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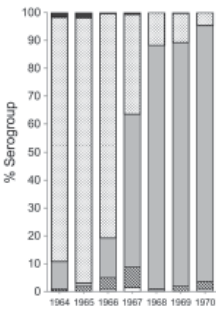

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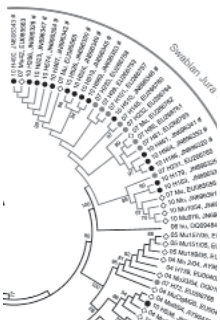

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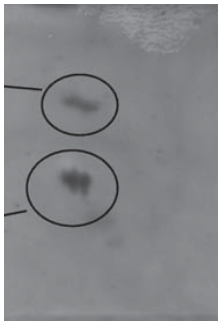

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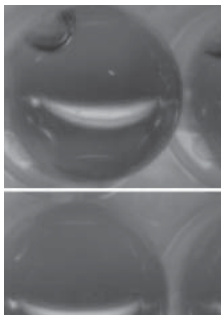

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Etymologia

Hepatitis E, a Vaccine-Preventable Cause of Maternal Deaths

Alain B. Labrique, Shegufta S. Sikder, Lisa J. Krain, Keith P. West, Jr., Parul Christian, Mahbubur Rashid, and Kenrad E. Nelson

Hepatitis E virus (HEV) is a major cause of illness and of death in the developing world and disproportionate cause of deaths among pregnant women. Although HEV vaccine trials, including trials conducted in populations in southern Asia, have shown candidate vaccines to be effective and well-tolerated, these vaccines have not yet been produced or made available to susceptible populations. Surveillance data collected during 2001–2007 from >110,000 pregnancies in a population of ≈650,000 women in rural Bangladesh suggest that acute hepatitis, most of it likely hepatitis E, is responsible for ≈9.8% of pregnancy-associated deaths. If these numbers are representative of southern Asia, as many as 10,500 maternal deaths each year in this region alone may be attributable to hepatitis E and could be prevented by using existing vaccines.

We sometimes remark that hepatitis E is so neglected that it fails to make the short list of neglected tropical diseases (*1*). Since the identification of hepatitis E virus (HEV) as a distinct viral agent in 1983 (*2*) and its subsequent cloning and sequencing in 1990–1991 (*3*), much has been learned about the still-perplexing epidemiology of this virus, which is now the leading cause of acute viral hepatitis globally (*4*). The landmark feature of HEV genotype 1, which predominates in populations in the greater Ganges floodplains of southern Asia, is increased deaths in pregnant women (*5*). The poorly understood pregnancy-associated case-fatality rate of hepatitis E, ranging from 7% to 40%, was noted in the earliest confirmed hepatitis E epidemic in 1955 (*6*) and has since been repeatedly confirmed by studies of sporadic cases and outbreaks.

Rein et al. (*7*) recently published their modeled estimates of the global incidence of HEV infections and associated deaths in 2005, suggesting >20 million incident infections and 3.3 million cases of hepatitis E per year, and a 20% probability of death in infected pregnant women. Some of these models were based, in part, on rigorous epidemiologic data collected by our group in a large research population, the Matlab demographic surveillance site in rural Bangladesh (*8*,*9*). Establishing reliable estimates of national and regional disease burden has been challenging because of the lack of standard assays for hepatitis E testing and substantial variability in the quality of commercial assays (*10*,*11*). However, it is clear that populations who show the highest incidence of HEV-associated illnesses and deaths are not benefiting from existing HEV vaccines or public health interventions.

In the mid-1990s, success with HEV vaccination in primates led to phase I trials of a recombinant HEV vaccine in 88 adults at the Walter Reed Army Institute of Research (*12*). During 2001–2004, a phase II clinical trial supported by the US Army and GlaxoSmithKline (Brentford, UK) was conducted in 1,794 predominantly male military conscripts from Nepal (*13*), but this trial was embroiled in controversy from its inception (*14*,*15*). An earlier community trial had been forcibly terminated because political opposition claimed the vaccine would be unaffordable to populations in which the vaccine was to be tested (*16*). Ultimately, this vaccine showed an efficacy of 88.5% after the first dose, which increased to >95.5% after 3 doses (*13*). These data were not published until 2007, three years after the end of the trial.

Unfortunately, GlaxoSmithKline did not pursue the manufacture of this vaccine, despite a growing body of evidence that confirms the substantial incidence of

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HEV infections in resource-limited sub-Saharan Africa, Asia, and South America and recent data that indicate an unrecognized silent epidemic of autochthonous HEV infections and illnesses across most of eastern Asia and central and western Europe (*17–19*). Recent evidence from our group suggests that HEV may be circulating widely in the United States, although the attributable disease incidence seems low (*20*).

In 2005, researchers at Xiamen University in Fujian, China, began working on a new recombinant HEV vaccine to address growing concerns about the increasing regional incidence of HEV infections. HEV 239 was tested in a randomized, controlled trial involving 112,604 participants in Jiangsu Province, where genotypes 3 and 4 are predominant. The vaccine showed an efficacy >99% in preventing clinical Hepatitis E among persons who completed the full 3-dose series of HEV 239 (n = 48,693) compared with placebo (a commercial hepatitis B vaccine, n = 48,663) (*21*). On January 23, 2012, we learned that this vaccine has been licensed for production and sale by the State Food and Drug Administration of China (*22*).

The initial use of this vaccine will probably be in high-risk populations within China. However, the manufacturer (Xiamen Innovax Biotech Co. Ltd., Xiamen, China) has expressed its intent to make the vaccine available in other regions (*23*,*24*). Demonstration of the effectiveness and safety of this vaccine in diverse settings is urgently needed. The vaccine has yet to be tested in high-need populations in which genotype 1 predominates. However, the fact that the capsid protein used in the vaccine was derived from the genotype 1 Burma reference strain (*25*) gives reason for optimism that it will be effective in these populations.

Data on the effectiveness of this vaccine in pregnant women are scant because of the exclusion of pregnant women in the trial in China. Post hoc analysis of 68 trial participants whose pregnancies were detected after receiving 1–3 doses of vaccine or placebo did not raise any obvious concerns (*26*). However, targeted safety, immunogenicity, and efficacy trials in pregnant women are warranted. Given the years of commercial inactivity after the determination of efficacy of previous HEV vaccines, the fact that an HEV vaccine may soon be commercially available in ≥1 HEV-endemic country is a major milestone on the road toward protecting vulnerable women in disease-endemic areas from HEV infection, fetal loss, and neonatal death or even maternal deaths.

To further underscore the urgency of action in this domain, we now highlight some findings from our ongoing work within the JiVitA Maternal and Child Health and Nutrition Research Site in Gaibandha, Bangladesh. Since 2001, we have been conducting prospective pregnancy surveillance in a northwestern rural population, as part of ongoing randomized community trials that aim to reduce maternal and neonatal deaths (*27–29*). In 3 consecutive trials, we have enrolled >110,000 pregnant women from a catchment population of ≈650,000. The details of this field site and these research activities have been reported (*28*,*29*). Study activities were reviewed and approved by the Johns Hopkins Bloomberg School of Public Health Committee on Human Research and by the Bangladesh Medical Research Council.

In this context, 1,091 deaths in women of reproductive age (245 during pregnancy or within 42 days postpartum) were recorded during August 2001–August 2007 by our surveillance system. Verbal autopsies by trained physicians identified hepatitis, hepatic failure, or jaundice as the primary cause of death in 19 (7.8%) of 245 pregnancy-related deaths and 61 (7.2%) of 846 nonpregnant deaths recorded. Overall, hepatitis-like illness was suggested as a direct or underlying cause in 24 (9.8%) of 245 pregnancy-related and 81 (9.6%) of 846 non–pregnancy-related deaths (Table). In another recent study, Khatun et al. (*30*) conducted verbal autopsies for 260 neonatal and 93 maternal deaths in populations in slums of urban Dhaka, Bangladesh. In that setting, viral hepatitis was reported to be the underlying cause for 11% of maternal deaths. In addition, an epidemic of HEV was documented in the slums at the time of the study (*30*).

Verbal autopsy results and other epidemiologic evidence also implicate hepatitis E as the probable cause of most deaths from sporadic hepatitis-like illness in the rural JiVitA cohort, with sudden onset of symptoms in the days or weeks before death. Although our surveillance system did not enable laboratory-based differential diagnosis of individual cases, hepatitis A, B, and C are

Table. Contribution of hepatitis and hepatitis-like symptoms as direct or underlying causes of death in women of reproductive age, northwestern Bangladesh, 2001–2007

Cause of death	Pregnancy-related deaths,* no. (%)	Non–pregnancy-related deaths, no. (%)	All deaths in enrolled women of reproductive age, no. (%)
All	245 (100.0)	846 (100.0)	1,091 (100.0)
Direct			
Hepatitis	19 (7.8)	61 (7.2)	80 (7.3)
Underlying			
Hepatitis	3 (1.2)	10 (1.2)	13 (1.2)
Jaundice	2 (0.8)	10 (1.2)	12(1.1)
Combined	24 (9.8)	81 (9.6)	105 (9.6)

*Defined as deaths of women who were pregnant or within 42 d of termination of the pregnancy, irrespective of the cause of death.

unlikely to play a major role in deaths in this population of women. Clinical studies of acute liver failure in Bangladesh have shown HEV to be the main etiologic agent responsible (*31*–*33*).

Serosurveys in rural Bangladesh have demonstrated nearly universal exposure to hepatitis A virus during childhood, with subsequent life-long immunity, implying an extremely low probability of symptomatic hepatitis A virus infection during pregnancy. Hepatitis C virus is rare in low-risk rural populations (prevalence of IgG against hepatitis C virus ≈1.5%) (*8*). Hepatitis B virus is endemic to rural Bangladesh, but the incidence of infection and illness are also low (*9*). Therefore, hepatitis E remains the most likely etiologic agent for these fatal hepatitis-like illnesses in pregnant women.

If these numbers are, as we suspect, representative of women of reproductive age and of the incidence of hepatitis throughout Bangladesh, as many as 1,180 of the ≈12,000 deaths that occur during pregnancy and soon after childbirth each year in Bangladesh (*34*) may be attributable to hepatitis E. If we apply these proportions to southern Asia, <10,500 of the estimated annual 109,000 pregnancy-related deaths in southern Asia (*35*) may be attributable to hepatitis E. Furthermore, the substantial contribution of hepatitis-like illness to deaths in nonpregnant women of reproductive age in this population deserves careful attention.

Hepatitis E should no longer be considered an obscure, newly emerging virus. More than 3 decades of study attest to its global dispersion and mortality rate. Nonetheless, etiologic surveillance across countries must be strengthened. Our population-based surveillance data suggest that ≈10% of deaths observed in pregnancy and in women of reproductive age in nonepidemic conditions could be attributable to HEV. With the availability of 2 tested efficacious vaccines, we must consider judicious and timely implementation of such interventions, where appropriate, to avoid a substantial portion of preventable deaths in these resource-limited settings.

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Effectiveness and Timing of Vaccination during School Measles Outbreak

Axel Antonio Bonačić Marinović, Corien Swaan, Ole Wichmann, Jim van Steenbergen, and Mirjam Kretzschmar

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Learning Objectives

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

- Assess measles vaccination and the prognosis of measles infection
- Distinguish the maximum amount of time permissible to initiate an outbreak response vaccination in order to prevent a large outbreak of measles
- Distinguish the maximum amount of time permissible to initiate an outbreak response vaccination in order to significantly affect the rate of measles infection at all
- Analyze the peak incidence rates of measles infection during a school outbreak

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Despite high vaccination coverage in most European countries, large community outbreaks of measles do occur, normally clustered around schools and resulting from suboptimal vaccination coverage. To determine whether or when it is worth implementing outbreak-response vaccination campaigns in schools, we used stochastic outbreak models to reproduce a public school outbreak in Germany, where no vaccination campaign was implemented. We assumed 2 scenarios covering the baseline vaccination ratio range (91.3%–94.3%) estimated for that school and computed outbreaks assuming various vaccination delays. In one scenario, reacting (i.e., implementing outbreak-response vaccination campaigns) within 12–24 days avoided large outbreaks and reacting within 50 days reduced outbreak size. In the other scenario, reacting within 6–14 days avoided large outbreaks and reacting within 40 days reduced the outbreak size. These are realistic time frames for implementing school outbreak response vaccination

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campaigns. High baseline vaccination ratios extended the time needed for effective response.

Measles is a highly contagious disease that causes illness and death in developing and industrialized countries. Measles has an estimated basic reproduction number (R_0) range of 12–40 (*1*–*5*), meaning that 1 case introduced into a susceptible (naive) population will produce, on average, that number of secondary cases. Worldwide, measles is the main vaccine-preventable cause of death among children; >31 million cases occur every year, and case-fatality rates for industrialized countries are ≈0.1%–0.2% (*6*). In 2001, the World Health Organization (WHO) and the United Nations published a global strategic plan for measles (*7*); the aims of the plan are to sustainably reduce deaths from measles and to interrupt measles virus transmission in countries and regions with elimination objectives.

In 2002, the WHO Region of the Americas was declared free from endemic measles transmission, which was achieved by implementing immunization programs with very high vaccination coverage (>95%). This goal has not been achieved in the WHO European region, for which the target year for measles elimination was 2010. Measles is so highly contagious that the average vaccination coverage in Europe (80%–95%) (*8*) is not high enough to prevent outbreaks among nonvaccinated persons. The new target year for measles elimination in the WHO European region is 2015 (*9*).

