database (http://crispr.u-psud.fr/crispr).

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DOI: 10.3201/eid1707.100740

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Typhoon-related Leptospirosis and Melioidosis, Taiwan, 2009

To the Editor: Global climatic changes have resulted in more natural disasters worldwide. These natural disasters can then cause outbreaks of emerging infectious diseases, including leptospirosis and melioidosis (*1*–*7*). In 2009, the moderate-strength Typhoon Morakot, with a maximum cumulative rainfall amount up to 3,059.5 mm, damaged Taiwan. After this natural disaster, unusual epidemics of leptospirosis and melioidosis occurred. The main objective of this study was to clarify whether these epidemics have resulted from this natural disaster.

Information about past typhoons that affected Taiwan was collected from the website of the Taiwan Meteorological Bureau (http://photino.cwb.gov.tw/tyweb/mainpage.htm; www.cwb.gov.tw) during January–August, 2009. The influential period of Morakot was in the 32nd week (August 5–August 10) in 2009. To evaluate the effects of this specific natural disaster, we divided the period into 2 intervals for analysis. The early period (before the typhoon) was from the 28th through the 32nd weeks, and the latter period (after the typhoon) was from the 33th through the 37th weeks in 2009. Information regarding 16 typhoons from 2000 through 2009 was further collected to evaluate effects of typhoon level, rainfall level, and maximum cumulative rainfall amounts on case numbers of leptospirosis and melioidosis after a typhoon.

The historical records of numbers of leptospirosis and melioidosis cases for analysis were obtained from the database collected weekly by the Centers for Disease Control, Taiwan. The information was referred to the website of the Taiwan Center for

Disease Control (http://nidss.cdc.gov.tw/). To assess geographic variations, the age-adjusted incidence rates per 100,000 persons of leptospirosis and melioidosis were calculated in each city and county in Taiwan from February through September 2000–2009. SPSS version 15.0.0 software (SPSS Inc., Chicago, IL, USA), ArcGIS (ArcMap, version 9.3; ESRI Inc., Redlands, CA, USA), and SaTScan version 8.0 (www.satscan.org) were used for statistical analysis.

As shown by Mann-Whitney U test, frequencies of leptospirosis and melioidosis cases before the typhoon were significantly lower than those after the typhoon (all $p<0.05$). Furthermore, more leptospirosis and melioidosis cases were observed during the posttyphoon period in 2009 than during 2006–2008 (all $p<0.05$) (Figure).

Using Pearson correlation test to evaluate the effect of cumulative rainfall from Morakot, we found a positive correlation for leptospirosis ($r = 0.54$, $p<0.05$) and for melioidosis ($r = 0.52$, $p<0.05$). Effects of typhoons on numbers of leptospirosis and melioidosis cases in the late stage of a typhoon were further analyzed by using records of typhoon level and maximum 24-hour cumulative rainfall during 2000–2009. After weighting typhoon levels with scores (strong typhoon: 5 points; moderate typhoon: 3 points; mild typhoon: 1 point), we found that typhoon level with higher weight was significantly correlated with more cases of leptospirosis and melioidosis ($r = 0.81$ and 0.87, respectively; all $p<0.05$). The results further suggested that, when the 24-hour cumulative rainfall was >500 mm, significantly more meliodosis cases were observed ($p<0.05$). Although not statistically significant, the number of leptospirosis cases was positively correlated with 24-hour cumulative rainfall ($r = 0.71$; $p = 0.14$).

Using the Anselin local Moran statistic to evaluate geographic variations of leptospirosis and melioidosis after Morakot, we identified significantly higher incidence rates of melioidosis in Tainan, Kaohsiung, and Pingtung Counties in southern Taiwan ($p<0.01$). Nevertheless, no melioidosis cases were observed in Taitung, the county in the same latitude (20°N) but in eastern Taiwan. No significant geographic variation was found in the occurrence of leptospirosis. However, a high incidence of leptospirosis was observed in Pingtung, where flooding caused by Morakot was most serious (maximum cumulative rainfall >2,500 mm).

This study found that epidemics of leptospirosis and melioidosis possibly resulted from the moderate Typhoon Morakot. The findings implied that the effect of typhoon strength on the case numbers of leptospirosis and melioidosis could be less than that of rainfall level and maximum cumulative rainfall amount. Of major importance, the number of melioidosis cases was positively correlated with rainfall level >500 mm. The study further indicated that typhoon strength level and total amount of rainfall must be studied separately to determine their effects on epidemics of infectious diseases. The current typhoon classification system is only related to its intensity, which might not be always associated with total rainfall. The results of this study also implied that epidemic of melioidosis was more likely to be restricted to some geographic regions; this finding was not observed for epidemics of leptospirosis.

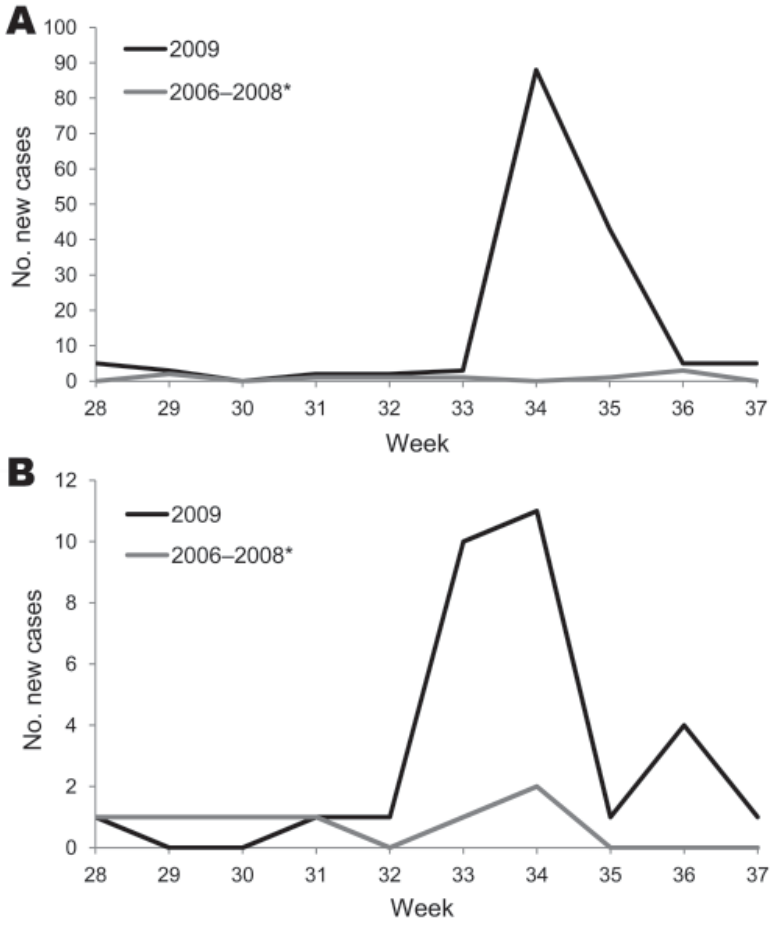

Figure. Comparison of epidemic curves during 2009 and 2006–2008. A) Epidemic curves of leptospirosis. B) Epidemic curves of melioidosis. 2006–2008* indicates that the curve was made by plotting the average weekly numbers.

In conclusion, this study suggests that natural disasters, such as typhoons, that engender large amounts of rainfall could result in epidemics of leptospirosis and melioidosis. More in-depth studies need to be conducted. Efforts need to be taken in advance to prevent possible transmission of these infectious diseases after typhoons.

Acknowledgment

We appreciate the administrative support that we received from the Centers for Disease Control, Taiwan.

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DOI: 10.3201/eid1707.101050

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Exposure to Lymphocytic Choriomeningitis Virus, New York, USA

To the Editor: Lymphocytic choriomeningitis virus (LCMV) is an arenavirus carried by the house mouse, *Mus musculus*. Human infections can range from mild febrile illness to severe encephalitis and disseminated disease (*1*). Infection during pregnancy is associated with teratogenic effects, including congenital hydrocephalus and chorioretinitis (*2*).

The overall occurrence of human exposure to LCMV is not known. Two large US serosurveys suggest that 3%–5% of persons tested had previous LCMV exposure as measured by immunoglobulin (Ig) G (*3*,*4*). In 2002, LCMV-associated congenital subependymal calcifications, hydrocephalus, and chorioretinitis were confirmed for 2 children in central Syracuse, Onondaga County, New York, USA. In 2009, the Centers for Disease Control and Prevention confirmed another case of LCMV-associated congenital hydrocephalus and chorioretinitis in a child from the same neighborhood. For each of the 3 cases, the mother's history included exposure to mice during pregnancy. One mother also had a pet guinea pig, which had negative results for LCMV by serologic testing and reverse transcription PCR of kidney tissue (*5*).

Congenital LCMV is rarely reported to public health departments or in the literature. Therefore, to better understand the magnitude of LCMV exposure in the general population of Onondaga County, we conducted a serosurvey. The American Red Cross provided the Wadsworth Center of the New York State Department of Health with blood or serum samples collected from persons ≥16 years of age at blood drives during August 2009. Information about date of birth, sex, and county and ZIP code of residence was provided. A subset of samples from blood donors residing in Onondaga County were tested at the Centers for Disease and Prevention by ELISA for LCMV IgM and IgG as described (*4*). State and federal institutional review board approval was obtained for this study.

Samples from 562 blood donors were tested. Mean age of donors was 48 years (median 50 ± 15 SD, range 17–79 years). LCMV IgG was detected in 2 (0.4%) samples (titer ≥400) and was undetectable in all other samples. LCMV IgM was not detected in any samples. Of the 25 donors who reported residing in 1 of the 2 ZIP codes as the case-patients with congenital LCMV, none had positive test results.