The only means of protection against measles are prior infection or vaccination. Several studies have focused on the effectiveness of mass outbreak-response vaccination campaigns for controlling measles outbreaks in settings where incidence and morbidity and mortality rates are high. Although some studies suggest that mass outbreak-response vaccination campaigns will not stop measles epidemics because of the rapid spread of the disease (*10*–*12*), other studies, which used more recent data, show that outbreak-response vaccination campaigns can successfully reduce illness and death (*13*–*17*). The current WHO guidelines recommend mass outbreak-response vaccination campaigns when a measles outbreak is confirmed in settings with a goal of reducing deaths from measles (*18*) and where most measles cases occur in children <5 years of age. There is no recommendation, however, for implementing outbreak-response vaccination campaigns in settings where incidence and morbidity and mortality rates are low, such as in the WHO European region. In these settings, because of the high baseline vaccination ratio (BVR), a shift in age distribution of measles cases toward older nonvaccinated school children is often noted (*19*). Consequently, outbreaks initially spread within relatively closed populations of children, such as schools or daycare centers, because of the high rate of social contact among nonvaccinated children in these establishments. Therefore, we focused our study on outbreak-response vaccination campaigns that targeted establishments with children where a measles outbreak was occurring in settings with high BVRs.

A delay from detection of an outbreak to implementation of an outbreak-response vaccination campaign to onset of an effective immune response of those vaccinated is inevitable. Because measles is highly contagious, many children might become infected during that delay. Thus, whether vaccinating children against measles during a school outbreak would substantially affect the outcome of a newly forming epidemic is in doubt (*20*). In this study, we used stochastic models to estimate the expected size of an outbreak in a school, depending on the delay between detection and implementation of complete school outbreak-response vaccination campaign.

Methods

We based our stochastic model design on a combined compartmental and individual-based approach (Figure 1). To set our baseline models, we calibrated the model parameters to fit the data from a real school measles outbreak during which no outbreak-response vaccination campaign was implemented. Although detection of an outbreak depends on notification and surveillance policies, we defined the beginning of the outbreak as the day on which the index case-patient showed clear signs of the disease because that is an objective starting point for the outbreak. We also defined the vaccination delay as the interval between the beginning of the outbreak and the day that the vaccination campaign was implemented at the school. For simplicity, we assumed that all nonvaccinated students were vaccinated on the day of implementation of the campaign. We used the calibrated model parameter values (Table) and investigated the outbreak size distribution by varying the vaccination delay. We computed 100,000 simulation runs for each model with different vaccination delays, ranging from day 0 (the same day that the outbreak began) to 150 days, in intervals of 2 days; day 0 meant that the school outbreak-response vaccination campaign was implemented at the beginning of the outbreak.

The Model

We used a compartmental approach to describe the part of the population not yet infected. We considered 2 subgroups—susceptible and vaccinated—to represent the nonvaccinated and vaccinated school populations, respectively, at the beginning of the outbreak. Susceptible persons were assumed to have been completely unexposed (naive) to measles virus and to have a high probability of becoming infected (P_{inf}) if they contacted an infectious person. Vaccinated persons were assumed to have acquired

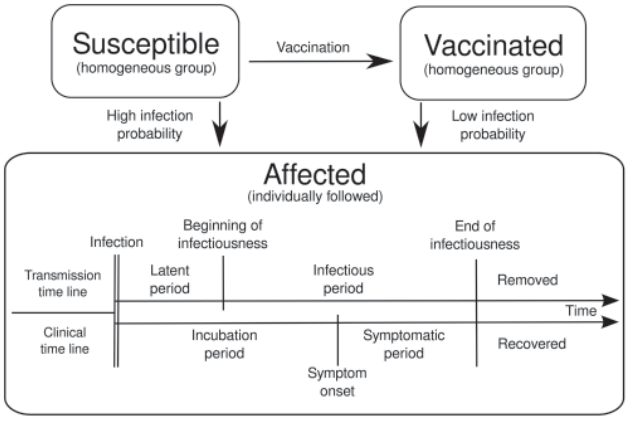

Figure 1. Schematic diagram of stochastic outbreak models to estimate the expected size of a measles outbreak in a school, depending on the delay between detection and implementation of a complete school outbreak-response vaccination campaign. Susceptible persons (susceptibles) become affected if they are infected and become vaccinated after vaccination is implemented. Vaccinated persons (vaccinated) can also be infected but with lower probability than susceptible persons. Those who become affected are followed individually, each with their own transmission and clinical time lines.

protection from measles virus by vaccination and to have a reduced probability of becoming infected ($P_{inf,vac}$>0) to account for an imperfect vaccine.

For susceptible (or eventually a vaccinated) persons who became infected, the model took an individual-based approach and followed each person from the time of infection until the end of the simulation. Persons who had had measles before the outbreak were included as affected but were already immune to infection from the beginning of the simulation. Each affected person had individual transmission and clinical time lines, depending on time since infection, which varied from one person to another.

Disease History Time Lines

From the moment a person became infected, and therefore affected, 2 parallel time lines were updated in 1-day steps. The time lines describe the disease history: transmission and clinical (Figure 1).

The transmission time line describes when the affected person became infectious, after a latent period of duration (D_{lat}), and when that person ceased to be infectious because the person recovered after the infectious period of duration (D_{inf}) had passed. Durations of these periods were generated randomly from their respective probability distributions (Table). After an affected person recovered, that person was unable to become infected again because of acquired life-long immunity. The transmission time line describes the events that dictate the dynamics of the epidemic, but in practice these events are difficult to observe.

In contrast, the clinical time line describes events that can actually be observed. This time line indicates the moment of the rash onset, after the incubation period (D_{inc}), and the moment when the affected person recovered from the disease, after the symptomatic period (D_{symp}) had passed. The values of these periods for each affected person are drawn from the probability distributions indicated in the Table. For simplicity, we assumed that the symptomatic period ended at the same time that the infectious period ended.

Persons who had been infected before the outbreak were considered recovered at the start of our simulations. That is, they were at the end of their disease time lines and did not contribute to the final size of the outbreak.

Table. Definitions of measles outbreak model parameters with assumed values and probability distribution*

Model parameter	Notation	Value/distribution	Reference
Duration of incubation period (from distribution)	D_{inc}	Log normal (2.3,0.2); 7–14 d after infection, mode 10 d.	(*21–23*)
Duration of latent period (distribution)	D_{lat}	(D_{inc} – 4) + normal (0.7); latent period ends ≈4 d before symptom onset.	(*21–23*)
Duration of infectious period (from distribution)	D_{inf}	(D_{inc} + 4 – D_{lat}) + normal (0.7); infectious period ends ≈4 d after symptom onset.	(*21–23*)
Duration of symptomatic period (from distribution)	D_{symp}	D_{lat} + D_{inf} – Dinc; assumes that symptomatic period ends at same time as infectious period.	(*21–23*)
Duration of period to build up immunity after vaccination (from distribution)	D_{imm}	13.2 + normal (3.0); approximates measles-specific IgM positivity rates of 2% and 61% after 1 and 2 weeks of vaccination, respectively.	(*24*)
Number of daily contacts per person (from distribution)	n_{cont}	20 + NegBin (0.155,2.2).	(*25*)
Infection probability of a susceptible person after contact with an infectious person	P_{inf}	0.12 in the R_0≈16 scenario; 0.2348 in the R_0≈31 scenario.	This article (Model Calibration)
Vaccination effectiveness	VE_S	0.9975.	(*5*)
Infection probability of a vaccinated person after contact with an infectious person	$P_{inf,vac}$	P_{inf} (1 − VE_S); 3×10^{-4} in the R_0≈16 scenario; 5.869×10^{-4} in the R_0≈31 scenario.	This article (Vaccination)

*NegBin, negative binomial probability distribution; R_0≈16, scenario in which basic reproduction number R_0≈16 is considered; R_0≈31, scenario in which basic reproduction number R_0≈31 is considered.

Infection

We assumed that every person in the school mixed with everybody, i.e., homogeneous mixing, and considered daily contact distribution as shown in the Table. Therefore, each day the number of contacts that an infectious affected person had (n_{cont}) was drawn from this distribution. From these contacts, the number of newly infected susceptible and vaccinated persons was computed according to the infection probabilities, P_{inf} and $P_{inf,vac}$, respectively.

Vaccination

The percentage of vaccinated persons at the beginning of the outbreak was determined by the BVR, and we assumed that vaccination-acquired protection does not wane with time. The high effectiveness of the measles vaccine (*5*,*26*) implies that outbreaks occur mostly among those who are not vaccinated. The intervention strategy consisted of vaccinating all school children without documented measles vaccination; the goal was 100% vaccination coverage. This strategy influences only the susceptible persons by reducing their probability of becoming infected to $P_{inf,vac} = (1\text{-}VE_S)$ P_{inf}, where VE_S is the vaccine effectiveness value (Table). Susceptible persons are not protected at the moment of vaccination because the immune response to the vaccine takes time to develop. These persons remain susceptible for a period ([D_{imm}]; probability distribution is indicated in the Table) after the vaccination day and then become part of the vaccinated group.

Isolation

Usually, persons with measles stay home while recovering from the disease. Therefore, we assumed that after disease symptoms developed (after the incubation period D_{inc}), infected persons stopped attending school. This absenteeism prevents further contact at school and further spread of the disease, even if the affected person remains infectious at home. For this study, we ignored the possibility of siblings attending the same school.

Model Calibration

We calibrated our models by using data from a retrospective cohort study conducted at a public day school in Duisburg, Germany, where a large measles outbreak had occurred in 2006 (*19*,*27*). The first cluster in this epidemic had occurred at that school, providing a typical scenario for the early behavior of an outbreak. The outbreak data consisted of a population of 1,250 schoolchildren 10–21 years of age (median 14 years), of which a high proportion (91.3%–94.3%), were vaccinated; 62 students had a history of measles before the outbreak, and a total of 55 cases were confirmed (*5*,*27*). Because no outbreak-response vaccination campaign had been implemented, we calibrated the infection probability (P_{inf}) in our models to describe this situation. Assuming a BVR of 91.3%, calculated by Wichmann et al. (*27*), we adjusted the infection probability (P_{inf}) in our models so that the size distribution of large outbreaks from our simulations peaked at 55 cases. The calibrated P_{inf} value in combination with our assumed contact distribution (Table) translated into a basic reproduction number of $R_0 \approx 16$, which is consistent with reported basic reproduction number estimations for measles (*1–4*). The calibrated P_{inf} value in combination with a BVR of 91.3% yielded roughly an effective reproduction number (R_{eff}) as follows: $R_{eff} \approx R_0[1-(BVR/100\%)] = 1.4$. In theory, if BVR is high enough that R_{eff} is <1, then 1 case generates on average <1 secondary case, leading to herd immunity effects and no large outbreaks (*1–4*,*28*).

In a later study, van Boven et al. (*5*) used a Bayesian method to estimate the BVR at 94.3%. Considering this BVR, a higher infection probability (P_{inf}) is needed in our models to reproduce the observed outbreak size, which translates to a higher basic reproduction number, $R_0 \approx 31$, needed to produce an outbreak in a population with such a high BVR. The conditions in this scenario yield an R_{eff} of ≈1.8, indicating that our simulated outbreaks spread more quickly and had higher attack rates than in the less contagious case of $R_0 \approx 16$ and $R_{ef} \approx 1.4$. To obtain equivalent outbreak sizes, the attack rate (percentage of affected susceptible persons) has to be higher in a population in which BVR is larger because the number of susceptible children is smaller.

Figure 2 shows the final size distribution histograms for $R_0 \approx 16$ and $R_0 \approx 31$. The typical bimodal distribution predicted by stochastic models appears in both scenarios (*28*) but is most pronounced for the case of $R_0 \approx 31$. The bimodal distribution arises because it is always possible that chance events will cause a new outbreak to die out before becoming large. We interpreted the local minimum in both distributions of Figure 2 (i.e., an outbreak size of 20) as a natural but arbitrary limit to distinguish between small and large outbreaks. Therefore, although both distributions show a most probable large outbreak size of 55 persons, in the $R_0 \approx 16$ model, 39% of the simulated outbreaks instances become large outbreaks, and in the $R_0 \approx 31$ model, 64% of the instances become large.

Results

Figure 3 shows the final size distribution of outbreaks as a function of the model vaccination delay indicated in the *x*-axis for the $R_0 \approx 16$ model. Because of the stochastic nature of the model, as a result of chance events, some outbreaks died out before the intervention was implemented, as could happen in real life. To study the effect of the intervention, we considered only those outbreaks that were still developing at the moment of vaccination. The longer it took to implement the vaccination campaign, the more the

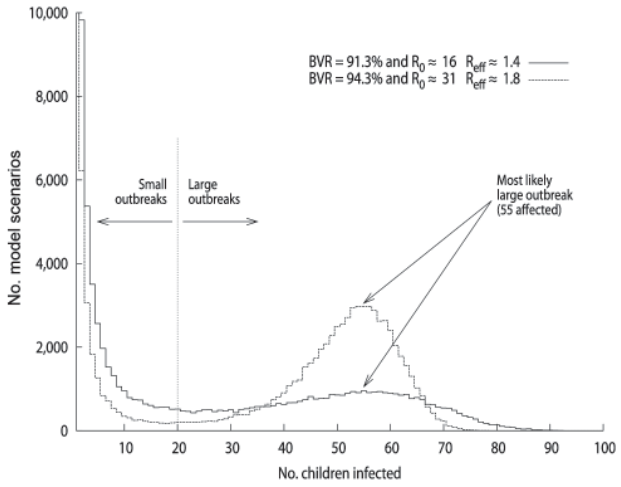

Figure 2. Measles outbreak size histograms calculated with calibrated models. The y-axis indicates the number of model instances counted in their corresponding outbreak size histogram bin, indicated in the x-axis. The dotted line indicates the limit from which large and small outbreaks are defined. BVR, baseline vaccination ratio; R_0, reproduction number, R_{eff}, effective reproduction number.

outbreak size distributions shifted toward larger outbreaks. Outbreaks were expected to remain small (<20 infected children) if the vaccination delay was 12–24 days. Although the reaction must be quick to avoid an outbreak involving >20 children, a reaction as late as 50 days reduced the final size of large outbreaks in 95% of the simulations. With vaccination delays of >80–90 days, the expected outbreak size was similar to that when no vaccination campaign was implemented.