Given our findings, little evidence supports a high level of human exposure to LCMV in Onondaga County. Compared with previously reported seroprevalences of 3%–5%, the proportion of persons exposed to LCMV was lower than expected (*3*,*4*). The same serologic assay was used in this study and the 2 previous US serosurveys, suggesting that the different results are not an artifact of different assays. Additionally, persons tested in the current survey were older than those tested in previous serosurveys (median 50 vs. 23 [*3*] and

40 years [*4*], respectively). Because IgG against LCMV can persist for years, seroprevalence would be expected to be higher for an older population as a result of more chances for exposure. Also, a serosurvey of >1,000 hospitalized persons from upstate New York in the 1970s detected no positive antibody titers (*6*), consistent with our findings.

Our serosurvey had a few limitations. Blood samples from an entire county cannot detect potential household- or neighborhood-scale areas of increased risk for LCMV exposure, which may be related to focal distribution of populations of LCMV-infected house mice. Serosurveys of house mice have previously shown evidence for clustering of LCMV-infected individuals (*7*); however, the prevalence of LCMV in house mice in Onondaga County is unknown. Additionally, because blood donors were volunteers, the population sampled did not necessarily reflect the population at risk for LCMV exposure. Despite these considerations, the low prevalence of LCMV antibodies suggests low occurrence of LCMV exposure in this population.

Although little is known about frequency of human exposure and infection, LCMV seems to be rare with a propensity for inducing severe disease. LCMV infection has been associated with high incidence of clinical disease, including a pet hamster–associated outbreak in 1973–1974 that resulted in at least 181 cases and 46 hospitalizations in 12 states (*8*).

LCMV-related disease is reportable in only 3 states (Wisconsin, Massachusetts, Arizona) and 1 city (New York, New York) and is considered to be widely undertested and underdiagnosed. A recent survey of health care providers in Connecticut found that LCMV diagnostic tests were not requested for all patients suspected to have LCMV infection (*9*); thus, missed diagnoses are possible. Additional studies are needed to understand the incidence of LCMV-related disease and LCMV seroprevalence in the general population (*10*).

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DOI: 10.3201/eid1707.101349

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Tickborne Relapsing Fever Caused by *Borrelia persica*, Uzbekistan and Tajikistan

To the Editor: Tickborne relapsing fever (TBRF) is caused by several *Borrelia* species and transmitted by argasid soft ticks of the genus *Ornithodoros*. The disease is endemic to many parts of the world, especially Africa (*B. duttonii* and *B. crocidurae* most prevalent), and the Mediterranean basin (*B. hispanica* most prevalent). In Eurasia, TBRF is mainly caused by *B. persica* (*1*–*3*). We report a patient who returned to France with *B. persica* infection after visiting Uzbekistan and Tajikistan.

In September 2008, a 32-year-old man sought care at the Saint Louis Hospital in Paris, France, for a fifth relapsing episode of fever. Three weeks earlier (July 31–August 18), he had trekked through Uzbekistan and Tajikistan and spent 7 nights in a tent in Uzbekistan. The day before his return to France, he had fever (39.5°C) that lasted 5 days without other symptoms. After a 4-day interval, fever recurred for 1 day, then relapsed 3 other times (every 2 days with fever for 12 hours). Laboratory

investigations performed earlier by his general physician on August 19 and 20 showed lymphopenia (0.76 × 10^9 cells/L and 0.44 × 10^9 cells/L), thrombocytopenia (94 ×10^9 cells/L and 80 ×10^9 cells/L), and C-reactive protein level 300 mg/L (reference level <6 mg/L).

Physical examination when the man was afebrile found no clinical abnormalities. He did not recall tick bites, and no lesion was seen on his skin. Laboratory investigations on September 2 indicated persistant lymphopenia (1.0 × 10^9 cells/L), C-reactive protein 96 mg/L, and mild cholestasis (alkaline phosphatase level 125 UI/L and gamma glutamyl transferase level 92 UI/L). Thick and thin Giemsa-stained blood smears showed neither *Plasmodium* spp. nor *Borrelia* spp. However, quantitative buffy coat analysis (QBC; Becton Dickinson, Le Pont de Claix, France) showed numerous spirochetes, which prompted a rereview and careful analysis of the slides, during which spirochetes were infrequently seen. PCR and sequencing of the 16S rRNA gene, performed as previously described, from a whole blood sample identified these bacteria as *B. persica* (*1*).

According to current recommendations in France, the patient was given doxycycline, 200 mg/d, for 10 days. During the first 12 hours, he was monitored for a Jarish–Herxheimer reaction, which was not observed. By the end of therapy, inflammatory syndrome and lymphopenia had resolved.

Despite high-level sequence conservation, identification of *Borrelia* spp. by sequencing the 16S rRNA gene is reliable and useful for clinical practice (*1,4–6*). The sequence obtained from the patient reported here was identical (100% identity over 1,472 bp) to the *B. persica* HM161645 reference sequence available from GenBank and sampled from the Galilee region of Israel. It differed by 2 nt (99.86% identity) from *B. persica* U42297 and another unpublished sequence (*B. persica* 11/95), each from Iran. *B. persica* 11/95 was obtained from blood from a rodent collected in Iran and examined at the Institut Pasteur of Iran. We submitted the 2 *B. persica* sequences (1 from the patient reported here and 1 from the 11/95 sample) to GenBank (accession nos. HQ610930 and HQ610931, respectively). We confirmed identification of *B. persica* from the patient reported here by sequencing of both *fla*B and intergenic spacer domains (data not shown), as described (*7,8*).

Although recently reviewed, the epidemiology of TBRF in the area of the former Union of Soviet Socialist Republics has not been extensively described (*3*). In most of these countries, the infection is mainly attributed to *B. persica* and transmitted by *Ornithodoros tholozani* ticks, which live in caves, soil, wall crevices, houses, and cow sheds. Mammalian reservoirs, if any, are not known (*3*). However, other agents have been reported, such as *B. latyschewii* (transmitted *by O. tartakovskyi* ticks, which inhabit rodent burrows) in Kazakhstan, Uzbekistan, and Turkmenistan and *B. caucasica* (transmitted by *O. verrucosus* ticks) in the western shore of the Caspian Sea (Armenia, Azerbaijan, and Georgia) (*9,10*). Other soft ticks (*O. nereensis* and *O. alactagalis*) have been described in central Asia.

To our knowledge, TBRF cases caused by *B. persica* in Uzbekistan and Tajikistan have been rarely reported. The clinical illness of the patient reported here did not differ substantially from that of patients in Israel, Iran, or Jordan, where the infection is more frequently detected. The illness appears benign without chills, headache, vomiting, arthralgia, epistaxis, or hematuria. However, lymphopenia, which resolved rapidly, has not been described for other TBRF cases.

Our report highlights that TBRF is endemic to countries of the former Union of Soviet Socialist Republics. Physicians should consider this diagnosis for febrile patient returning from this area. Efforts to prevent tick bites should be emphasized. Accurate microbiological diagnosis comprises molecular detection or quantitative buffy coat analyses, each of which enhances sensitivity. However, in most disease-endemic countries, diagnosis is based only on examination of direct blood smears, which can lead to false-negative results and underestimation of the actual extent of this infection (*1*).

Acknowledgments

We thank Nuria Marti-Ras for the sequence of the *B. persica* sample from the Institut Pasteur of Iran (sequence 11/95) and François Rodhain and Guy Baranton for providing information about TBRF epidemiology in the region and reviewing the manuscript.

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DOI: 10.3201/eid1707.101894

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Toxoplasmosis and Horse Meat, France

To the Editor: *Toxoplasma gondii* parasites are obligate intracellular apicomplexans that can infect virtually all warm-blooded animals; felids are definitive hosts. The most common sources of human infection are ingestion of tissue cysts in undercooked meat or of food or water contaminated with oocysts shed by felids and transplacental transmission. Acquired toxoplasmosis in immunocompetent humans is frequently asymptomatic but is associated with cervical or occipital lymphadenopathy in ≈10% of patients. Severe or fatal outcomes for immunocompetent patients have been attributed to the virulence of specific *T. gondii* genotypes (*1*). We describe 3 cases of toxoplasmosis caused by atypical strains probably acquired by from ingestion of raw horse meat imported from Canada and Brazil.

Patient 1, a 74-year-old man, was hospitalized locally (Antibes-Juan Les Pins, southern France) in March 2009 for asthenia and persistent febrile bronchitis. His medical history included severe smoking-related chronic obstructive pulmonary disease and coronary artery disease. He received broad-spectrum antimicrobial drugs and methylprednisolone. On day 23 after admission, he was transferred to our teaching hospital in Nice because of clinical deterioration and persistent fever. Disseminated toxoplasmosis was diagnosed on the basis of serologic evidence of recent primary *T. gondii* parasite infection and quantitative PCR detection of high *Toxoplasma* DNA levels in peripheral blood. Despite specific antitoxoplasma therapy with sulfadiazine and pyrimethamine, he remained febrile, his respiratory function worsened, and he died on day 27.

Patient 2, a 24-year-old pregnant woman, was hospitalized in Draguignan, France, in December 2009 for full-term delivery. Three weeks earlier, routine serologic testing showed *T. gondii* parasite infection seroconversion. The newborn's and mother's ophthalmologic examinations were unremarkable. Congenital toxoplasmosis was diagnosed on the basis immunoglobulin M in the infant's serum, positive quantitative PCR of samples from the placenta, and strain isolation after inoculation of mice with a placental preparation. Sulfadiazine and pyrimethamine were started.

We performed a retrospective epidemiologic investigation of an unusual case of toxoplasmosis that occurred in March 1991. Patient 3, a 21-year-old pregnant woman living in the Nice area, was treated with spiramycine because routine serologic testing had shown *T. gondii* parasite infection seroconversion at 22 weeks' gestation. Amniocentesis showed *T. gondii* tachyzoites in amniotic fluid by microscopic examination. At 26 weeks' gestation, the woman underwent termination of pregnancy for ultrasonography-detected fetal severe abnormalities. Fetal necropsy showed numerous cerebral, cardiac, and hepatic abscesses with *T. gondii* tachyzoites. A few days after pregnancy termination, the woman experienced cervical lymphadenopathy, which lasted 3 years. She reported having eaten raw horse meat regularly during her pregnancy.

Genetic analyses with microsatellite markers of the *Toxoplasma* spp. strains isolated from the 3 patients found 3 different atypical genotypes. Atypical strains are common in South America but unusual in France, where >95% of reported strains collected from human and animal toxoplasmosis cases belonged to the type II clonal lineage (*2*,*3*). Hence, isolation of an atypical *Toxoplasma* genotype from a patient in France strongly suggests contamination by a non-European strain, either during residence abroad or after ingestion of imported meat.

Epidemiologic investigation of our case-patients that included questioning relatives, patients, and butchers found that eating raw horse meat imported from Canada (patient 1) or Brazil (patient 2) was the most likely source of the parasites. The geographic origin of the horse meat eaten by patient 3 is unknown.

Moreover, an atypical *T. gondii* strain was isolated after mouse inoculation with horse meat from the first patient's butcher. In all 3 cases, close relatives encouraged the patients to eat raw horse meat regularly because the practice is traditionally thought to reinforce health. Human toxoplasmosis cases associated with horse meat consumption are rarely reported but are probably underestimated (*2*). In the European Union, France and Italy account for more than two thirds of all horse meat eaten, predominantly raw, thereby increasing the likelihood of infection by various parasites, including *Trichinella* spp. and *Toxoplasma* spp. (*4*). Under natural conditions, serologic prevalence of *T. gondii* parasites in horses worldwide may range from 0% to 80% (*5*). Many factors could account for this variation, including the sensitivity and specificity of the serologic test, ages of animals, geographic area and hygienic condition of farm management (*5*). The only prevalence survey of horses slaughtered for food that we are aware of was conducted in Canada and the United States and found antibodies to *T. gondii* parasites in 124 (6.9%) of 1,788 serum samples (*6*).

T. gondii tissue cysts in meat are immediately killed by reaching an internal temperature of 67°C in all parts of meat during cooking (*7*). Deep freezing (≤–12°C for at least 3 days) of meat before cooking is recommended because it reduces the risk for infection by inactivating most tissue cysts (*7*). These precautions are often not applied to horse meat because these imported carcasses are usually shipped as "fresh meat" and frequently eaten raw. Eating raw horse meat imported from non-European countries may expose consumers to high inocula of highly virulent atypical *Toxoplasma* spp. strains, which may cause life-threatening primary infection (case-patient 1) or severe congenital toxoplasmosis with atypical outcome of acquired toxoplasmosis in the mother (case-patient 3). Risk assessment for toxoplasmosis from horses slaughtered for food and imported into the European Union, as was recently done in France for ovine meat, is urgently needed (*3*).

Acknowledgments

We thank Veronique Blanc and Lionel Lerousseau for their collaboration.

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DOI: 10.3201/eid1707.101642

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Israeli Spotted Fever, Tunisia

To the Editor: Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* was the first rickettsiosis described in Tunisia. *R. conorii* was thought to be the only species existing in this country. However, authors reported other rickettsioses either from spotted fever group or typhus group (*1*,*2*). In Sfax, a town in southern Tunisia, physicians noted patients with severe forms of MSF and suspected the presence of other species or a virulent *R. conorii* strain. We report 2 cases of Israeli spotted fever (ISF) from Sfax that were confirmed by detection of rickettsial DNA in skin biopsy specimens.

In September 2009, two previously healthy men, 45 and 46 years of age (patients 1 and 2, respectively), were hospitalized in the infectious disease department of Hedi Chaker University Hospital (Sfax, Tunisia). They came

from suburban areas 30 km apart. The men were admitted with histories of fever of a few days' duration and cutaneous maculopapular rash. Patient 1 had fever of 38°C, chills, headache, and arthromyalgia without hemodynamic abnormalities. Patient 2 was admitted with fever of 41°C, conjunctivitis, and cardiovascular collapse; he was treated in an intensive care unit for 1 day. No inoculation eschar was found on either patient. Biological findings for the 2 patients showed a leukocyte count within normal ranges, anemia, thrombopenia, high levels of C-reactive protein, and elevated liver enzymes. Both patients had contact with dogs, but neither patient reported a tick bite. The patient with more severe illness worked in the livestock importation industry; his illness developed 5 days after his return from a 2-week trip to Libya. The patients received 200 mg doxycycline per day for 10 days and improved rapidly.

Skin biopy specimens from the rash and whole blood samples were obtained from the 2 patients. PCRs targeting outer membrane protein (*omp*) *A* and *B* genes were done by using previously described primers (*3*). A negative control (sterile water and DNA from a sterile biopsy specimen) and a positive control (*R. montanensis* DNA) were included in each test. Amplicon sequencing confirmed *R. conorii* ISF strain DNA in the 2 skin samples and in the blood sample of patient 1. For both patients, the sequence homology to *R. conorii* ISF strain DNA was 99% for *ompB* gene (833 pb) and 100% for *ompA* (596 pb) (GenBank accession nos. AF123712.1 and AY197564.1, respectively). Serologic testing performed by a microimmunofluorescence assay yielded negative results for the first blood samples. A second blood sample was tested only for patient 2 and showed immunoglobulin M titers of 64 and immunoglobulin G titers of 128.

We demonstrated human infection caused by *R. conorii* ISF strain in Tunisia. This strain has been recently suggested for classification with 3 others as a subspecies within the species *R. conorii* on the basis of multilocus sequence typing (*4*). ISF was first described in Israel where it is endemic (*5*). The disease appears to be more widely spread in the Mediterranean countries than first believed because cases from Italy and Portugal have been reported (*6*,*7*). Recently, a patient from Switzerland with confirmed ISF was suspected to be infected in Libya (*8*). One patient in our study may also have been infected during his stay in Libya. Thus, geographic distribution of ISF seems to be extended to all Mediterranean countries and not limited to Israel, Italy, and Portugal. Its distribution areas probably overlap with those of MSF because the 2 infections share the same vector, the dog tick (*Rhipicephalus sanguineus*) (*9*). Although a history of tick bite could not be documented from the recorded anamnesis data, contact with dogs was noted in our cases. Furthermore, the 2 cases were diagnosed during the same month (September), corresponding to seasonal fluctuations generally observed for MSF in our region. Although 1 of our patients reported recent travel, the second patient affirmed he had not left his locality; thus, endemicity of ISF in our region in Tunisia is possible.

De Sousa et al. reported the differences between patients infected with *R. conorii* Malish and ISF strains (*10*). The characteristic eschar at the site of the tick bite was markedly less noted in ISF. The absence of this eschar has been also described in other studies (*9*,*10*). In our report, patients were treated with a delay of 10 and 6 days and neither patient died, but 1 patient did experience severe illness. Our observations suggest that the supposed ability of the ISF *Rickettsia* sp. to cause more severe illness is not ascribed to late diagnosis but may be due to more virulent strains, as suspected by De Sousa (*10*).

Finally, PCR applied to whole blood and tissue samples was more effective in diagnosing these cases earlier than serology because antibodies appear to have slow kinetics. Physicians should be alert to the possibility of ISF in febrile patients in our region, especially because fatal outcomes of this infection have been reported (*8*).

Acknowledgment

We thank Didier Raoult for providing antigens used in the serologic analysis and the *R. montanensis* DNA used as a positive control in PCR.

All work was financed by our research laboratory "MPH, Habib Bourguiba University Hospital."

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DOI: 10.3201/eid1707.101648

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Catabacter hongkongensis Bacteremia with Fatal Septic Shock

To the Editor: *Catabacter hongkongensis* is a newly described anaerobic agent that is likely an intrinsic component of normal gut flora; it was first reported by Lau et al. in 2007 (*1*). We report a fatal case of infection caused by *C. hongkongensis* that was identified by 16S rRNA sequence.

A man 52 years of age was admitted to an intensive care unit in France for septic shock. He was a retired service member, smoker, and alcohol drinker. He had a history of hypertension but no previously known gastrointestinal disease. He sought treatment for acute abdominal pain and diarrhea of several hours' duration. On admission, he had persistent abdominal pain with generalized abdominal distension, arterial hypotension, and hypoxemia but was not febrile. Two sets of anaerobic and aerobic blood cultures were performed at a 1-hour interval, and empiric treatment with amoxicillin/clavulanic acid and gentamicin was started. Biochemical screening showed severe metabolic acidosis, acute renal insufficiency, and systemic inflammatory response syndrome. An abdominal radiograph revealed massive pneumoperitonitis. Laparotomy showed multiple lesions and intestinal perforation at the ascending and first part of the transverse colon, with a large amount of purulent fluid in the peritoneal cavity. A complete colectomy was performed, with rectum closure and end ileostomy. Despite fluid resuscitation and catecholamine infusions, hemodynamic instability worsened rapidly and led to the patient's death.