We also considered the scenario described by van Boven et al. (*5*), who based the $R_0 \approx 31$ estimate on the same school outbreak with a high BVR (94.3%). The high R_0 value of 31 implies that the infection is more contagious than that in our $R_0 \approx 16$ model, leading to a higher effective reproduction number. Figure 4 shows that the outbreak size distributions shifted toward larger outbreaks if the vaccination delay was longer, similar to the $R_0 \approx 16$ model. However, because of the higher infectiousness of the disease, outbreaks spread more quickly than in the models with $R_0 \approx 16$. This provided only a small time frame of 6–14 days to implement the vaccination campaign if the outbreak size was to be kept small (<20 persons). In 95% of the simulations, a vaccination campaign implemented as late as 40 days after start of the outbreak reduced the final size of the outbreak. There was almost no difference between implementing an outbreak-response campaign after ≈60 days and not implementing one at all.

From those outbreaks that did not die out before the vaccination campaign was implemented, we computed the percentage of those that would become large. This percentage can be interpreted as the probability that an outbreak will become large. The dependency of this percentage on the vaccination delay is shown in Figure 5, where a range limited by the larger and smaller R_0 cases is considered. The large outbreak percentage was reduced to 31%–59% if the outbreak-response vaccination campaign was implemented with a delay of 28 days, and it was reduced further if the campaign was implemented earlier: 17%–42% with a 21-day delay, 7%–23% with a 14-day delay, and 1%–7% with a 7-day delay. Although the percentage of large outbreaks became increasingly larger as delay to vaccination became larger, later implementation of outbreak-response vaccination campaigns might still substantially reduce the size of large outbreaks (Figures 3 and 4).

To extend our study to different settings, we ran simulations in the same school setting and assumed various BVRs, the lowest being 80%, comparable to the current situation in Europe (*8*) (Figure 6). Outbreaks with a higher R_0 developed more quickly under the same initial conditions and did not become large if the BVR was high

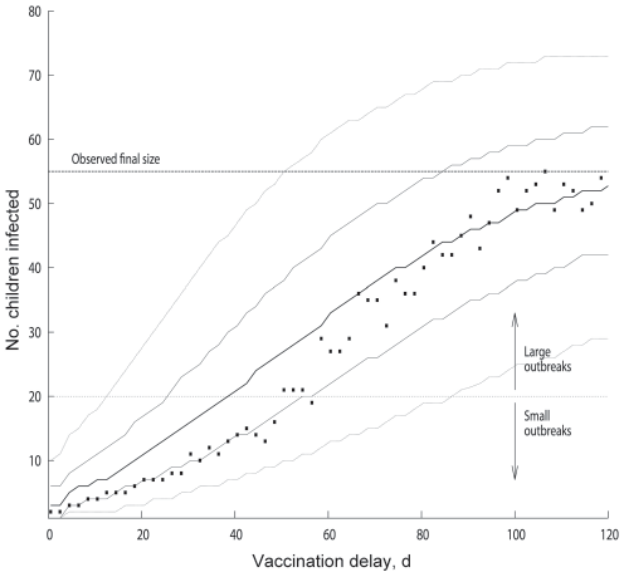

Figure 3. Distribution of measles outbreak sizes as function of vaccination delay for models with basic reproduction number (R_0) of ≈16 and baseline vaccination ratio (BVR) of 91.3% (effective reproduction number ≈1.4). We considered the outbreaks that were still ongoing at the day of implementation of the outbreak-response vaccination campaign and not those that had spontaneously died out earlier by chance. For every given vaccination delay, the squares indicate the most likely large outbreak size, and the thick solid line indicates the median outbreak size value. The thin solid lines indicate 25th and 75th percentiles, and the tiny dotted lines indicate 5th and 95th percentiles of the outbreak size distribution as a function of vaccination delay. The dashed line shows the outbreak size from the observed data, and the dotted line indicates the chosen limit to separate large and small outbreaks.

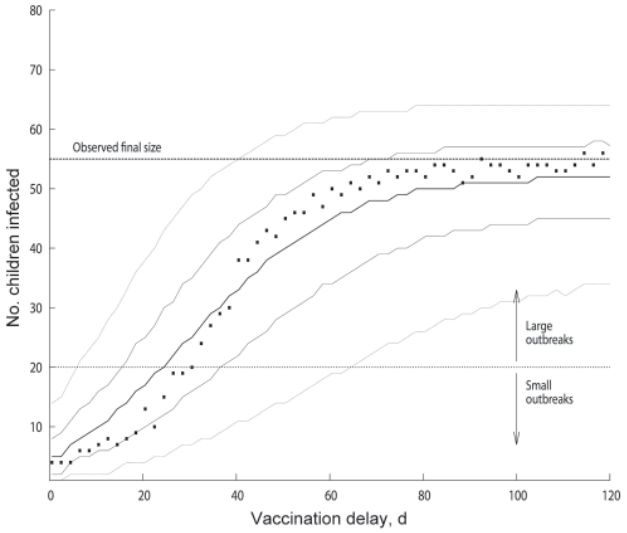

Figure 4. Distribution of measles outbreak sizes as function of vaccination delay for models with basic reproduction number (R_0) of ≈31 and baseline vaccination ratio (BVR) of 94.3% (effective reproduction number ≈1.8). We considered the outbreaks that were still ongoing at the day of implementation of the outbreak-response vaccination campaign and not those that had spontaneously died out earlier by chance. For every given vaccination delay, the squares indicate the most likely large outbreak size, and the thick solid line indicates the median outbreak size value. The thin solid lines indicate 25th and 75th percentiles, and the tiny dotted lines indicate 5th and 95th percentiles of the outbreak size distribution as a function of vaccination delay. The dashed line shows the outbreak size from the observed data, and the dotted line indicates the chosen limit to separate large and small outbreaks.

enough to achieve herd immunity (>93.8% and >96.8% for R_0≈16 and R_0≈31, respectively). For lower BVRs, the final size was larger and outbreaks spread more quickly because of a larger effective reproduction number. Therefore, the time frame to react is smaller; e.g., if BVR = 80% and R_0≈16, a 1-week delay is already long enough to expect large outbreaks, but a reaction within 3–4 weeks might substantially reduce the outbreak size. Some outbreak sizes were larger than the number of nonvaccinated children in the population, which is explained by imperfect vaccine producing an effect similar to a lower BVR.

We considered a school of 1,250 children, but there are many smaller institutions. In a simulation for a school of 500 children, while conserving the proportions of children with a history of measles and BVR, the boundary separating large and small outbreaks changed to 13 persons. However, results regarding the timing of vaccination remained approximately the same.

Discussion

We calculated results for 2 reproduction number values, 31 and 16, which belong to a range that is high when compared with published estimates of the basic reproduction number of measles (R_0≈8–18) (1–4). This comparison indicates that our results are rather conservative with regard to how quickly the intervention should be implemented. However, it must be noted that our results apply to schools in Europe for which BVRs were average (>80%) before the outbreak (*8*).

When we considered institutions with smaller populations than that considered in our study, timing for vaccination remained roughly the same. This finding is explained by the association between the time to react effectively and the generation interval of infection, which is the average time it will take for a newly infected person to infect someone else. The generation interval will depend mostly on the within-host disease development, as long as children have enough contacts to transmit the disease, even in schools with ≈300 children (*25*).

We considered our contact structure to be homogeneous mixing. More intricate contact networks, such as those considering clustering in the different classrooms of the school, popularity of some children, and household contacts with siblings attending the same school, might better resemble reality. Some network structures, contact rates, and superspreading events can influence the speed and growth of an epidemic (*29*). However, our baseline simulations had peak incidence during weeks 6–11 (16th and 84th percentiles, respectively), mode 7 weeks, for the R_0≈31 scenario; and weeks 6–14, mode 8 weeks, for the R_0≈16 scenario. These data are consistent with the real outbreak described by Wichmann et al. (*27*), indicating that

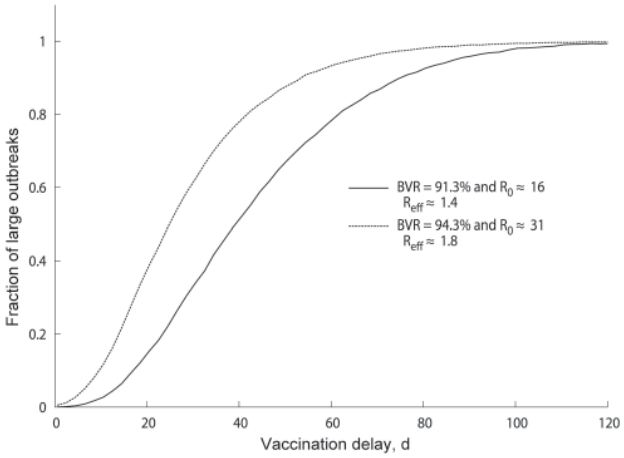

Figure 5. Percentage of measles outbreaks that become large for the indicated models. We considered those outbreaks that are ongoing at the moment of implementation of the vaccination campaign, indicated by the vaccination delay in the x-axis. BVR, baseline vaccination ratio; R_0≈16, scenario in which basic reproduction number R_0≈16 is considered; R_0≈31, scenario in which basic reproduction number R_0≈31 is considered; R_{eff}, effective reproduction number.

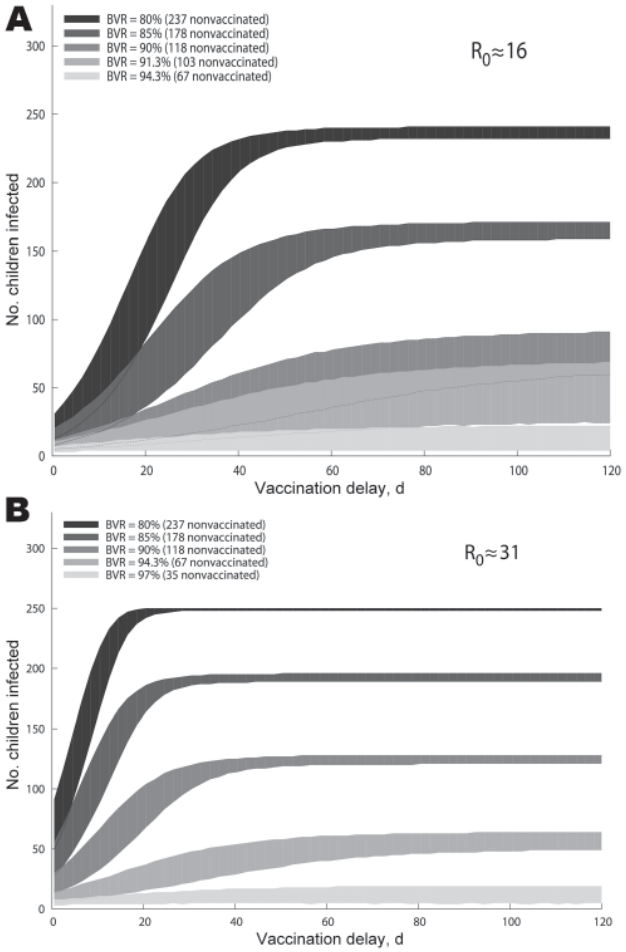

Figure 6. Measles outbreak size ranges as function of vaccination delay, for models with basic reproduction number R_0≈16 (A) and R_0≈31 (B) in the same school setting with various baseline vaccination ratios (BVRs). The ranges shown are between the 95th and 50th percentiles of the outbreak size distribution as a function of vaccination delay and are calculated for each BVR indicated.

the rate of disease spread in our simulations was similar to that in the real outbreak.

At the moment of vaccination, a person might already be infected but not yet symptomatic or might become infected soon after vaccination, before immunity has had time to develop. We assume that in these cases the disease progresses in the same way it does in nonvaccinated persons. However, there is evidence that vaccinating during the incubation period might mitigate the symptoms of measles infection (*30*). The vaccine might act as postexposure prophylaxis, potentially reducing the infectiousness of the vaccinated persons.

In many schools in Europe a BVR >80% can be found, but in communities with rather low BVRs (e.g., because of religious or philosophic beliefs), measles virus spreads much quicker. For example, in an outbreak originating in an anthroposophic community in Austria during 2008, with a BVR of 0.6%, of the 123 cases in the anthroposophic school of that community, 96% occurred during the first 4 weeks of the outbreak (*31*), indicating that most cases were either second or third generation (the index case is considered the first generation). An outbreak-response vaccination campaign during such a rapidly spreading outbreak would be effective in a school setting only if implemented within 1 average generation interval ≈9 days) to avoid third-generation infection, which is logistically difficult and requires 100% compliance. However, because school outbreaks are often part of larger community outbreaks, vaccination activities focused on susceptible persons should be promoted in the broader community at any time to prevent further spread of the disease, independent from the time of a school outbreak.

In our study, we assumed 100% compliance to the vaccination strategy. But high compliance to a vaccination campaign cannot be expected in regions with low BVRs associated with religious or philosophical beliefs that are opposed to vaccination. The compliance needed for an intervention to be effective should ensure herd immunity (≈95% vaccinated children). Other complementary measures can be implemented to control measles outbreaks at schools when no vaccination compliance is expected. For example, a measure such as the temporary exclusion of students who lack documented vaccination or whose parents do not agree to vaccination of their children might have a limited effect on preventing further spread of measles by itself (*20*,*32*). However, a combination of timely vaccination of all susceptible students at school who agree to be vaccinated and temporary exclusion of those who do not would be the most effective way to control a measles outbreak in a school.

In conclusion, we computed the possible outcomes of a measles outbreak in a school according to the vaccination delay, assuming that all potentially susceptible children in the school were vaccinated on the same day. We found that it is possible to reduce the number of cases during a measles outbreak in a school by applying a schoolwide vaccination strategy within a realistic time frame. Subsequently, because disease tends to spread in schools during the early stages of a city outbreak, reducing the effects of school outbreaks should help reduce the extent of the outbreak in the larger community. We also showed that BVRs that are high (>80%) but not high enough to achieve herd immunity (≈95%) keep the number of susceptible persons low, reduce the size of an outbreak, and reduce the speed at which the disease spreads, thereby increasing the time frame for mounting an effective intervention.

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Evaluation of Diagnostic and Therapeutic Approaches for Suspected Influenza A(H1N1)pdm09 Infection, 2009–2010

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Learning Objectives

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

- Analyze the use of diagnostic testing in cases of influenza-like illness
- Evaluate the use of antiviral medications for outpatient cases of influenza-like illness
- Evaluate the use of antiviral medications for inpatient cases of influenza-like illness
- Assess the care of patients with influenza-like illness and lower respiratory tract infections

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To assess adherence to real-time changes in guidelines for influenza diagnosis and use of oseltamivir during the 2009 influenza A(H1N1) pandemic, we reviewed medical records of patients with confirmed or suspected influenza-like illness (ILI) and those with no viral testing in a large Los Angeles (California, USA) hospital. Of 882 tested patients, 178 had results positive for influenza; 136 of the remaining patients received oseltamivir despite negative or no results. Oseltamivir use was consistent with national recommendations in >90%. Of inpatients, children were less likely than adults to have ILI at testing and to receive oseltamivir if ILI was found. Of outpatients, children were more likely to have positive test results; 20% tested did not have ILI or other influenza signs and symptoms. Twenty-five of 96 test-positive patients and 13 of 19 with lower respiratory tract disease were, inappropriately, not treated. Variations between practice and national recommendations could inform clinical education in future influenza seasons.

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In April 2009, the novel influenza A(H1N1) pandemic influenza virus (influenza A[H1N1]pdm09) was identified as the cause of influenza outbreaks. Influenza

disease caused by this strain rapidly spread, and in June 2009, the World Health Organization (WHO) declared a global pandemic. Disease activity peaked during May–June 2009, again in October 2009, and essentially disappeared by May 2010 (*1*–*3*). As with previous pandemics, the strain reemerged in the United States during the subsequent 2010–2011 influenza season and accounted for ≈25% of characterized strains (*4*).

During the pandemic, the Centers for Disease Control and Prevention (CDC) issued several guidances for healthcare providers for the identification and treatment of patients with suspected influenza A(H1N1)pdm09 disease (Figure 1). Several rapid influenza diagnostic tests for identification of the 2009 H1N1 strain were available, but their poor sensitivity soon became clear (*5*–*7*). CDC recommended that the neuraminidase inhibitor oseltamivir be used as a first-line treatment during the pandemic (*8*). Available data suggested that the drug was clinically effective, but only when given within <48 hours of symptom onset (*9*–*11*). These guidelines changed during the course of the pandemic as real-time epidemiologic, virologic, and clinical data emerged (*8*,*12*–*15*).

CDC initially recommended priority use of antiviral drugs for only hospitalized patients and those at increased risk for influenza-related complications. This recommendation reflected the knowledge that most persons infected with A(H1N1)pdm09 virus had self-limited, mild-to-moderate disease; that commercial and stockpiled supplies of oseltamivir were limited; and that the development of resistance was a concern, particularly since no other effective and easily administered antiviral drugs were available (*15*–*18*). Questions remained, however, with regard to the overall risks and benefits and appropriate dosage of the drug for very young and obese patients. In September 2009, CDC advised that rapid influenza diagnostic tests be prioritized for patients who were hospitalized or for whom a diagnosis of influenza could inform clinical decision making. Furthermore, CDC reinforced the idea that presumptive treatment should be administered to this group of patients and expanded the target group for treatment to include outpatients with risk factors for severe disease, even when test results were unknown (*5*). Clinical judgment was clearly a key factor in the clinical management of patients with possible A(H1N1) pdm09 disease.

Much has been published with regard to the epidemiology, virology, and clinical spectrum of A(H1N1) pdm09 illness (*19*,*20*), but no information is available with regard to diagnostic and therapeutic decision making of physicians or their adherence to national guidelines for ill patients. We conducted this study to evaluate the adherence of physicians to contemporaneous national guidelines for diagnosis and use of oseltamivir among patients with suspected or confirmed A(H1N1)pdm09 virus infection in the inpatient and outpatient settings.

Methods

The study population included all persons who accessed care from May 1 to December 31, 2009, at Harbor–UCLA Medical Center (HUMC) in Los Angeles, California. HUMC is a 538-bed, urban, academic, teaching hospital; it serves a diverse population, which is ≈55% Latino, 11% Caucasian, 24% black, 4% Asian, and 4% Pacific Islander.

We conducted a retrospective cohort study to evaluate 3 issues: 1) adherence of clinicians to national recommendations for use of osteltamivir among patients with suspected or confirmed influenza virus infection; 2) appropriateness of patient selection for diagnostic testing; and 3) the likelihood of clinicians to prescribe antiviral drug therapy for persons with known influenza-like illness (ILI) or lower respiratory tract infection (LRTI), 2 conditions for which CDC specifically recommended antiviral drug therapy. For the first 2 objectives, we identified child and adult inpatients and those seen in the emergency department with A(H1N1)pdm09 disease by using 4 overlapping data sources, including the following: 1) prospectively collected electronic A(H1N1)pdm09 virus laboratory-based surveillance data obtained by the HUMC clinical virology laboratory and the Infection Prevention and Control Department; 2) electronic, pharmacy-based oseltamivir utilization data; and 3) data on point-of-care testing performed in the emergency department. These data were combined, and we reviewed the medical records of all patients with a positive laboratory test for influenza in the outpatient setting and of inpatients who

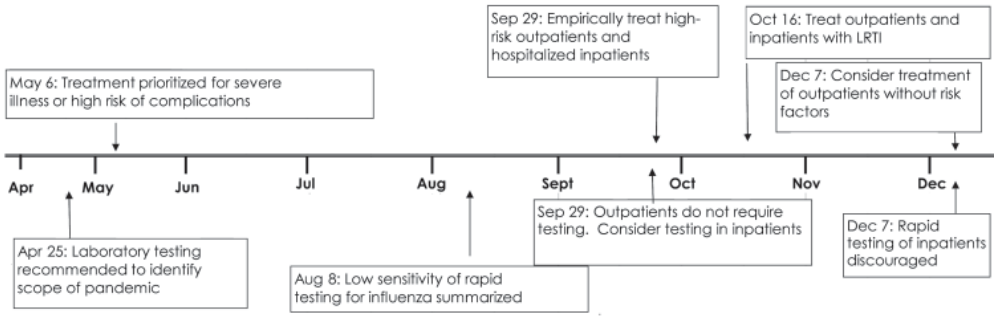

Figure 1. Centers for Disease Control and Prevention (CDC) guidance during the 2009 pandemic of influenza A(H1N1)pdm09 disease. LRTI, lower respiratory tract infection.

had a laboratory test that was positive for influenza virus or were prescribed oseltamivir. Approval for human subjects research was obtained from the Los Angeles Biomedical Research Institute.

We performed a comprehensive review of medical records by using a standardized data collection instrument to identify demographic information and clinical characteristics of patients with the illness, including symptoms and signs and results of viral diagnostic testing and chest radiographs. Use of and indications for oseltamivir, including dose and duration of use, were recorded and, if oseltamivir was not prescribed, reasons for not using the drug were noted. We also recorded whether the patient exhibited risk factors for complications and death (from a preselected list that included concomitant cardiopulmonary, renal, liver, endocrine, blood, or metabolic disorders; immunosuppressive conditions; aspirin therapy; and neurologic conditions), diagnoses at admission or discharge, and length of stay.

We defined suspected influenza as illness in any patient for whom oseltamivir was prescribed by the treating clinician. We defined confirmed influenza disease as illness in a patient with a positive laboratory test result for the virus. To evaluate adherence to guidelines, we used the contemporaneous CDC definition for ILI (fever and cough with or without sore throat) and defined severe illness as requiring intensive care, a documented oxygen saturation of <92%, or both.

To assess the likelihood of clinicians to prescribe antiviral drug therapy for persons with known ILI or LRTI, we identified all inpatients and outpatients with possible upper or lower respiratory tract influenza disease by using International Classification of Diseases, Ninth Revision (ICD-9) diagnostic codes as follows: 079.89 (viral infection), 079.99 (viral infection not otherwise specified [NOS]), 460 (nasopharyngitis, acute), 462 (pharyngitis, acute), 465.8 (infectious upper respiratory, multiple sites, acute), 465.9 (infectious upper respiratory, multiple sites, acute NOS), 466.0 (bronchitis, acute), 466.19 (bronchiolitis, acute, due to other infectious organism), 478.9 (disease, upper respiratory /NOS), 480.1 (pneumonia caused by respiratory syncytial virus), 480.8 (pneumonia caused by virus), 480.9 (viral pneumonia unspecified), 484.8 (pneumonia in other infectious disease), 485 (bronchopneumonia, organism NOS), 486 (pneumonia, organism NOS), 487.0 (influenza with pneumonia), 487.1 (influenza with respiratory manifestation), 487.8 (influenza with manifestation), 488.1 (influenza caused by identified novel H1N1 influenza virus), 490 (bronchitis NOS), 780.6 (fever), 784.1 (pain, throat), 786.2 (cough) (*21*). The validity of the ICD-9–based ascertainment was assessed by using prospective emergency department triage ILI surveillance data collected beginning October 21, 2009, through the end of the study period.

From this group, we randomly selected 100 persons, stratified by age (50 persons ≤18 and 50 >18 years of age) by using SAS 9.2, Proc Samplesurvey (SAS Institute, Cary, NC, USA). Using medical record review, we then identified persons with ILI (defined above) or LRTI, defined by the presence of at least 1 specific lower respiratory tract sign, including tachypnea, retractions, or hypoxia (oxygen saturation <92%), and/or abnormal auscultatory findings (crackles/crepitations or wheezing), and/or unequivocal and abnormal radiographic findings.

We performed descriptive analyses of the above variables by using SAS version 9.2. Testing of proportions was performed by using χ^2 or Fisher exact test as appropriate. All reported p values are 2-tailed and were considered significant if $p<0.05$.

Results

Entire Cohort

We identified 882 patients who were tested for influenza virus during the study period, among whom 178 (20%) tested positive. An additional 136 received oseltamivir but were not tested or had a negative laboratory test result for influenza virus. Overall, 232 (74%) of 314 patients had ILI, and 82 (26%) of 314 had a positive test result for influenza virus but did not meet the CDC-defined criteria for ILI. Of these 82, 36 (44%) had other signs or symptoms consistent with influenza, such as headache, myalgia, nausea, or diarrhea. We identified 218 (69%) inpatients among the 314 patients with confirmed or suspected influenza. Of those 314 patients, 55 (18%) were <2 years of age, 129 (41%) were 2–18 years of age, 89 (28%) were 19 to <50 years of age, 32 (10%) were 51 to <65 years of age, and 9 (3%) were >65 years of age. An underlying medical condition was recognized in 88 (48%) children (most commonly, asthma) and in 95 (52%) adults (most commonly, immunosuppression).

Oseltamivir was prescribed for 86 (66%) of 130 children and 89 (87%) of 102 adults with ILI. Oseltamivir was prescribed at the correct dosage and duration of therapy for 229 (95%) of 240 patients, and 216 (90%) of 240 patients received the drug <48 hours after symptom onset. Another 16 received the drug within 72 hours of disease onset. Severe illness was identified in 132 (42%) of 314 patients, 118 (89%) of whom received oseltamivir (Figure 2).