Microbiological analysis of abdominal fluid revealed the presence of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus* spp., but no anaerobic agent. On day 3 postincubation, 1 of the 2 anaerobic blood cultures grew a motile gram-positive bacillus, which grew only in strictly anaerobic conditions. Phenotypic analysis showed catalase production but not indole positivity or nitrate reduction. Standard phenotypic tests were performed with the rapid ID 32A and api20A strips (bioMérieux, Marcy l'Etoile, France). The numeric profiles obtained were 0002000010 and 4030121, respectively. The bacteria produced acid from arabinose, glucose, mannose, and xylose, was negative for glycerol fermentation and leucine arylamidase and positive for rhamnose fermentation. Despite these results, phenotypic tests failed to identify the isolate. Antimicrobial drug susceptibility was determined by disk diffusion method and Etest for MICs. The isolate showed susceptibility to metronidazole (MIC <0.016 μg/mL), vancomycin, and colistin (MIC <0.016 μg/mL) and resistance to penicillin (MIC 2 μg/mL), gentamicin, netilmycin, kanamycin, amikacin, and cefotaxime (MIC >32 μg/mL) according to Eucast clinical breakpoints (www.eucast.org). No other bacteria were isolated in the blood cultures.

Genetic analysis was performed by 16S rRNA gene sequencing of a 1,265-bp fragment by using DG74 and RDRO80 primers (*2*). The nucleotide sequence obtained was compared with known sequences in GenBank by multiple sequence alignment using the ClustalW program (*3*). It was 100% identical to *C. hongkongensis* (GenBank accession no. AY574991).

The first 4 case-patients with *C. hongkongensis* infection were described by Lau et al. in 2007 (*1*). Two of these patients lived in Hong Kong and the 2 others in Canada. As in our case, only 1 patient died. Since there was a high degree of phenotypic and genetic difference with other anaerobic agents, the authors proposed a new genus and species and affiliation with a new family, *Catabacteriacae.* The 2 isolates from Canada differed from the 2 others by being negative for glycerol fermentation and positive for rhamnose fermentation and leucine arylamidase, similar to our case, except for leucine arylamidase, which in our case was negative. In the previously reported cases, *C. hongkongensis* was susceptible to metronidazole, vancomycin, and kanamycin; variably susceptible to penicillin (MICs 0.5–4.0 μg/mL); and resistant to colistin and cefotaxime (*1*).

Whether *C. hongkongensis* belongs to the intestinal flora, as do *Bifidobacterium*, *Eggerthella*, *Eubacterium*, and *Lactobacillus* spp., remains undetermined. Codony et al. recently investigated by real-time PCR the presence of *Catabacteriaceae* in 29 water samples in the vicinity of Barcelona, Spain. Four samples were positive, demonstrating presence of this organism in the European environment and its probable enteric origin (*4*).

Because our patient sought treatment with severe infection associated with isolation of other pathogenic bacteria, whether blood infection by *C. hongkongensis* may be responsible for such a fatal outcome is unknown. Nevertheless, we can exclude sample contamination by this anaerobic bacteria for the 2 following reasons. First, anaerobic contaminants are rare in blood cultures and generally involve *Propionibacterium acnes*. Furthermore, the rapid growth of the present isolate in blood cultures within 3 days suggested a relatively high bacterial load in the blood sample.

Our report confirms that *C. hongkongensis* can be found in blood culture associated with gastrointestinal disease and may reflect intestinal perforation. Identification may be difficult. Isolation of motile gram-positive anaerobic bacillus together with catalase positivity should lead to suspicion of *C. hongkongensis* in clinical laboratories. Full identification of this pathogen requires 16S sequencing. Environmental reports have demonstrated the presence of this organism in human wastewater in Europe, which suggests that it may be universally present as part of the normal human gastrointestinal flora.

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DOI: 10.3201/eid1707.101773

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Endemic Angiostrongyliasis, Rio de Janeiro, Brazil

To the Editor: The nematode *Angiostrongylus cantonensis* (rat lung worm), a zoonotic parasite that can accidentally infect humans and cause eosinophilic meningoencephalitis, has the Norway rat (*Rattus norvegicus*) as one of its most frequent definitive vertebrate hosts (*1*). Adult worms live in the pulmonary arteries of the definitive hosts, which excrete first-stage larvae in their feces. Intermediate hosts, such as snails and slugs, are infected by first-stage larvae, which reach the infective third stage after 2 molts. Third-stage larvae are then ingested by rats as they feed on the intermediate hosts, thus closing the life cycle. Humans become infected by eating raw or undercooked snails and slugs and through paratemic hosts and vegetables contaminated with infected snail mucus (*2*).

In Brazil, the first 3 documented cases of eosinophilic meningoencephalitis occurred in 2007 in 2 cities in the southeastern state of Espírito Santo (*3*). In 2009, a new case was reported in Pernambuco in the northeast region (*4*). Only intermediate hosts have been found naturally infected with rat lung worm in Brazil. Infected terrestrial and freshwater snails of the species *Achatina fulica*, *Sarasinula marginata*, *Subulina octona*, and *Bradybaena similaris* in Espírito Santo; *A. fulica* and *Pomacea lineata* in Pernambuco; and *A. fulica* in Rio de Janeiro and Santa Catarina have been reported (*3*,*5*,*6*). Thus, because of the recent cases of eosinophilic meningoencephalitis in Brazil and the occurrence of naturally infected *A. fulica* snails in Rio de Janeiro, we investigated the existence of potential natural reservoirs for the parasite in São Gonçalo.

São Gonçalo (22°48′26.7″S, 43°00′49.1″W) is a densely populated city (≈1 million inhabitants) with a tropical Atlantic climate (14°C–35°C) that is part of the metropolitan region of Rio de Janeiro. Two collections were made in March and June 2010. Forty live traps (20 Tomahawk [Tomahawk Live Trap Company, Tomahawk, WI, USA] and 20 Sherman [H.B. Sherman Traps Inc., Tallahassee, FL, USA] traps) were placed along two 30-m transects for 4 consecutive nights (Brazilian Institute of Environment and Renewable Natural Resources license no. 2227–1/2010) in an urban area where *A. fulica* snails had been collected in high numbers. Twenty-seven Norway rats (16 males) were captured. We collected 265 adult lung worms from the pulmonary arteries of the captured animals, fixed the worms in 70% ethanol, and taxonomically identified them as *A. cantonensis* on the basis of the large size of the spicules and the patterns of the bursal rays (*7*). Voucher specimens have been deposited in the Helminthological Collection of the Oswaldo Cruz Institute (no. 35712). Nineteen (74%) rats were infected; mean intensity and mean abundance were 13.52 ± 2.36 and 9.81 ± 1.96, respectively.

To confirm the morphologic identification of the *Angiostrongylus* specimens obtained, a DNA bar coding approach was used. DNA was extracted from 3 ethanol-preserved adult worms previously recovered from the pulmonary arteries of a naturally infected Norway rat, PCR-amplified, sequenced for a partial region of the COI gene (*8*), and subsequently compared with available GenBank *Angiostrongylus* spp. sequences. The three 360-bp COI sequences obtained (GenBank accession no. HQ440217) were Clustal-aligned (www.clustal.org) with homologous COI fragments of *A. cantonensis* (GenBank accession no. GQ398121), *A. vasorum* (GenBank accession nos. EU493162, EU493163, EU493166, EU493167), and *A. costaricensis* (GenBank accession no. GQ398122) and subjected to phylogenetic analysis. *Ancylostoma tubaeforme* (GenBank accession no. AJ407940) was used as the outgroup. Haplotypes for *A. vasorum* isolates from Brazil (*A. vasorum* 5421, 5641, and 5642) were reconstructed from published information (*9*) and included in the alignment. We used MEGA4 (www.megasoftware.net) to construct a neighbor-joining phylogenetic tree based on Kimura 2-parameter (K2-p) distances (Figure). The 3 *A. cantonensis* specimens from São Gonçalo, Rio de Janeiro, yielded a single haplotype, which formed a clade with the *A. cantonensis* haplotype from the People's Republic of China with low genetic distance (K2-p 0.038) and high bootstrap support (98), thus confirming the morphologic identification. Comparisons with the other 2 *Angiostrongylus* species yielded higher genetic distance values (K2-p 0.120, with *A. vasorum*, and 0.149, with *A. costaricensis*).

These results indicate that *A. cantonensis* lung worm infection is enzootic among the exotic Norway rat population in the region studied. The natural infection rate of 74% is the second highest reported among 14 severely *A. cantonensis* infection–endemic regions (*2*). These findings, together with the observation of dense populations of *A. fulica* snails in urban areas of the country (*10*), call attention to the risk for disease transmission to humans, given that Norway rats also are likely to be present in these areas.

Local residents should be informed about disease transmission and prevention, and physicians should consider *A. cantonensis* lung worm infection in the differential diagnosis when appropriate. Although public health authorities should consider implementation of surveillance and control strategies to reduce the populations of snail and rat hosts, a better understanding is needed of the epidemiologic significance of these findings, which can be attained through studies to identify human cases of eosinophilic meningitis in the region.

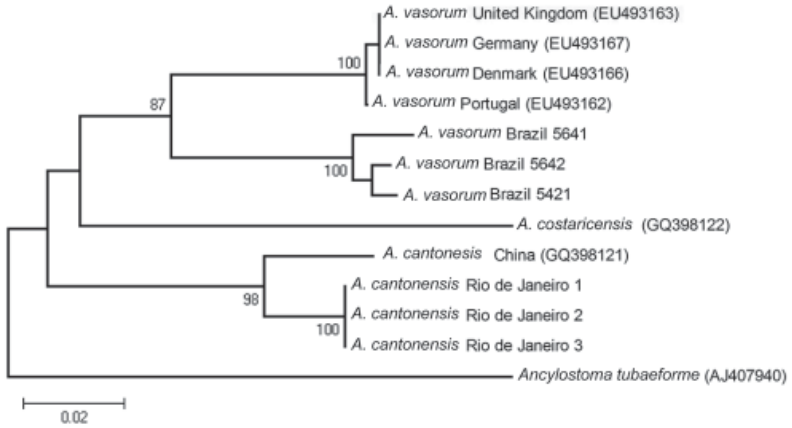

Figure. Neighbor-joining phylogenetic tree based on Kimura 2-parameter (K2-p) distances that includes all *Angiostrongylus* COI sequences in GenBank and the sequences obtained from 3 *Angiostrongylus* specimens recovered from the pulmonary arteries of a naturally infected Norway rat (*Rattus norvegicus*) from São Gonçalo, Rio de Janeiro, Brazil, 2010. The specimens yielded 1 haplotype, which clustered together with the *A. cantonensis* haplotype from the People's Republic of China with a low genetic distance (K2-p 0.038). Scale bar indicates 0.02 K2-p genetic distance.

Acknowledgments

We thank the anonymous reviewers whose comments and suggestions helped improve an earlier version of the manuscript.

This study was supported by the Brazilian Research Council.

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DOI: 10.3201/eid1707.101822

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Aircraft and Risk of Importing a New Vector of Visceral Leishmaniasis

To the Editor: Kala-azar, or visceral leishmaniasis, is a parasitic disease that leads to fever, anemia, and hepatosplenomegaly. Death is the usual outcome when infection is not treated. The majority of infections are caused by the protozoan *Leishmania donovani*, restricted to India and eastern Africa, but the most widespread are caused by *L. infantum*, found from People's Republic of China to the New World, where it infects humans, dogs, and wild canids. All Mediterranean countries are affected by *L. infantum*, where most patients are co-infected with HIV. Several species of sand flies transmit the disease (*1*).

During the 1980s, urban transmission of kala-azar became a major problem in Brazil. More than 3,000 cases are reported annually, and the disease has spread from northeastern Brazil westward to the Amazon region, as well as to the industrialized southeast. Several as yet unproven explanations for the urbanization of kala-azar in Brazil have been proposed (*2*), but whatever the reason, it is associated with proliferation of *Lutzomyia longipalpis* sand flies, which, in turn, are strongly associated with human environments. The vector can easily spread by entering buses or trains looking for food at night or for hiding places at dawn. Invasion of new areas by sand flies through transportation of ornamental plants has been observed (R. Brazil, pers. comm.), possibly by insect eggs or larvae being carried in organic matter.

Kala-azar has now reached the temperate Brazilian south and Argentina. This spread of the disease warns us of the danger of introduction in other temperate areas. Europe is particularly vulnerable because of the existing natural transmission of *L. infantum*. This risk is increased by recently created daily direct flights to Lisbon from Fortaleza, Natal, Brasília, and Belo Horizonte (Figure), Brazilian cities where epidemics of the disease have occurred. Lisbon is suitable to canine infection, and >10% of dogs may be infected (*3*). The climate is a barrier for the introduction of many vectors outside their normal range, such as *Anopheles gambiae* mosquitoes in temperate zones (*4*,*5*), but the threshold of change for *L. longipalpis* sand flies is minimal. The Mediterranean area is as dry as northeastern Brazil, where the disease is now highly endemic. Furthermore, the annual average temperature and

Figure. Commercial air transport routes between Lisbon, Portugal, and cities in Brazil that could make possible the accidental importation into Europe of *Lutzomyia longipalpis* sand flies, a vector of visceral leishmaniasis.

cooler months in Lisbon (at 38°44′N) are only 3–4°C lower than those of São Borja, Rio Grande do Sul state, the southernmost city where *L. longipalpis* transmits kala-azar, and even warmer than Chajarí, Argentina (at 30°46′S, ≈500 km from Buenos Aires and only 8° farther from a pole than Lisbon), at the highest southern latitude where this vector is found (*6*).

Human kala-azar is less common in Europe, possibly because sand flies there are less anthropophilic. If aircraft introduce anthropophilic *L. longipalpis* sand flies in Lisbon, the situation could change dramatically, and kala-azar might become a major urban disease in Europe. The International Health Regulations recommends disinfection of aircraft by preflight and blocks-away spraying with pyrethroids (*7*). However, significantly reduced susceptibility to pyrethroids in wild populations of *L. longipalpis* sand flies was recently described in Brazil (*8*). Centuries after its introduction to South America by Iberian colonizers, kala-azar may make its way back to Europe with a more forceful vector—this time by air, not by sea. To reduce this risk, much information needs to be known about the biology of *L. longipalpis* sand flies, such as minimum temperature tolerance, mechanisms of urban spread, presence in aircraft, and role in inducing more severe disease.

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DOI: 10.3201/eid1707.102002

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Enzootic Angiostrongyliasis, Guangdong, China, 2008–2009

To the Editor: The nematode *Angiostrongylus cantonensis* was discovered in pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China, by Chen in 1935 (*1*). This parasite has a complex life cycle (*2*) and causes cerebral angiostrongyliasis after ingestion of infective larvae found in freshwater and terrestrial snails and slugs, paratenic hosts (such as freshwater fish, shrimp, frogs, and crabs), and contaminated vegetables (*3*).

During 2000–2006, a total of 7 outbreaks of angiostrongyliasis were reported in the People's Republic of China, including an outbreak in Zhaoqing, Guangdong Province (*4–6*). We conducted a survey of *A. cantonensis* nematodes in mollusks and rodents in Qingyuan, Guangong Province, during August 2008–October 2009.

Qingyuan is located in northern Guangdong Province (23°31′–25°12′N, 111°55′–113°55′E). It is the largest city in the province. Qingyuan borders Zhaoqing on the west and Guangzhou on the south. Its climate is subtropical monsoon, and it has an average annual temperature of 20.7°C. The city has an area of 19,152.89 km^2 and a population of 3.87 million. Nematode hosts were obtained in 3 counties in Qingyuan: Qingxin, Fogang, and Lianzhou.

During August 2008–October 2009, we captured 288 rats of 7 species (257 *Rattus norvegicus*, 13 *R. flavipectus*, 7 *R. losea*; 6 *R. rattus*, 3 *Bandicota indica*, 1 *R. rattus alexandrinus*, and 1 *Mus musculus*). Rats were examined for adult *A. cantonensis* nematodes in pulmonary arteries and right heart cavities.

Among the 288 rats examined, 27 (9.4%) from 3 species were infected with *A. cantonensis* adults in their cardiopulmonary systems (Table). Infected rodents were found in all 3 counties. The 27 infected rats were 25 *R. norvegicus*, 1 *R. losea*, and 1 *M. musculus*. *R. norvegicus* rats were most frequently captured in the 3 counties, and this rodent had the highest prevalence of infection. Infected *B. indica* rats in Lianzhou and *M. musculus* rats in Qingxin were also found, but the total numbers of infected animals and the prevalences are lower than that for *R. norvegicus* rats. On the basis of these findings, we conclude that *R. norvegicus* rats are the major definitive host for *A. cantonensis* nematodes in Qingyuan.

Specimens from 510 snails (144 *Pomacea canaliculata*, 306 *Achatina fulica*, and 60 *Bradybaena despecta*) were digested with pepsin for isolation of *A. cantonensis* larvae (*7*). Metastrongylid larvae were found in 21 (4.1%) of 510 examined snails. Prevalence rates of *A. cantonensis* in *P. canaliculata*, *A. fulica*, *and B. despecta* were 8.3%, 2.0% and 5.0%, respectively. Differences between the 3 prevalence rates were significant (χ^2 9.604, $p<0.05$). Prevalence rates in the 3 counties are shown in the Table.

All 3 species of infected snails were found in Qingxin and Fogan Counties. *P. canaliculata* and *B. despecta* snails were found infected in Qingxin County. However, only *A. fulica* snails were found infected in Fogang. These findings are similar to those of studies conducted in Guangdong Province (*8–10*).

Distributions of snail species among the 3 sites differed. Although all 3 species were found in Qingxin and Fogang Counties, only *A. fulica* snails were found in Lianzhou County. Lower temperatures in this county may contribute to this uneven distribution. Our failure to detect infected snail hosts in Lianzhou County was unexpected, and further surveys are needed to identify parasite hosts in this area. Our findings suggest that the 3 species may play a major role as intermediate hosts for *A. cantonensis* nematodes in human infections.

Qingyuan is a natural focus for *A. cantonensis* nematodes. Residents in the study area frequently eat raw or undercooked snails and slugs, unaware that these animals may contain infective larvae of *A. cantonensis* that can cause eosinophilic meningitis. Therefore, to protect local residents from parasite infections, inhabitants of this region must be given relevant information about *A. cantonensis* nematodes. Control measures to control spread of this parasite must also be implemented.

Table. Prevalence of infection with *Angiostrongylus cantonensis* in 3 snail species and rodents in 3 counties in Qingyuan, Guangdong Province, China, 2008–2009

	Host species, no. positive/no. tested (%)			
County	*Pomacea canaliculata*	*Achatina fulica*	*Bradybaena despecta*	Rodents
Qingxin	12/135 (8.9)	0/32 (0)	3/41 (7.3)	22/137 (16.1)
Fogang	0/9 (0)	6/152 (3.9)	0/19 (0)	2/117 (1.7)
Lianzhou	Not found	0/122 (0)	Not found	3/34 (8.8)
Total	12/144 (8.3)	6/306 (2.0)	3/60 (5.0)	27/288 (9.4)

Acknowledgment

We thank Wen Yang for assistance in improving the manuscript.