Inpatients

Of 218 inpatients who received a diagnosis of or treatment for influenza, 107 (49%) were children, and 111 (51%) were adults. Laboratory testing was performed

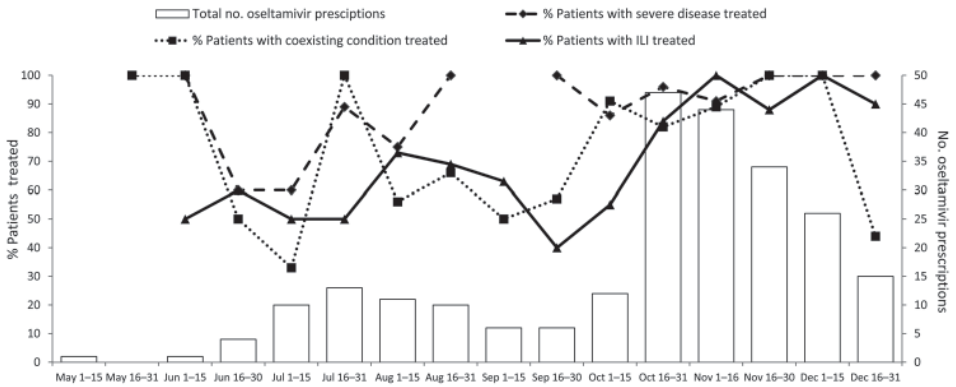

Figure 2. Total number of patients treated with oseltamivir by category, presence of influenza-like illness (ILI), and disease severity, Los Angeles, California, USA, 2009.

for 177 (81%) inpatients, and 74 (42%) were positive for influenza virus (Table). Oseltamivir was administered to 198 (91%) of 218 inpatients, among whom 110 (50%) had a negative test or no laboratory testing performed. Of the remaining 88 with a positive test result, 5 did not receive oseltamivir because the patient refused, the patient was "well appearing," or patient's onset of symptoms occurred >48 hours before they received a diagnosis.

Of the inpatients, we identified 68 (64%) of 107 children and 86 (77%) of 111 adults who had ILI at the time of laboratory testing ($p<0.04$). Oseltamivir was given to 58 (85%) of the 68 children with ILI and 84 (98%) of 86 adults with ILI ($p<0.02$). Oseltamivir was prescribed for 145 (94%) of 155 inpatients with an underlying medical condition and for 118 (91%) of 129 patients with severe illness.

The median interval from illness onset to initiation of antiviral treatment was 2 days (range 1–8). The dosage or duration of therapy, or both, was incorrect for 11 (5%) inpatients; for 6 inpatients, no adjustment was made for renal insufficiency. Of those 6 inpatients, 2 had chronic renal insufficiency after a transplant, 1 had diabetic nephropathy, and 3 had pneumonia and renal insufficiency. Three obese patients received a doubled dose of oseltamivir.

Receipt of the vaccine against influenza A(H1N1) pdm09 virus was documented in 61 (28%) of 218 patients, but 59 (97%) of them received the vaccine at hospital discharge. Only 1 patient had received the seasonal influenza vaccine before admission, and none received vaccine at discharge.

Outpatients

We identified 664 patients who underwent rapid influenza diagnostic testing, of whom 77 (19%) of 398 children and 19 (7%) of 266 adults tested positive ($p<0.001$). Twenty percent of tests were carried out on patients without CDC-defined ILI and for whom no other indication was present. As noted in Figure 3, only 11%

Table. Patients who underwent testing or treatment for influenza by category, Los Angeles, California, USA, 2009*

Test results and treatment	Inpatients†	Outpatients‡
Influenza diagnostic test		
Patients tested for influenza		
Total	177/218 (81)	664/664 (100)
Adults	79/111 (71)	398/398 (100)
Children	98/107 (92)	266/266 (100)
Positive influenza test result		
Total	74/177 (42)	96/664 (14)
Adults	18/79 (23)	19/398 (5)
Children	56/98 (57)	77/266 (29)
ILI among patients with a positive test result		
Total	44/74 (59)	77/96 (80)
Adults	14/18 (78)	16/19 (84)
Children	30/56 (54)	61/77 (79)
Oseltamivir prescribed		
Patients with positive influenza test result	53/74 (72)	22/96 (23)
Patients with coexisting condition	145/155(94)	15/28 (54)
Patients with severe influenza disease	118/129 (91)	0/3 (0)
Median time from illness onset to treatment, d	2 (1–8)	2 (1–5)

*Values are no./total no. (%) unless otherwise indicated. ILI, influenza-like illness.
†For inpatients who received a diagnostic test for influenza, N = 218; for outpatients who received a diagnostic test, N = 664. For inpatients who received oseltamivir, N = 218. In the outpatient setting, study cohort was identified through diagnostic testing only. Use of oseltamivir was evaluated only among those for whom a diagnostic test result was positive (N = 96).

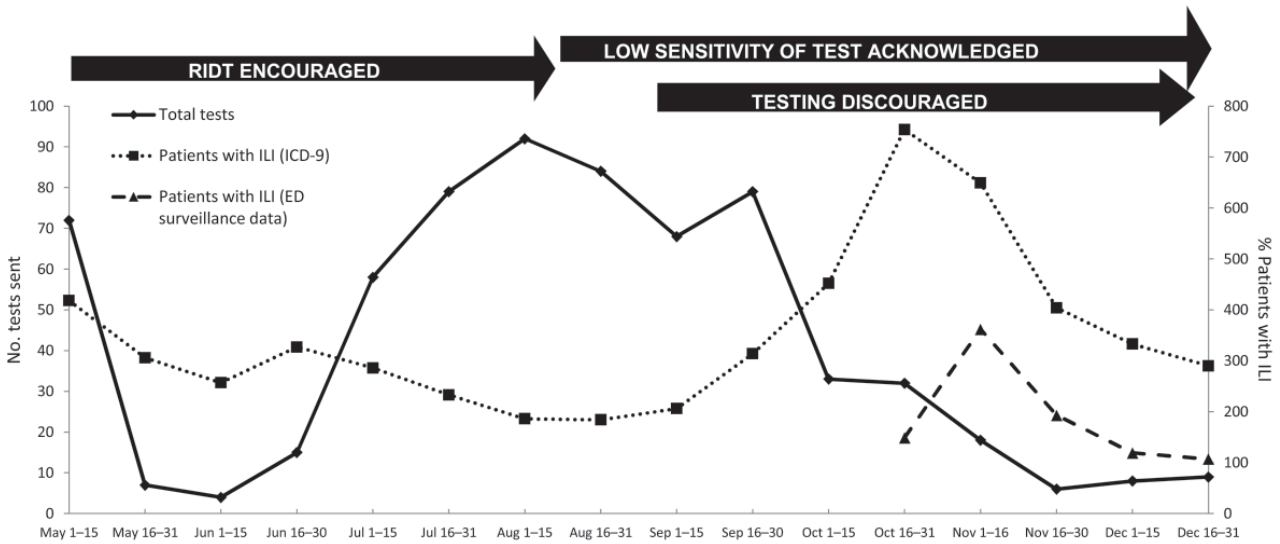

Figure 3. Rapid influenza diagnostic testing (RIDT) performed for outpatients with influenza-like illness (ILI), Los Angeles, California, USA, 2009.

(73/664) of these tests were performed >2 weeks after CDC actively discouraged their use.

Oseltamivir was prescribed for 37 (48%) of 77 outpatient children and 5 (26%) of 19 adults who tested positive for influenza (p>0.05), all at the appropriate dose and duration. As recommended, 35 (83%) of 42 received the drug <48 hours from symptom onset, and the remaining patients received the drug within 72 hours of symptom onset. Of 54 (56%) of 96 patients who tested positive and did not receive oseltamivir, 25 (46%) were not treated according to CDC guidelines, and 8 (15%) refused therapy. The reasons for not initiating oseltamivir therapy included onset of symptoms >48 hours previously and lack of an underlying medical condition. For 21 (39%) of the untreated patients, we found no documentation of the reason for withholding therapy.

We found 3 outpatients who had severe illness, none of whom received oseltamivir, and the reasons for withholding therapy could not be determined. Conversely, 16 (3%) of 522 patients with a negative test result received oseltamivir. The most common reasons documented for initiating therapy in this group included an underlying medical condition or concomitant diagnosis of pneumonia, ILI, or both, each consistent with CDC guidelines.

Therapy for Patients with ILI or LRTI Not Tested for Influenza Virus

We reviewed records of 50 randomly selected outpatients with ICD-9 codes for ILI who were not tested for influenza virus. Only 3 patients (6%) received oseltamivir (as recommended by CDC). Of the remainder who did not receive the drug, the duration of illness was >48 hours, the patient was "well appearing," or no underlying risk factors were found. The median time from illness onset to obtaining medical attention was 3.7 days (range 0–14 days); 22 (44%) sought treatment within 48 hours. Thirteen (26%) had an underlying medical condition (7 children and 6 adults). For each, however, there was an appropriate reason for withholding therapy, per CDC guidelines.

Among 50 outpatients with ILI and LRTI, 14 (28%) were admitted, 2 to the intensive care unit. The median time from illness onset to obtaining medical attention was 3 days (range 0–28 days); 31 (62%) of 50 sought treatment >48 hours after symptom onset. Eight of 25 (32%) children and 5 (20%) of 25 adults received oseltamivir, and 6 patients received the drug <48 hours from symptom onset. Oseltamivir was administered to 6 (38%) of 16 patients with severe illness and to 7 (25%) of 28 who had an underlying medical condition. The reason for not prescribing oseltamivir was documented in 5 charts, and the reasons included were that symptom onset was >48 hours from the visit to the hospital and that the patient was "well appearing." Overall, 13 (68%) of 19 patients with LRTI who sought treatment within 48 hours of illness onset did not receive oseltamivir as recommended by CDC.

Discussion

We believe that this study provides useful information with regard to the diagnostic and therapeutic behaviors of clinicians caring for patients with possible influenza virus infection. Although our data reflect physician behavior during the 2009–10 influenza A (H1N1) pandemic, the findings are likely applicable to any influenza year because diagnostic test performance, disease intensity, antiviral

agent resistance, and virus strain affect clinical decision making each year.

We were interested in 2 general concepts: practice performance when influenza was clinically suspected and the potential for missed therapeutic opportunities when it was not. For the former, we found that providers' practices were often consistent with CDC guidelines but notable deficiencies were also identified. In particular, a substantial proportion of potentially high-risk patients were not empirically treated, and a reason to withhold therapy could not be documented. This dynamic is similar to that for other medical conditions for which clinical practice guidelines are available: provider behavior at variance with the guideline may reflect available patient-level information or other immediate concerns (*22*,*23*). In any case, we have identified potential areas for targeted education of healthcare providers that should be supplemented by rapid dissemination and follow-up of national guidelines if and when they change over time.

We also found inconsistencies in the use of antiviral drug therapy, which was often at variance with contemporaneous guidelines. In our population, 25% of patients who received oseltamivir did not have ILI or another clear indication for treatment. During the pandemic, the drug was recommended for inpatients with ILI and outpatients with ILI and risk factors for severe illness if they had sought treatment within 48 hours of symptom onset (*5*). However, although too many outpatients without ILI received oseltamivir, too few (32%) received the drug despite having LRTI, a consistent indication for therapy. For most patients with LRTI, we could not identify a reasonable justification for withholding therapy. Not surprisingly, all of these patients received antibacterial agents, yet it remains unclear whether the clinicians actively considered influenza virus as a primary pathogen or risk factor for the presumed bacterial superinfection. Influenza virus infection and its association with secondary bacterial infection is well documented with influenza A(H1N1) pdm09 virus infection and with interepidemic disease (*24*–*27*). Treatment with antiviral drugs in this setting may lessen illness when superinfection exists (*26*,*28*). In this circumstance, greater recognition of the possibility of influenza virus infection and use of antiviral drug therapy may mitigate illness and lessen hospital costs (*29*,*30*).

We found that diagnostic practices were often inconsistent with contemporaneous guidelines. Nearly one third of patients were tested for influenza virus, despite the lack of ILI and ≈20% had no other indication for which testing might otherwise be justified (e.g., headache, myalgia). Previous work has shown that relatively few patients with influenza virus infection have systemic signs without fever, sore throat, or cough (*31*). Although changes in CDC recommendations were quickly disseminated to hospital clinicians by management memo, email, or face-to-face meetings, even more rapid communication and follow-up reminders may have enhanced adherence to guidelines.

We found that the dosage and duration of oseltamivir were generally consistent with CDC guidelines in ≈90% of all treated patients, and specifically for all outpatients. HUMC required the use of a preauthorization drug form that noted the appropriate age- and weight-based dose; an outpatient prescription for oseltamivir would not have been released without a completed form. Such tools have been shown to limit dosing errors (*32*,*33*). Also consistent with the CDC guidelines, >90% of hospitalized patients and patients with severe illness in our study received oseltamivir. Among outpatients, we noted that for ≈50%, an appropriate rationale for not providing oseltamivir was documented in the medical record.

Among the small number of dosing errors identified, >40% were related to inappropriate adjustment for renal insufficiency. More than 90% of oseltamivir is metabolized to oseltamivir carboxylate, 99% of which is eliminated by renal excretion, thus requiring dosage adjustment in this setting. Antimicrobial drug dosing errors are common (*34*,*35*), and a failure to adjust for renal impairment is a frequent underlying reason (*36*,*37*). Although controlled data are not available, oseltamivir has been associated with the development of thrombocytopenia, particularly when renal clearance is artificially lowered by concomitant administration of the drug probenicid (*38*). Attention should be given to patients' renal function, particularly in the elderly (diminished renal clearance) and in those for whom higher doses may be recommended, such as the severely ill or obese (*8*).

We identified clinical management differences between how clinicians prescribed treatment for adult patients and how they prescribed treatment for children. Children who were inpatients were significantly less likely to have ILI at the time of testing and to receive treatment for ILI. When testing was carried out, children were also more likely to test positive for influenza virus than were adults, possibly because of the higher virus load in this population. These data also may reflect more overall testing of children, particularly young children who are more likely than adults to have nonspecific signs and symptoms (lethargy, poor feeding, abdominal pain) (*39*,*40*). In addition, infants and young children may not articulate symptoms of ILI (e.g., sore throat), leading to increased nonspecific testing and treatment of this population.

The main strength of this study is the comprehensive nature of case ascertainment, which included laboratory-based information and review of all prospectively collected logs for emergency department point-of-care testing. However, some patients who underwent testing for

influenza virus may not have been noted in the outpatient log system. We appreciate that ICD-9 code data for ILI and LRTI may be nonspecific, but our prospectively collected ILI data (albeit for a limited portion of the surveillance period) validated the temporal trends for this diagnosis in the outpatient setting. We also did not include data from medical outpatient (nonemergency department) clinics where other patients with influenza may have been identified and treated, perhaps skewing our data to those who were more ill. As a retrospective study, our conclusions depend solely upon information documented in the medical record, which may be incomplete. Also, the use of an antiviral agent authorization form most likely improved the dosing practice, as has been shown in other settings (*32*,*33*). Last, our study population includes only a single academic medical center and therefore may not be representative of the region or the nation.

To our knowledge, similar studies of physician behavior with regard to influenza disease, and for A(H1N1) pdm09 disease in particular, have not been reported. We have identified variations in clinical practice in relation to national guidelines that suggest potential areas of education for future influenza seasons.

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Dr. Vijayan performed this work as a Fellow in Pediatric Infectious Diseases at Harbor–UCLA Medical Center. She is currently an assistant professor of pediatrics at the University of Florida, Gainesville. Her research interests include preventing infections, such as influenza and pertussis in mothers and their infants through maternal immunization, and diagnosis and management of travel- and migration-associated disease.

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Evaluation of Immigrant Tuberculosis Screening in Industrialized Countries

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In industrialized countries, tuberculosis (TB) cases are concentrated among immigrants and driven by reactivation of imported latent TB infection (LTBI). We examined mechanisms used to screen immigrants for TB and LTBI by sending an anonymous, 18-point questionnaire to 31 member countries of the Organisation for Economic Co-operation and Development. Twenty-nine (93.5%) of 31 responded; 25 (86.2%) screened immigrants for active TB. Fewer countries (16/29, 55.2%) screened for LTBI. Marked variations were observed in targeted populations for age (range <5 years of age to all age groups) and TB incidence in countries of origin of immigrants (>20 cases/100,000 population to >500 cases/100,000). LTBI screening was conducted in 11/16 countries by using the tuberculin skin test. Six countries used interferon-γ release assays, primarily to confirm positive tuberculin skin test results. Industrialized countries performed LTBI screening infrequently and policies varied widely. There is an urgent need to define the cost-effectiveness of LTBI screening strategies for immigrants.

Tuberculosis (TB) in industrialized countries has reemerged as a public health concern after decreases in incidence during the 20th century. Over the past 30 years, although industrialized countries have shown country-specific quantitative changes (decrease, stabilization, or increase) in overall TB notifications, they share a similar underlying shift in TB epidemiology: decreasing incidence in the native population and an increasing incidence in foreign-born persons (*1*,*2*).

This disproportionate epidemiology is driven primarily by interaction of reactivating latent TB infection (LTBI) and high or increasing immigration levels. This interaction is demonstrated by the small proportion of clustered cases among foreign-born persons, which is lower than that among native-born persons, in molecular epidemiology studies from diverse industrialized settings (*3*). This interaction is also demonstrated by TB acquired before immigration and high or increasing levels of immigration from countries with a high incidence of TB in sub-Saharan Africa, Asia, South America, and northern Africa to industrialized countries that have a low incidence of TB (*4*,*5*).

Surveillance data from several industrialized countries show that a high proportion of active TB cases in foreign-born persons occurs in the first 5 years after arrival (new entrants) (*6*,*7*). The high level of foreign-born persons with TB in industrialized countries potentially jeopardizes national TB control programs and has reopened the debate about how industrialized, immigrant-receiving countries should screen immigrants (*8*,*9*). Although industrialized countries have national policies on immigrant screening, little contemporary comparison (*10*) of critical elements of these policies has been made.

We conducted an international evaluation of screening practices for TB among immigrants in industrialized countries. We also compared critical elements of national guidance, including whether screening identified cases of active TB or LTBI, which groups were targeted for screening, when screening was conducted, and which screening tools were used.

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Methods

Ethics

No patient-specific data or personal identifiers were used. Our study was an analysis of routine data collected as part of service evaluation.

Sampling Frame

All 31 industrialized (high-income) member states, as of 2010, of the Organisation for Economic Co-operation and Development (OECD) (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/pdfs/12-0128-Techapp.pdf) were included in the study (*11*). These countries have an estimated population of 1.0 billion persons, of whom 109.5 million (10.95%; 95% CI 10.94%–10.95%) are immigrants (*12*). Six of the top 10 immigrant-receiving countries are industrialized OECD countries (*12*). In 2009, median TB incidence in these countries was 7.4 cases/100,000 population (interquartile range [IQR] 6.0–10.6 cases/100,000), and a median of 46.9% (IQR 30.1%–65.0%) cases were in foreign-born persons (*1*,*13*).

Questionnaire

An 18-point questionnaire (online Technical Appendix Table 2) based on a published evaluation of screening practices in the United Kingdom (*14*) was formulated to obtain information on immigrant screening practices in each industrialized country. Data were collected during May–December 2010 by abstracting published, publicly available, national immigrant TB screening guidelines or, more frequently, by contacting (by email) persons involved in local TB control programs and screening of immigrants for TB (usually the TB control program director). Replies were received electronically, and nonresponders were emailed 2 reminders. No person-specific data were collected on the questionnaire.

Country-specific Data

Information for 2009 (or the most recent publically available data) on country-specific TB incidence was used. Countries with low and high TB incidence were classified as having <15 cases/100,000 and ≥15 cases/100,000, respectively, according to national TB reports (*15*–*20*). The proportion of cases among foreign-born persons were obtained from the World Health Organization global database (*21*), and net imigration rates were obtained from the OECD migration database (*22*).

Statistical Analysis

Data were analyzed quantitatively, although certain open answers were categorized by investigators. Categorical responses were summarized by using proportions and 95% CIs, and comparisons were made by using the Fisher exact test. Continuous data were non-normally distributed and summarized as medians and IQRs and compared by using the Mann-Whitney U test. Analyses were performed by using STATA version 12.0 (StataCorp LP, College Station, TX, USA). A p value <0.05 was considered significant.

Results

Response Rate and Profile of Responding Countries

Data were obtained from 29 (93.5%) of 31 industrialized OECD countries (online Technical Appendix Table 1). For these 29 countries in 2009, median TB incidence was 7.6 cases/100,000 (IQR 6.4–10.6), 46.8% (IQR 29.7%–64.6%) of cases were in foreign-born persons, and median net annual number of immigrants was 30,623 (IQR 12,322–77,206). There was no significant difference between responder and nonresponder countries for median TB incidence (p = 1.00), median proportion of cases in foreign-born (persons = 0.68), or median net number of immigrants (p = 0.36).

Coverage and Extent of Immigrant Screening for Active TB

Twenty-five (86.2%) of 29 countries (95% CI 67.3%–96.0%) had a system for screening immigrants for active TB (Table 1; online Technical Appendix Table 3); this system was compulsory in 19 (76.0%) of 25. Sixteen (64.0%) of 25 screened all legal immigrants and 4 (16.0%) of 25 screened selected legal immigrants. A higher number (24/25, 96.0%; p = 0.02) screened refugees/asylum seekers than all legal immigrants. Five (20.0%) of 25 countries restricted screening for active TB to refugees/asylum seekers. Countries that screened immigrants for active TB (either refugees/asylum seekers or legal immigrants) were less likely to have a high incidence of TB (≈15 cases/100,000) (50% vs. 95.7%; odds ratio 0.05, 95% CI 0.003–0.59, p = 0.018).

Timing of Screening for Active TB

Countries differed in when they screened for active TB (Table 1; Table 2, Appendix, wwwnc.cdc.gov/EID/article/18/8/12-0128-T2.htm); several countries tailored screening according to type of immigrant (refugee/asylum seeker vs. legal immigrant). Nine (36.0%) of 25 countries screened prearrival, 5 (20.0%) of 25 screened at arrival, and 23 (92.0%) of 25 screened postarrival. Of the 23 that screened postarrival, 8 (34.8%) of 23 also screened prearrival and postarrival but reserved screening postarrival mainly for asylum seekers/refugees.

Demographic Characteristics of Immigrants Selected for Active TB Screening

Specific immigrants, in terms of age and country of origin, targeted for active TB screening are shown in Table

Table 1. Screening practices for detecting active TB in immigrants in 29 industrialized OECD countries*

Screen for active TB	No. countries positive/ no. tested (%)
Yes	25/29 (86.2)
Compulsory	19/25 (76.0)
Timing of screening†	
Prearrival	9/25 (36.0)
At arrival	5/25 (20.0)
Postarrival	23/25 (92.0)
Type of immigrants screened	
All legal‡	16/25 (64.0)
Selected legal§	4/25 (16.0)
Refugees/asylum seekers¶	24/25 (96.0)
Selection criteria for immigrants screened	
Age	2/25 (8.0)
All ages	23/25 (92.0)
TB cases/100,000 population in country of origin	6/25 (24.0)
>15	1/6 (16.7)
>40	2/6 (33.3)
>50	2/6 (33.3)
>100	1/6 (16.7)
Region of origin#	19/25 (76.0)
All	5/19 (26.3)
All except EU, North America, Australia, and New Zealand	11/19 (57.9)
Other**	4/19 (21.1)
Screening tools used in children	
Clinical examination	6/25 (24.0)
Clinical examination and TST	8/25 (32.0)
Clinical examination and chest radiograph	0/25 (0.0)
Clinical examination, TST, and chest radiograph	2/25 (8.0)
TST	3/25 (12.0)
TST and chest radiograph	3/25 (12.0)
Chest radiograph	3/25 (12.0)
Screening tools used in adults	
Clinical examination	1/25 (4.0)
Clinical examination and TST	1/25 (4.0)
Clinical examination and chest radiograph	9/25 (36.0)
Clinical examination, TST, and chest radiograph	2/25 (8.0)
TST	1/25 (4.0)
TST and chest radiograph	2/25 (8.0)
Chest radiograph	9/25 (36.0)

*OECD, Organisation for Economic Co-operation and Development; TB, tuberculosis; EU, European Union; TST, tuberculin skin test.
†Numbers do not add up to the total because some countries screened at >1 location.
‡Countries in which all legal immigrants (if they meet screening criteria) are screened (see online Technical appendix, wwwnc.cdc.gov/EID/pdfs/12-0128-Techapp.pdf) for more detailed categorization and definitions.
§Countries in which only selected categories of legal immigrants (if they meet screening criteria) are screened (see online Technical Appendix for more detailed categorization and definitions).
¶Countries in which refugees/asylum seekers are screened (see online Technical Appendix for more detailed categorization and definitions).
#Numbers do not add up to the total because the Czech Republic screened refugees/asylum seekers from all countries but legal immigrants only from selected countries.
**Congo, Czech Republic, Israel, Kenya, Moldova, Mongolia, Nigeria, North Korea, Pakistan, Slovenia, South Korea, Tajikistan,Turkmenistan, Uzbekistan, and Vietnam.

1 and Table 2, Appendix. Twenty-three (92.0%) of 25 countries screened all age groups for active TB.

There was more variability in countries of origin that were targeted for screening. Six (24.0%) of 25 countries determined which immigrants should be screened on the basis of TB incidence in their country of origin. Incidence thresholds at which screening was initiated ranged from >15 cases/100,000 to >100 cases/100,000, although >40 cases/100,000 and >50 cases/100,000 were most commonly used.

Nineteen (76.0%) of 25 countries selected migrants for screening on the basis of the country from which they originated. Immigrants from countries with high incidence of TB were most commonly screened; 11 (57.9%) of 19 countries screened immigrants arriving from any region except for the European Union (EU), North America, Australia, and New Zealand.

Methods of Screening New Immigrants for Active TB

Among countries that screened for active TB, screening methods are shown in Table 1 and online Technical Appendix Table 4, although screening at these locations was reserved. In children and certain adults, such as pregnant women, for whom chest radiography is generally avoided, the most common methods of initial screening for active TB were clinical examination plus tuberculin skin test (TST) and clinical examination alone.

Adults and older children were assessed by using similar methods, although countries differed in the minimum age at which they used chest radiographs to screen for active TB (range birth to >18 years). Overall, screening by clinical examination plus chest radiograph and chest radiograph alone were the most frequent methods of screening adults for active TB.

Coverage and Extent of Immigrant Screening for LTBI

The proportion of industrialized OECD countries that screened immigrants for LTBI is shown in Table 3. Sixteen (55.1%) of 29 countries screened immigrants for LTBI. Of these 16 countries, 11 (73.3%; compulsory in 7 [(63.6%] of 11), 2 (13.3%; compulsory in 0 [0.0%] of 2), and 15 (93.8%, compulsory in 8 [53.3%] of 15) screened for LTBI in all legal migrants, selected legal migrants, and asylum seekers/refugees, respectively (12 countries screened >1 immigrant group) (online Technical Appendix Table 3). There was no difference in TB incidence, proportion of cases among foreign-born persons, and net migration when we compared countries that screened and did not screen for LTBI.

Timing of Screening for LTBI

As with screening for active TB, countries differed in when they screened for LTBI (Table 3), which depended

on the status of the immigrant (Table 4, Appendix, wwwnc.cdc.gov/EID/article/18/9/12-0128-T4.htm). Two (12.5%) of 16 countries screened prearrival and 2 (12.5%) of 16 screened at arrival, although screening was reserved primarily for asylum seekers and refugees. LTBI screening was most frequently conducted postarrival in the host country (16/16, 100%).