This study was supported by grants from National Basic Research Program of China (973 Program 2010CB530004 entitled Mechanisms of Immunoregulation in *Angiostrongylus cantonensis*) to Z.-D.W., the National Natural Science Foundation of China, and the Guangdong Provincial People's Government of the Joint Natural Science Fund (U0632003) to X.-M.Z.

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DOI: 10.3201/eid1707.100714

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Malaria, Oromia Regional State, Ethiopia, 2001–2006

To the Editor: In Ethiopia, malaria is unstable and commonly occurs as intraannual and interannual epidemics. Transmission is associated with altitude, temperature, and rainfall, generally peaking twice a year, after the 2 rainy seasons (March–May and July–September) (*1*). Cases are caused by *Plasmodium falciparum* and *P. vivax*. *Anopheles arabiensis* mosquitoes are the main vector for both species. Although malaria is the most common communicable disease in Ethiopia (*2*), few longitudinal case data has been published (*3*).

We report a retrospective analysis of outpatient data for July 2001–June 2006 obtained from all secondary and tertiary government-run health facilities (152 health centers and 25 hospitals) in Oromia Regional State. Oromia has 17 administrative zones and 297 districts. Data were reported monthly on paper forms by health facility staff at district level to the Oromia Regional Health Bureau Zonal Health Offices, which aggregated zonal data before forwarding them to the Oromia Regional Health Bureau Malaria Control Department.

Data obtained included number of outpatient cases (i.e., patients attending the health facility grouped by age <5 years and age ≥5 years); number of clinical malaria cases (i.e., patients with fever grouped by age and sex); number of clinical cases confirmed by microscopy; and number of cases caused by *P. falciparum* and *P. vivax*. If no outpatient data were reported, the case number was changed from zero to missing. The data were entered into Microsoft Excel (Microsoft, Redmond, WA, USA) and analyzed by using Stata version 9.0 (StataCorp LP, College Station, TX, USA).

During 2001–2006, a total of 8,786,088 outpatient consultations were reported. A total of 905,467 and 562,996 clinical and confirmed malaria cases, respectively, were reported. Patients were predominantly seen at health centers rather than at hospitals, with 80.2% clinical and 72.2% confirmed malaria cases seen at health centers. Clinical malaria accounted for 10.3% of outpatient consultations in all facilities. However, this percentage varied between years (6.1%–16.0%) and zones (1.3%–21.9%) (online Technical Appendix Figure 1, www.cdc.gov/EID/content/17/7/1336-Techapp.pdf).

Of clinical malaria cases, 16.5% were in children <5 years of age (range between years [RBY] 14.0%–18.3%, range between zones [RBZ] 10.9%–61.0%) and 54.3% were in male patients (RBY 52.2%–55.6%, RBZ 50.1%–66.8%). Of clinical malaria cases, 49.2% were confirmed by microscopy (RBY 37.1%–58.0%, RBZ 15.3%–98.4%), and 58.5% (RBY 46.4%–63.4%, RBZ 12.1%–82.4%), and 41.2% (RBY 36.3%–53.4%, RBZ 17.6%–87.9%) of confirmed cases were caused by *P. falciparum* and

P. vivax, respectively. Of confirmed cases, 0.4% were caused by mixed *Plasmodium* infections (RBY 0.2%–0.5%, RBZ 0.0%–1.1%). The average incidence of clinical malaria per 100,000 population per month ranged from 14 in February 2002 to 122 in November 2003, and there was considerable variation between months, years, and administrative zones (online Technical Appendix Figure 2).

We found that up to 29.0% of outpatient visits to health facilities in certain administrative zones during high transmission years were for malaria. The incidence of malaria is likely to be underestimated because only ≈30% of the population accessed health facilities at that time (*4*). There appeared to be only 1 annual peak of transmission in September–January (online Technical Appendix Figure 1). Clinical and confirmed disease varied between zones; 5 of the 15 zones in Oromia (East Hararge, East Shoa, East Wellega, Jimma, West Hararge) reported >75% of the clinical cases seen at health facilities during 2001–2006. Malaria incidence varied between years: clinical and confirmed cases increased in 2003, the last epidemic year recorded in Oromia (*5*), before decreasing to 2001 levels in 2004 (online Technical Appendix Figure 1).

The *P. falciparum* to *P. vixax* ratio changed geographically and temporally (online Technical Appendix Figure 1), and increases in the proportion of *P. falciparum* cases coincided with the peak malaria transmission season. In the epidemic year of 2003, the proportion of *P. falciparum* cases was larger than in other years, and children <5 years of age were disproportionately affected (online Technical Appendix Figure 1). Contrary to previous reports (*6*), our data did not indicate a change in the *P. falciparum* to *P. vivax* ratio after artemether/lumefantrine was introduced in 2005.

Health facility data can have many caveats (*7*), including concerns about data representativeness (e.g., if only a small number of facilities are assessed); data validity, particularly if, as was the case during that time, only limited diagnostic quality assurance was available (*8*); and analytical approaches used. Our analysis comprised all Oromia secondary and tertiary facilities; only 3.4% of health centers and 13.0% of hospitals surveyed had no data, suggesting that given the extensive data reported, these missing data would have only marginally affected the temporal and spatial trends observed.

Our data complement those of recent cross-sectional surveys (*9*) and provide a useful baseline to assess scale-up of malaria prevention and control efforts. Unlike cross-sectional and small-scale facility surveys (*6*), our comprehensive longitudinal monthly data monitored disease trends spatially and temporally, showing that malaria still represented a major health services problem until 2006.

Acknowledgments

We thank the Oromia Regional Health Bureau district and zonal staff for providing logistic support and collecting data, and Jimee Hwang for comments on the manuscript.

The Oromia Regional Health Bureau supported meetings to collect district-level data from health offices and facilities.

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DOI: 10.3201/eid1707.100942

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Foodborne Illness Acquired in the United States

To the Editor: The updated estimates of foodborne illness in the United States reported by Scallan et al. probably overestimate the occurrence of illness caused by unspecified agents because they did not account for the apparent sensitivity of the population survey to the occurrence of norovirus (*1*,*2*). The number of illnesses attributed to unspecified agents was derived from the simultaneous processes of extrapolation and subtraction: extrapolation from the population survey to create a base of diarrheal illnesses and subtraction of known agents from this base. Scallan et al. averaged illness rates from 3 successive population surveys to come up with a rate of 0.6 episodes of acute gastroenteritis per person per year. However, the individual rates were 0.49 (2000–2001), 0.54 (2002–2003), and 0.73 (2006–2007). The 2006–2007 survey was conducted at the time of widespread norovirus activity. The estimated rate of population illness was strongly correlated with the number of confirmed and suspected norovirus outbreaks reported to the Centers for Disease Control and Prevention Foodborne Disease Outbreak Surveillance System during each of the survey periods (300, 371, and 491, respectively; R^2 0.97, $p<0.0001$). No other known agents were correlated with the population survey rates, and the total numbers of outbreaks were inversely correlated with the population survey data.

The strength of the correlation between norovirus outbreaks and survey results suggests that the population survey is sensitive to norovirus activity and that norovirus may account for much of what is considered to be unspecified. The fact that the highest observed population rate was ≈50% greater than the lowest rate suggests that annual variation in norovirus activity may account for a considerable proportion of what otherwise seems to be unspecified. More thorough and timely investigation and reporting of outbreaks could facilitate the development of models to evaluate the number of illnesses and update them annually.

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DOI: 10.3201/eid1707.110019

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To the Editor: The report by Scallan et al. provides a valuable update regarding estimated annual illnesses, hospitalizations, and deaths caused by recognized foodborne pathogens, most of which cause diarrheal disease, in the United States (*1*). However, absent from this study, and from most previous reviews of foodborne illness, was attention to possible extraintestinal disease, especially antimicrobial drug–resistant infections caused by food-source *Escherichia coli* and associated resistance elements.

A growing body of molecular and epidemiologic evidence suggests that a substantial fraction of extraintestinal *E. coli* infections in humans, particularly those involving antimicrobial drug–resistant strains, might be caused by *E. coli* from food animals (*2*). Extraintestinal pathogenic and antimicrobial drug–resistant *E. coli* commonly contaminate retail meat products (*3*,*4*); rates of contamination and resistance associated with "no antibiotics" production methods, labeling, and markets are lower (*4*). In a study of women with acute urinary tract infection, frequent consumption of chicken and pork was associated with isolation of antimicrobial drug–resistant *E. coli* from urine (*5*).

Extraintestinal *E. coli* infections, which include urinary tract infections and sepsis, are more common and result in more hospitalizations and deaths than do infections caused by the classic foodborne pathogens. For example, each year in the United States, an estimated 40,000 deaths are associated with sepsis caused by extraintestinal *E. coli* infection (*6*); only <1,400 deaths are caused by all major classic foodborne pathogens combined (*1*). Therefore, if even a modest fraction (e.g., 5%–10%) of all extraintestinal *E. coli* infections in humans are of foodborne origin—which seems highly plausible, considering the molecular evidence (*2*)—the extent of associated disease may equal or exceed that attributable to the classic foodborne pathogens as estimated by Scallan et al. Greater recognition of this possibility by the public health system is needed so that appropriate attention can be devoted to this neglected, invisible foodborne disease threat.

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DOI: 10.3201/eid1707.110256

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In Response: We welcome the suggestions by Hedberg (*1*) and Johnson (*2*) on how future estimates of foodborne illness in the United States could be improved (*3*,*4*). We agree with Johnson that our estimates of foodborne illness probably reflect underrecognition of the extent of extraintestinal disease (*2*). Our estimates of foodborne illness caused by major pathogens included 7 pathogens that cause conditions other than gastroenteritis. However, our estimates of foodborne illness caused by unspecified agents relied on reports of acute gastroenteritis. We did not include illness caused by known foodborne agents that do not typically cause gastroenteritis, such as ciguatoxins and some mushroom toxins, for which insufficient data were available to estimate agent-specific episodes of illness (*4*). We agree that urinary tract infections with *Escherichia coli*, most of which come from the patient's own gut flora and for which the original source may be food, may fall into this category and that the number of such illnesses is large (*5*). Moreover, there are probably unknown or unrecognized agents in the food supply that cause illness other than gastroenteritis. We recognize the need to think of ways to include more of these agents—known and unspecified—in future estimates.

Hedberg noted that rates of acute gastroenteritis in the 3 FoodNet population surveys correlate with the number of reported foodborne norovirus outbreaks and suggested that we may have consequently underestimated illnesses caused by norovirus and overestimated illnesses caused by unspecified agents (*1*). Although such an association between FoodNet survey findings and reported norovirus outbreaks is possible, it should be treated with caution. The annual number of foodborne norovirus outbreaks reported during our study was probably influenced by improvements in diagnosis and surveillance. The steady increase in suspected and confirmed foodborne norovirus outbreaks during 1998–2003 was accompanied by a decrease in foodborne outbreaks of unknown etiology, suggesting that the higher number of norovirus outbreaks reported during 2006–2007 resulted, at least in part, from improved diagnosis. In addition, nonfoodborne outbreaks caused by a new norovirus strain increased during 2002–2003 and were not reflected in the population survey by an increased rate of gastroenteritis (*6*). The surveys varied in how questions were worded and ordered, especially the 2006–2007 survey compared with earlier surveys, and higher rates of acute gastroenteritis might be related to these variations. Moreover, the 2006–2007 increase in gastroenteritis reported by the surveys was driven by increases in diarrhea rather than vomiting (the latter is more suggestive of norovirus illness). The limited number of comparison data points—only 3—and the fact that the data were from different populations (all states vs. 10 FoodNet sites) during slightly different time periods (FoodNet surveys were conducted over 12 months but not in 1 calendar year) also warrant conservative assessment of the association between annual rates of acute gastroenteritis and number of foodborne norovirus outbreaks. Because of uncertainties in both measures, we preferred a rank-based method; the Spearman ρ gives an exact p value of 0.33.

To estimate average annual number of illnesses, we used data from several years (2000–2008) and probability distributions. The fraction of acute gastroenteritis estimated to be attributable to norovirus was wide (6%–26%), so it probably encompassed year-to-year variations in incidence. Not only is norovirus estimated to be the most common known cause of foodborne illness, but it is the illness for which we have the least surveillance information. Better data for norovirus are needed. Studies to examine the association between norovirus activity and reported rates of acute gastroenteritis of unknown cause could be useful. Although the most recent FoodNet survey of gastroenteritis was conducted in 2006, more surveys await funding.

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DOI: 10.3201/eid1707.110572

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Comment on Zoonoses in the Bedroom

To the Editor: In response to Chomel and Sun (*1*), we would like to correct potentially misleading representations of risk factors for parasitic diseases. The authors correctly described risk for Chagas disease from exposure to infected insect vectors but included Chagas disease in the table, "Zoonoses acquired by close contact with pet, 1974–2010." The bloodborne protozoan that causes Chagas disease is transmitted not by contact with an infected mammal but by contact with a vector insect that has bitten an infected mammal (*2*).

For some parasitic zoonoses, contact with pets may not be a major source of infection. Molecular studies indicate that risk for human infection with *Giardia* and *Cryptosporidium* spp. from dogs and cats may be lower than previously believed. Infections with these parasites are usually with species-specific genotypes. Human infections with assemblages C, D (dog specific), and F (cat specific) of *G. duodenalis* have not been confirmed. Infections with assemblages A or B have been reported for humans and other animal species, including dogs and cats, but no direct transmission has been documented (*3*,*4*). Most human cryptosporidial infections are caused by *C. hominis* and *C. parvum* (*5*); a smaller percentage are caused by *C. canis* and *C. felis.*

Human infection with *Toxocara canis* or *T. cati* occurs when embryonated eggs are ingested; however, embryonation requires 2–4 weeks in the environment, suggesting that the risk from eggs in pet fur may be less than risk from exposure to eggs in contaminated soil. Other more serious zoonotic parasitic disease risks from contact with pet feces, including toxoplasmosis, are mentioned only briefly, if at all.

Physicians need information that accurately communicates zoonotic parasitic disease risks to their patients. However, inaccurate or overstated risk communication can also lead to unnecessary prevention efforts and misdirected concerns about dogs and cats as sources of disease.

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DOI: 10.3201/eid1707.110317

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In Response: We agree with Montgomery et al. (*1*) that Chagas disease is not directly transmitted by dogs to humans. However, we emphasize that Chagas disease is in the southern United States even if risk for infection is extremely low. A study in Mexico found direct correlation of seropositivity between humans and dogs, suggesting that testing dogs may help identify prevalence of *Trypanosoma cruzi* infection among humans. They stated, "Dogs may be domestic reservoir hosts and help maintain human transmission of *T. cruzi*" (*2*).

For toxocariasis, indeed only embryonated eggs are infectious. In a study in the Netherlands (*3*), ≈25% of *Toxocara* eggs found on fur were fertilized, but none were viable after 6 weeks; presence of embryonated eggs on dog fur is uncommon but can occur.

We did not mention all zoonoses that could be transmitted in a bedroom, such as toxoplasmosis or ringworm, because we could not identify publications specifically documenting contamination in that environment. We can, however, cite examples of other infections, such as *Cheyletiella blakei* dermatitis in a woman who shared her bed with a recently acquired cat (*4*). We also reiterate the potential risk for human infection by the plague bacillus (*Yersinia pestis*) as a result of bed sharing, as illustrated by the case reported from Oregon in 2010 (*5*).

Although the risk of contracting a zoonosis in the bedroom is low, it remains possible. Bed sharing with pets should be avoided, especially for those who are immunocompromised, young, or elderly.

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DOI: 10.3201/eid1707.110590

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References were misnumbered in the article Complete Sequence and Molecular Epidemiology of IncK Epidemic Plasmid Encoding $bla_{CTX\text{-}M\text{-}14}$ (J.L. Cottell, et al.). The article has been corrected online (http://www.cdc.gov/eid/content/17/4/645.htm).

etymologia

Melioidosis

[me″le-oi-do′sis]

From the Greek *melis*, distemper of asses, *oeidēs*, resemblance, and *osis*, a suffix indicating an abnormal condition or disease. Alfred Whitmore, a British pathologist serving in Burma, and his assistant C. S. Krishnaswami first described melioidosis in 1912. The infection became known as Whitmore's disease. In 1925, Ambrose T. Stanton and William Fletcher, the researchers who identified *Burkholderia pseudomallei* as the infection's causative agent, renamed the infection melioidosis because of its clinical resemblance to glanders.

Source: Dorland's Illustrated Medical Dictionary. 31st edition. Philadelphia: Saunders, 2007; Stanton AT, Fletcher W. Melioidosis, a disease of rodents communicable to man. Lancet. 1925;205:10–3. doi:10.1016/S0140-6736(01)04724-9

Salum Kambi (b. 1970). *The Village Hut* (2008) Acrylic on canvas (60.32 cm × 60.32 cm). Courtesy of U*Space Gallery (www.uspacegallery.com), Atlanta, Georgia, USA

The Tortoise and the Hut

Polyxeni Potter

"The men of old were born like the wild beasts. In woods, caves, and groves, they lived on food gathered in the fields," wrote Marcus Vitruvius Pollio more than 2,000 years ago in his well-known account of classical traditions in architecture. One day, a dense group of trees, agitated by winds and storms, caught fire. Humans nearby ran from the flames but then, attracted to the warmth, began to draw near. They tried to keep the flames going and brought others to see them. As they shared the discovery, they gestured, made sounds with their voices, and started to articulate words. Society and language had begun.

"As they kept coming together in greater numbers into one place, they began… to construct shelters" dug in on the sides of mountains, roofed with boughs, or made of twigs and mud like bird nests. They observed each other's efforts and made improvements. "At first they wove their walls with upright forked props and drove twigs between them…. When in winter-time the huts could not withstand the rains, they made their roofs sloping and projected and, smeared with clay, the ridged roofs drew off the rain water."

Such were the origins of architecture, rooted in human needs and natural surroundings: a pitched roof made of living materials shaped to more or less resemble a turtle. And apart from its obvious economy and efficiency, the dwelling has endured as a symbol of ecologic equilibrium, made to conform to nature and fit in with the landscape. From the construction of huts, humans progressed to other arts and sciences. With time, harmonious alignment of dwelling with nature and form with function has not always kept pace. Yet, spatial relationships, the way forms interact with those who use them and their surroundings, have remained a main consideration not only in architecture but all the arts and no less so in painting. This consideration as it pertains to color is very prominent in the work of East African painter Salum Kambi, whose work *The Village Hut* graces this month's issue.