Demographic Characteristics of Immigrants Selected for LTBI Screening

Details of which immigrant subgroups were targeted for LTBI screening are shown in Table 3 and online Technical Appendix Table 4. In 16 countries that screened for LTBI and imposed age criteria, persons of a wide range of ages (birth to <40 years of age) were screened. Children and young adults were most commonly targeted for screening although 8 (50.0%) countries imposed no upper age limit for screening.

Selection of immigrants for screening of LTBI as determined by TB incidence in the country of origin or by specific countries of origin was conducted in 5 (31.3%) of 16 and 13 (81.3%) of 16 countries, respectively. Selection criteria in the United States and Ireland used TB incidence and country of origin (Table 4, Appendix). The incidence threshold at which immigrants were screened for LTBI ranged from >20 cases/100,000 to >500 cases/100,000. Among 13 countries that screened for LTBI on the basis of specific countries of origin, 5 (38.5%) screened immigrants arriving from countries with a high incidence of TB outside the EU, North America, Australia, and New Zealand, and 5 screened immigrants from all countries.

Methods of Screening Immigrants for LTBI

Screening methods used by 16 industrialized countries that screened immigrants for LTBI are shown in Table 4, Appendix. The most commonly used screening protocol was TST (11/16, 68.8%). Six (37.5%) countries used the interferon-γ release assay (IGRA) when diagnosing LTBI, 3 countries used a stepwise TST plus confirmatory (IGRA) approach, 2 countries advocated single-step IGRA, and 1 country (United Kingdom) recommended TST and confirmatory IGRA (for persons ≤35 years of age) and single-step IGRA (for persons 16–35 years of age).

Table 3. Immigrant screening practices for latent TB infection in 16 industrialized OECD countries*

Screen for latent TB	No. positive countries/ no. tested (%)
Yes	16/29 (55.2)
Location of screening	
Prearrival	2/16 (12.5)
At arrival	2/16 (12.5)
Postarrival	16/16 (100.0)
Selection criteria based on age	
No age cutoff†	6/16 (37.5)
Age cutoff values for screening, y	10/16 (62.5)
<5	1/10 (10.0)
<15	2/10 (20.0)
<35	3/10 (30.0)
<40	1/10 (10.0)
Other	3/10 (30.0)
TB incidence in country of origin	5/16 (31.3)
TB cases/100,000 population at screening‡§	
>20	1/5 (20.0)
>40	2/5 (40.0)
>50	1/5 (20.0)
>100	1/5 (20.0)
>500	1/5 (20.0)
Specific country of origin§	13/16 (81.3)
Countries screened	
All	5/13 (38.5)
All except EU, North America, Australia, and New Zealand	5/13 (38.5)
Other¶	3/13 (23.1)
Screening tools used for LTBI#	
TST	11/16 (68.8)
TST and confirmatory IGRA	4/16 (25.0)
IGRA	3/16 (18.8)

*OECD, Organisation for Economic Co-operation and Development; TB, tuberculosis; EU, European Union; LTBI, latent TB infection; TST, tuberculin skin test; IGRA, interferon-γ release assay.
†All ages screened.
‡Numbers do not add up to the total because certain countries (e.g., Ireland) screened at different incidence thresholds for children and adults.
§Numbers do not add up to the total because certain countries base screening criteria on incidence threshold and country of origin.
¶Ethiopia, sub-Saharan Africa, Asia, Africa, and Eastern Europe.
#Numbers do add up to the total because in some countries (e.g., United Kingdom and United States), >1 screening method is allowed or recommended.

Discussion

Increased attention is being given to TB among immigrants as a public health issue in industrialized countries (*8*), which underscores use of our data in determining how to best augment current TB control programs. Our international evaluation of immigrant screening policies among industrialized OECD countries indicated that although screening for active TB is frequently performed, LTBI screening is less common. Moreover, screening that is performed for active TB and LTBI varies among countries. Our results indicate that heterogeneity exists in screening location, selection criteria for which immigrant subgroups to screen, and screening methods used.

The primary objective of immigrant screening in industrialized countries appears to be to diagnose active TB, either before immigration or soon after arrival in the host country. Although diagnosing and treating infectious TB reduces transmission, data from numerous settings suggest that yields for active TB diagnosed at or around the time of migration are low: 0.35% in recent meta-analyses and lower in UK studies (*23–26*).

Although most countries in our study screened for active TB, screening was not universal, and countries differed in which immigrants they screened. Among countries that screened for active TB, asylum seekers and refugees were most commonly targeted for screening. Although

these groups are at high risk for having TB infection and disease (because of poor social circumstances, inadequate housing, poor nutrition, and stress of migration), and yields and effects from screening are likely to be higher among them (*23*), they constitute only 2.1% of all immigrants to industrialized OECD countries and are likely to have a smaller role in TB epidemiology (*12*). However, a limitation of current national surveillance data is that it does not stratify TB cases among foreign-born persons by immigration status, which makes it impossible to know what proportion of TB cases arise from documented versus undocumented or illegal immigrants.

There was evidence of heterogeneity for specific countries of origin and TB incidence in countries of origin that were selected for active TB screening. Immigrants from countries with high incidence of TB were generally targeted. However, this targeting was partly modulated because free movement of citizens between EU member states indicated that citizens of certain EU nations with a high incidence of TB (particularly where countries based the decision to screen solely on country of origin) were not eligible to be targets for screening when migrating within the EU, although they would be targeted when migrating to countries (mainly non-EU) that based screening policy on TB incidence. In those industrialized countries that used TB incidence as the selection criteria, screening was performed at incidence thresholds from >15 cases/100,000 to >100 cases/100,000.

It is unclear why countries had such different policies although setting the threshold at a higher level may have increased the yield of screening. The specific immigrant subpopulations targeted (either by country of origin or TB incidence in country of origin) may reflect unique migration patterns to each OECD country (*5*), which may stem from colonial, historic, or linguistic links, financial resources, the current health care system, and infrastructure to deal with immigrants. A possible limitation of current screening protocols is that they may not target immigrants from regions with a high incidence of TB who arrive in industrialized, low-incidence settings, acquire citizenship, and then move to a country with a low incidence of TB (although this group might be small).

Similar variation was observed in screening tools used to diagnose active TB. Younger children were often screened by clinical examination with or without a TST, although making a diagnosis of active TB in children is often difficult when based on such limited evidence (*27*). Adults and older children were usually screened by chest radiograph, although the lower age limit at which chest radiographs were permissible varied from birth to 18 years of age. The wide range likely reflects reluctance to unnecessarily expose children to radiation and different, more adult-like patterns of pulmonary disease seen in older children (*28*).

The prevalence of active TB at entry is small and imported active disease that is detectable among immigrants arriving in their country of destination is not driving the increasing disease incidence seen in foreign-born persons in industrialized OECD countries (*24*). Moreover, because epidemiologic data suggest a high and increasing proportion of extrapulmonary TB in foreign-born persons, chest radiographs would play a limited role in diagnosis (*29*,*30*). This finding would limit screening systems that many industrialized countries currently use for adults. The UK Health Protection Agency has reviewed port health regions and recommended urgent review of continued use of chest radiography as the initial diagnostic test for new entrants (currently underway) (*26*).

In contrast, TB epidemiology in OECD countries and molecular typing data indicate that reactivation of imported LTBI in the first few years after immigration is driving the increase in foreign-born persons with TB cases (*3*). Therefore, although screening for active TB is needed, without commensurate targeting of LTBI, screening is unlikely to control TB at a population level. We found that only half of industrialized countries screened immigrants for LTBI, and refugees/asylum seekers were most commonly targeted for screening. This finding indicates that screening legal immigrants for LTBI remains a low-priority TB control measure in industrialized countries, a potential gap that needs to be urgently addressed.

Among countries that screen for LTBI, there was heterogeneity in which immigrant subgroups were screened. For age, children and young adults were most commonly targeted because these groups have the highest risk for progression to active TB and are most likely to benefit from chemoprophylaxis. However, 47% of countries screened all age groups for LTBI, which suggests that in certain countries, older immigrants are given chemoprophylaxis, despite often-cited concerns about hepatotoxicity (*31*).

Similar variability was seen in which countries of origin of immigrants were targeted for screening. Among industrialized countries that selected immigrants on the basis of TB incidence in the country of origin, the TB incidence screening threshold ranged from >20 cases/100,000 to >500 cases/100,000. This wide variation likely reflects uncertainty about the optimal threshold at which to screen. Setting the incidence threshold too low would result in large numbers of immigrants needing to be screened. Thus, a low threshold would increase costs and likely overwhelm TB screening services, although many immigrants from lower-incidence countries (who have a low prevalence of TB) often do not contribute to TB incidence in industrialized countries. In contrast, if the incidence screening threshold

is set too high, few immigrants would be screened, which means that a large proportion of the immigrant population that has LTBI, and subsequently converts to active TB, would be missed (*32*).

The most cost-effective policy option is likely to be to target at an intermediate incidence that balances, most cost-effectively, the numbers of immigrants being screened (and therefore associated costs) against prevalence of LTBI in the immigrant population (*33*). However, in many OECD countries, making cost-effective policy decisions about immigrant screening for LTBI is hampered by gaps in evidence in several areas, including which immigrant groups to screen (depending on TB incidence/country of origin), which screening methods to use, and which location is best for screening. This policy may partly explain variability in screening models adopted by OECD countries. These gaps could be appropriately addressed by obtaining prospective, multicenter data on prevalence of LTBI in immigrants and assessing performance of screening tools and outcomes of screening in different locations. This policy would enable investigators to calculate yields and relative cost-effectiveness of screening at different incidence thresholds (as was conducted recently in the United Kingdom [*33*]), for different screening tools and in different locations, thereby enabling countries to formulate country-specific, evidence-based, immigrant screening policies.

LTBI screening methods also varied widely. The most commonly used screening method was TST. Although TST is widely used and inexpensive, it has poor specificity in *Mycobacterium bovis* BCG–vaccinated populations (e.g., immigrants arriving in industrialized countries), poor sensitivity in immunocompromised persons, and logistic drawbacks, including the need for a return visit and trained staff (*34*). Although data suggest that IGRAs have higher specificity and sensitivity than TST (*34*), their use was limited to 40% of industrialized countries as a confirmatory test for a positive TST result and increasingly as a single-step test to replace TST. This finding may reflect recent evidence that suggests that IGRAs are cost-effective and, if results are positive, can predict progression to active TB (*35*,*36*). However, the predictive power of IGRA for progression to active TB does not appear to be higher than that of TST (*37*). Empirical data are needed for relative performance of these tests in immigrant populations so that contemporary health economic analyses can conclude which screening modality is most cost-effective. Given the pivotal need for predictive power in improving cost-effectiveness of testing for LTBI (*33*), a more powerfully prognostic test would transform the cost-benefit equation for LTBI screening.

A major factor when considering the potential effect of screening for and treating LTBI is that suboptimal completion rates for chemoprophylactic regimens adversely affect efficacy of screening programs, thereby underscoring the need for adopting a patient-centered approach and new, faster-acting, drugs for LTBI (*38*). Given these potential drawbacks, an alternative approach, depending on patient preference and risk perception, could be to follow-up persons with LTBI for clinical signs over a defined period to rapidly identify and treat those with infections that become active. This approach is used in parts of the United Kingdom and the Netherlands (*39*).

Our study builds on previous research, which focused on fewer industrialized countries (*40*) and was conducted some years ago. However, it failed to capture recent changes in guidance (*40*), did not specifically focus on LTBI, and failed to identify the critical elements of immigrant screening programs, such as which immigrants were selected for screening (*10*,*40*).

Our study had several limitations. Information was gathered through a questionnaire with potential for recall/responder bias, although this limitation was minimized by clarifying ambiguous responses of responders or cross-referencing against national guidelines. In addition, our study only captured what screening is currently recommended, and thus presents an idealized situation of how screening should be conducted, which may be different from actual practice at the local level (*14*). Only a detailed assessment of national practice can determine the extent to which national guidance is followed.

TB in industrialized countries primarily occurs in foreign-born persons. Current immigrant screening policies in these countries focus primarily on identifying active TB. Although the contribution of active TB at the time of immigration is crucial, data from 2 large contemporary meta-analyses suggest that the prevalence of active disease in immigrants arriving from countries with a high incidence of TB remains relatively low (0.35%) (*23*,*24*), making cost-effectiveness and value of the current screening strategies uncertain. In contrast, epidemiologic data suggest that LTBI reactivation in immigrants plays a central role in determining national TB incidence. However, LTBI screening is paradoxically limited, and there is no consensus on which immigrants to screen and how to screen.

Addressing these issues is critical to effective TB control in industrialized countries, as is identification and treatment of persons with LTBI, and where control measures should be targeted while remaining vigilant about timely diagnosis and treatment for active disease. To address this problem effectively, robust evidence-based data are urgently needed to develop affordable, effective, and cost-effective policies on which immigrant subgroups to screen (*33*). Such policies will need to be developed in the context of nation-specific economic considerations,

including resource availability and the funds policy makers are willing to spend to control the incidence of active TB.