Kambi was born in Dar es Salaam, Tanzania, a culturally diverse nation with more than 120 ethnic groups and rich artistic traditions going back to prehistoric times. A talented youth, he sought opportunities everywhere. "I entered a competition related to research on chimpanzees in the Gombe Forest in Kigoma and was one of five artists selected as the winners and had the chance to meet former President Mwalimu Julius Nyerere." Kambi apprenticed with artist Mohamed Raza and his son, "They taught me a lot about art and painting." He worked with cartoonist Godfrey Mwampemba known as Gado. "Having no formal art training, I acquired my skills with the help of these fellow Tanzanian artists and while attending workshops in Dar es Salaam." For a time, he practiced his trade in Kampala, Uganda, but returned to Tanzania where he had several exhibits. He has also exhibited in the Netherlands, Germany, Finland, Sweden, Italy, Denmark, and the United States.

"For me, art is all about color. I use color to depict feelings… of people, landscapes, animals." Like the anonymous ancestors of the Sandawe people in central Tanzania, who painted or engraved images on rocks, Kambi depends entirely on color. They used predominantly a single primary color, relying exclusively on red, black, or white values. He uses oil and acrylic paints applied with a knife, a brush, or both to create striking abstractions that exude feeling. In more modern terms, his work recalls the fauvists, the French avant-garde movement of the early 20th century, and their spontaneous expressions in potent color used directly from the tube.

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DOI: 10.3201/eid1707.AC1707

"Fauve art isn't everything, but it is the foundation of everything," Henri Matisse believed. "When I put a green, it is not grass. When I put a blue, it is not the sky." Even if the subject is traditional—a human body, a piece of fruit, a mountain—fauve colors make it look outworldly and unfinished. Form does not determine color. Color is there to create a sensation, so it can be nonrepresentational and unnatural. Choice of color is not right or wrong but the subjective vision of the artist.

The Village Hut approaches a primeval subject, shelter, along these unconventional lines. The artist's palette transforms the ordinary with unexpected hues. He is not interested in likeness so much as intensity or the sky would not be red, the trees not black, the thatched roof not icy white. Constructed of cubes and strokes of color and framed by auxiliary structures, the compound rests comfortably against the brilliant backdrop and the slick, luminescent pathway. This dwelling seems inhabited, vibrant. This hut is a place.

More than 80% of the population of Tanzania is rural. Kambi's emblematic depiction of standard quarters in the countryside captures not just the dwelling itself with its tortoiselike functionality and simplicity but also its explosive milieu, rich with organic elements of the tropics capable of engulfing the makeshift structure at will. And while for architectural purposes the tortoise analogy works, in practice, the hut has none of the tortoise's inherently sealed structure isolating the animal inside the shell. Drafty and unstable, huts contain other creatures inside the building materials, which are generally infested with disease carrying insects. Defying the very essence of shelter as an exclusionary agent, rodents and arthropod vectors follow their hosts into the hut, becoming domestic and even changing hosts in the interest of their own survival.

While much of the world has moved from huts to homes with foundations, solid roofs, and walls made with bricks or stones, many still share the tortoise's compact quarters, albeit without the expert insulation. The more than 1.4 billion people who live in extreme poverty, unable to upgrade their habitations, remain vulnerable to the ecologic realities of tropical and subtropical diseases, some of them woefully neglected—dengue, visceral leishmaniasis, lymphatic filariasis. Also Chagas disease, whose agent *Trypanosoma cruzi* is transmitted by triatomine bugs living in the mud walls of far too many homes in Latin America—not to mention the ever-present scourge of malaria with its ubiquitous and very adaptable vector mosquitoes.

In the southwestern United States, rural environment and housing that did not exclude rodents have been associated with the spread of a novel (now 18 years old) hantavirus. In Argentina, with increased urbanization and encroachment on the subtropical ecosystems of the Paraná Delta, recognized cases of emerging *Rickettsia parkeri* rickettsiosis are likely to escalate. In the Caribbean region of Colombia, as rural populations increase, the potential for arenaviral diseases, whose agents are mainly hosted by rodents, could become a public health concern. In Mongolia, where plague is endemic, the most common source of human infection is contact with and consumption of marmots. In Africa, plague caused by *Yersinia pestis* is transmitted when infected fleas move from feral rodents species to those living in homes. Tick-borne relapsing fever, an infection endemic to many parts of the world, especially Africa and the Mediterranean basin, is mainly attributed to *Borrelia persica* and transmitted by *Ornithodoros tholozani* ticks. These vectors live in caves, soil, and wall crevices, as well as in homes and cow sheds.

The collective and communal nature of the hut's origins, the coming together of humans to build shelters, may eventually bring about an improved version of this abode. Not only in the interest of less restrictive and more functional dwellings for all but also as part of public health policy. Because the hut is intrinsically connected with the environment (as early builders knew and Kambi so effectively has shown in his painting), persistent and emerging infections transcend its porous walls and reach across the world. Therefore, public health efforts may one day turn the dilapidated and inadequate shelter into a tighter model, with screens, moats, and better insulation, more akin to the tortoise of the initial plan.

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Article Title

Neurognathostomiasis, a Neglected Parasitosis of the Central Nervous System

CME Questions

1. A traveler returning from Thailand is suspected of having neurognathostomiasis. Which of the following is least likely to be a food source for the infection?

A. Undercooked pork
B. Undercooked chicken
C. Raw fish
D. Undercooked frog

2. In the patient from question 1, which of the following is likely to be the most common presentation?

A. Intracerebral hemorrhage with headache and hemiparesis
B. Myelopathy with radicular pain and ascending paresis
C. Meningoencephalitis with photophobia and seizures
D. Subarachnoid hemorrhage with loss of consciousness

3. Which of the following is considered the most accurate test for the diagnosis of neurognathostomiasis?

A. Cerebrospinal fluid microscopy
B. Brain biopsy
C. Western blot serology
D. Spinal fluid culture

4. Which of the following treatments are believed to be most efficacious based on clinical trials?

A. Albendazole alone for 4 weeks
B. Albendazole with prednisolone
C. Ivermectin with albendazole
D. None of the above

5. Which of the following best describes the most recent reports (after 2000) of rates of poor outcomes associated with gnathostomiasis infection?

A. Over 50% mortality
B. Mortality or severe disability rate of 40%
C. Poor outcome in 30%
D. Poor outcome in 20%

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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Article Title

Hantavirus Pulmonary Syndrome, United States, 1993–2009

CME Questions

1. You are seeing a 50-year-old woman with a 2-day history of headache, myalgia, and fever to 39.5°C. While your differential diagnosis remains broad for this patient, what should you consider regarding the epidemiology of infection with hantavirus?

A. The majority of hantavirus pulmonary syndrome (HPS) cases in the United States are caused by the Sin Nombre virus
B. Hantavirus is primarily transmitted via droplet secretions from human to human
C. The incubation period of HPS is usually less than 48 hours
D. Human fecal-oral transmission is the principal source of hantavirus

2. The prevalence of HPS is highest and most variable over time in which region of the United States?

A. Eastern region
B. Southern region
C. Midwest region
D. Southwest region

3. The patient from Question #1 develops HPS. What does the current study suggest regarding her prognosis?

A. The case-fatality rate was 35%
B. Older age predicted a higher fatality rate
C. Patients in the Southwest were most likely to die from HPS
D. The mean time from symptom onset to death was 3.5 weeks

4. Based on the results of the current study, which of the following laboratory findings is associated with the most significant risk for mortality due to HPS?

A. Any thrombocytopenia
B. Reduced hematocrit
C. Elevated serum creatinine
D. Reduced serum albumin

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

EMERGING INFECTIOUS DISEASES

Upcoming Issue

Control and Prevention of Viral Gastroenteritis

Dengue Virus Infection in Africa

Early Warning System for West Nile Virus Risk Areas, California

Novel Surveillance Network for Norovirus Gastroenteritis Outbreaks

Spread of Measles Virus D4-Hamburg, Europe

Novel Human Reovirus Isolated from Children with Acute Necrotizing Encephalopathy

Risk Factors for Pandemic (H1N1) 2009 Seroconversion, Singapore

Influenza-like Illness and Health Care Worker Absenteeism during Pandemic (H1N1) 2009 Outbreak

Deaths associated with Adenovirus-14p1 Infections, Europe

Antibody against Arenaviruses in Persons with Acute Central Nervous System Disease or Undifferentiated Febrile Illnesses

Risk Factors for Hospitalization Because of Pandemic (H1N1) 2009 Influenza, Australia

Enterovirus 68 in Children with Severe Acute Respiratory infection, the Philippines

Asymptomatic Merkel Cell Polyomavirus Infection among Men, Pittsburgh, Pennsylvania

Incidence of Acute Gastroenteritis and Role of Norovirus, Georgia, USA, 2004–2005

Seroprevalence of Trichodysplasia Spinulosa–associated Polyomavirus

Human Polyomavirus Related to African Green Monkey Lymphotropic Polyomavirus

Pandemic (H1N1) 2009, Southern Brazil

High Concentrations of Human Aichi Virus in Patients with Acute Diarrhea

Surveillance Programs for Deaths Caused by Pandemic (H1N1) 2009

Complete list of articles in the August issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

July 8–10, 2011
International Society for Infectious Diseases Neglected Tropical Diseases Meeting (ISID-NTD)
Boston, MA, USA
http://ntd.isid.org

August 8–19, 2011
12th International Dengue Course
Havana, Cuba
http://www.ipk.sld.cu/cursos/dengue2011/index.htm

August 27–31, 2011
2011 Infectious Disease Board Review Course—16th Annual Comprehensive Review for Board Preparation
Ritz-Carlton, Tysons Corner
McLean, VA, USA
http://www.IDBoardReview.com

September 17–20, 2011
51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)
McCormick Place Chicago
Chicago, IL, USA
http://www.icaac.org

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

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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 (50–150 words) of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.