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Trends in Meningococcal Disease in the United States Military, 1971–2010

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Meningococci have historically caused extensive illness among members of the United States military. Three successive meningococcal vaccine types were used from 1971 through 2010; overall disease incidence dropped by >90% during this period. During 2006–2010, disease incidence of 0.38 (cases per 100,000 person-years) among members of the US military was not significantly different from the incidence of 0.26 among the age-matched US general population. Of the 26 cases in the US military, 5 were fatal, 15 were vaccine failures (e.g., illness in a person who had been vaccinated), and 9 were caused by *Neisseria meningitidis* serogroup Y. Incidences among 17- to 19-year-old basic trainees and among US Marines were significantly higher than among comparison military populations ($p<0.05$). No apparent change in epidemiology of meningococcal disease was observed after replacement of quadrivalent polysaccharide vaccine with conjugate vaccine in 2007. The data demonstrate that vaccination with meningococcal vaccine is effective.

Cases of meningococcal disease have long plagued the United States military, with incidences (defined as cases per 100,000 person-years) as high as 150 during World War I (*1*) and 80 during World War II (*2*). Corresponding incidences among the US general population were 9 and 16 during World War I and World War II, respectively (*2*). In 1969, the incidence among US Army basic trainees was 81 (*2*). Such elevated incidences relative to the non–age-matched US population were attributed to crowding and unhygienic conditions in unique environments. In particular, these factors imposed higher incidences of disease at military basic training centers. Contributing factors likely included the convergence of people from a wide geographic area and the extreme physical demands of basic military training.

During the past century, US military incidence of meningococcal disease has markedly decreased to converge with that of the (non–age-adjusted) US general population (Figure 1). Since the 1970s, the military has maintained a policy of universal meningococcal vaccination for all persons entering all branches of service. After the US Army's early 1970s introduction to all incoming personnel of a vaccine targeting *N. meningitidis* serogroup C, disease rates dropped by >90% (*1*,*4*,*5*). However, during 1971–1989, the Army's mean annual incidence remained significantly higher than that among the non–age-matched general population (3.6 vs. 1.02; $p<0.0001$; general population data from [*3*]).

In 1982, a quadrivalent polysaccharide vaccine (MPSV-4; Menomune, Sanofi Pasteur, Bridgewater, NJ, USA) was introduced; this vaccine targets serogroups A, C, W-135, and Y. No broad-coverage vaccine against serogroup B exists (*6*). During 1982–1989, meningococcal disease incidence among members of the military was 2.1 (*1*); for 1990–2009, rates among both the Army and the US general population dropped significantly, with Army rates not significantly different from those observed in the general population (0.5 vs. 0.7; $p = 0.19$; general population data from [*5*]).

Despite declining incidence during the past 4 decades, the elevated susceptibility to meningococcal disease among members of the US military makes this population of interest regarding the performance of current vaccines. Of particular interest is the performance of the newer conjugate vaccine, MCV-4 (Menactra; Sanofi Pasteur,

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Swiftwater, PA, USA), which gradually replaced MPSV-4 in the military during 2006–2008. During 2009–2010, virtually all vaccinations were with MCV-4.

We report the epidemiology of 26 cases of meningococcal disease that occurred in members of the US military during 2006–2010. Demographics, geographic location, clinical syndrome, vaccination, and death rates are reviewed. Historical and current trends in the military are evaluated and compared with those of the US general population.

Materials and Methods

Case Information Collection

The Naval Health Research Center (NHRC) monitors reports from the Armed Forces Health Surveillance Center (AFHSC) Defense Medical Surveillance System database (*7*) and from the EpiData Center at the Navy and Marine Corps Public Health Center. Possible cases of meningococcal disease are identified from the Defense Medical Surveillance System database by using diagnostic code 036 from the International Classification of Diseases, 9th Revision, or by reports from military and US civilian laboratories that have identified *N. meningitidis* infection in active-duty military members. Possible cases without a confirmatory laboratory diagnosis are defined as probable by the presence of the appropriate clinical syndrome, with laboratory identification of encapsulated, gram-negative diplococci that are not identified as *N. meningitidis*. A case is defined as confirmed if the patient has the appropriate clinical syndrome and a laboratory identification of *N. meningitidis* is made by either PCR or culture. PCR and direct fluorescent antibody testing were used in our laboratories to identify a specific serogroup. The NHRC obtains case information and a variety of patient specimen types from the treating hospital.

Statistical Analyses

Analysis of variance and Student *t* tests were used to compare incidences for which we did not have numerators or denominators over different periods within the Army and military populations and between the Army and military and the general population. The normal approximation was used for the comparison of incidences of >100 cases/year; for incidences <100 cases/year, Poisson modeling was used to evaluate differences between population strata (*8*). Demographic categories evaluated were patient age, sex, military rank, military branch, region, and military versus US general population; *N. meningitidis* serogroups were also compared.

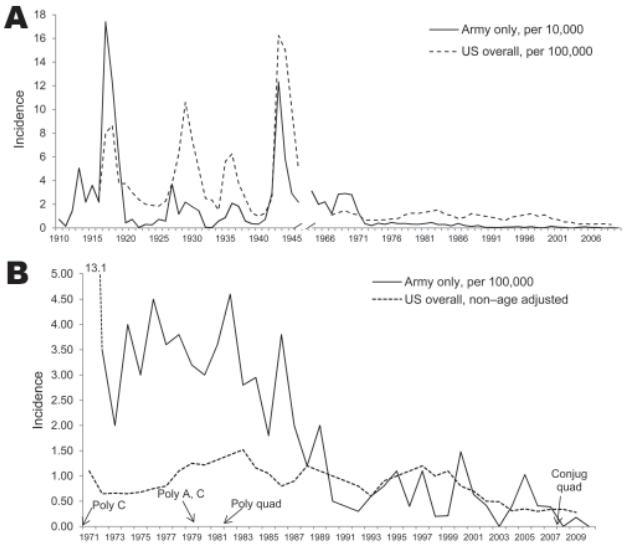

Figure 1. Timeline showing 100 years of meningococcal disease incidence in the US population compared with members of the US Army (A) and effects of introduction of meningococcal vaccines (B; years in which the vaccine types were introduced into the military indicated by arrows). Rates are unadjusted for age matching. Data for the US Army and the general population for 1910–1946 from Brundage and Zollinger (*2*). General population data for 1967–1977 from Brundage and Zollinger (*2*) and for 1978–2009 from the Centers for Disease Control and Prevention (*3*). US Army data for 1964–1998 from Brundage et al. (*1*), 1999–2010 from personal communication with the Armed Forces Health Surveillance Center, and 2006–2009 from Naval Health Research Center Meningococcal Disease Surveillance. The incidence in the Army from 1982–1989 of 2.1 cases per 100,000 person-years was deduced from Figure 1 in Brundage et al. (*1*) by using the percentage of each military division's percentage contribution to the total active-duty military populations during 1982–1989, with each year's percentages estimated from those of the 1990–1992 average. These percentages were stable within ±2 percentage points during 1990–2006. Poly, polysaccharide; C, *Neisseria meningitidis* serogroup C; A, *N. meningitidis* serogroup A; quad, quadravalent (*N. meningitidis* serogroups A, C, W-135, and Y); conjug; conjugate.

Results

Cases

Of the 84 possible cases of meningococcal disease within US military that were reviewed during 2006–2010, the NHRC confirmed 23 cases by culture, PCR, or both; 1 case was determined to be probable. The NHRC also confirmed 2 cases that had not previously been identified by the AFHSC. The result of the surveillance was 25 confirmed cases and 1 probable case, of which 5 were fatal (Table 1); *N. meningitidis* serogroup breakdown was 6 B, 7 C, and 10 Y. The large number of possible cases reported among the military that were not confirmed were likely a result of miscoding of an initial or subsequent diagnosis;

Table 1. Confirmed and probable cases of meningococcal disease among members of the United States military, 2006–2010*

Case no.	Year	Q	Death	Clinical syndromes	Military branch	Patient age group, y/sex	Patient race/ ethnicity	Patient military status	Vaccine type	Months from vaccination to illness	*Neisseria meningitidis* serogroup
1	2006	1	Yes	Men, sep	A	20–24/M	B	AD	MPSV-4	12	C
2	2006	1	No	Men	M	17–19/M	W	AD	MPSV-4	7	C
3	2006	3	No	Men	M	20–24/M	W	Trainee	MPSV-4	NA	UNK
4	2006	3	No	Men, sep	A	17–19/M	W	Trainee	MPSV-4	1	C
5	2006	3	No	Men	M	17–19/M	W	Trainee	NA	1	UNK
6	2006	4	No	Men	M	20–24/M	W	AD	MPSV-4	7	C
7	2007	3	No	Men	N	25–29/M	W	AD	NA	NA	UNK
8	2007	3	Yes	Sep	A	25–29/M	W	AD	MPSV-4	62	B
9	2007	3	No	Sep	CG	35–39/M	UNK	AD	MPSV-4	229	B
10	2007	3	Yes	Sep	A	17–19/M	W	AD	MCV-4	3	C
11	2007	4	No	Sep	N	17–19/M	B	AD	NA	NA	Y
12	2008	1	No	Sep	M	17–19/M	B	Trainee	MPSV-4	3	Y
13	2008	1	No	Men	M	17–19/M	W	AD	MPSV-4	5	Y
14	2008	1	No	Men	M	17–19/M	W	Trainee	MCV-4	2	B
15	2008	2	No	Men	M	17–19/M	W	AD	MCV-4	9	UNK
16	2008	2	No	Men, sep	M	17–19/M	W	Trainee	MCV-4	1	B
17†	2008	3	Yes	Sep	AF	35–39/F	A	AD	MPSV-4	161	B
18	2008	4	No	Sep	M	20–24/M	H	Trainee	MCV-4	1	Y
19	2008	4	Yes	Men, sep	N	20–24/M	W	Cadet	MPSV-4	15	Y
20	2009	1	No	Men, sep	N	20–24/M	W	AD	MCV-4	4	Y
21	2009	1	No	Men	A	20–24/F	UNK	AD	MCV-4	7	B
22	2010	1	No	Sep	M	20–24/M	B	Trainee	MCV-4	1	Y
23	2010	2	No	Men, sep	N	20–24/F	W	AD	MPSV-4	43	Y
24	2010	3	No	Men, sep	M	17–19/M	W	Trainee	MCV-4	3	Y
25	2010	3	No	Men	M	25–29/F	B	AD	MCV-4	8	C
26	2010	4	No	Men	AF	20–24/M	B	AD	MCV-4	7	C

*Q, quarter; men, meningitis; sep, sepsis; A, Army; B, black; AD, active duty; MPSV-4, polysaccharide; M, Marine Corps; W, white; NA, not available (highly unlikely that person was not vaccinated, but vaccination cannot be confirmed); UNK, unknown; CG, Coast Guard; MCV-4, conjugate; N, Navy; AF, Air Force; A, Asian; H, Hispanic/Latino.
†Probable case.

these included miscoding of a patient with viral or aseptic meningitis (n = 34), a final negative laboratory result after an initial diagnosis of meningococcal disease (n = 19), and a positive result on a nonsterile site only (n = 4).

Demographic and Clinical Variables

Relationships and covariations within demographics, military branch, military rank, geographic location, diagnosis, *N. meningitidis* serogroup, vaccination status, and fatalities were examined. Significant differences were found between stratifications within age ($p<0.05$), military branch ($p<0.05$), and rank ($p<0.05$) (Table 2).

Age

During 2006–2010, incidence of meningococcal disease in the military among those >17 years of age was not significantly different from that among the age-matched general population (0.38 vs. 0.26; $p>0.05$; general-population data from Centers for Disease Control and Prevention [CDC], unpub. data). No significant difference was found between the military and general population in the 17- to 29-year-old age group (0.55 vs. 0.40, respectively; $p>0.05$). The peak disease incidence in military personnel occurred among the 17- to 19-year-old age group and was significantly higher than that in their general-population counterparts (2.34 vs. 0.62; $p<0.05$; general population data from CDC, unpub. data). This difference was not present in older age categories; incidences among both military personnel and civilians decreased with age.

In succeeding age ranges (20–24, 25–29, and 35–39 years and ages 17–39 combined), the incidence of meningococcal disease among the military was comparable to that in the general population (general population data from CDC, unpub. data). No cases occurred in the military in the 34–39 and >40 age ranges.

Military Branch and Rank

The mean incidence of meningococcal disease for US military branches ranged from 0.12 for the Air Force to 1.35 for the Marine Corps (Table 2). Of the 11 cases occurring in the 17- to 19-year-old age group, 8 were Marine Corps basic trainees, who are usually 18–21 years of age. Incidence for the Marine Corps was significantly higher than that of the other military branches ($p<0.05$).

Basic trainees as a group had an incidence of 1.18, significantly higher than enlisted and officer personnel ($p<0.05$; Table 2). Service branch and rank, however, are confounded. Marine Corps basic trainees represented 35% (9/26) of the total military cases but only 2% of the military population.

Geotemporal Clustering

While ≈1 million active-duty military personnel (including all recruits) serve in the United States and an additional 400,000 serve around the world, no geographic clustering of cases was observed. In addition, no significant temporal clustering or seasonality of cases was observed.

Serogroup and Vaccination Status

The historical and recent proportions of *N. meningitidis* serogroups per year are shown in Figure 2. Among the 26 cases that occurred during 2006–2010, *N. meningitidis* serogroup was determined for 22; 6 (27%) were B, 7 (32%) C, and 9 (41%) Y (Figure 2). In contrast, in the general population during 2006–2009, 24% were B, 45% C, and

Table 2. Cases of meningococcal disease (n = 26), by *Neisseria meningitidis* serogroup, within demographic and clinical categories, United States military, 2006–2010*

Characteristic	*N. meningitidis* serogroup, no. (%) cases					% Cases	% Total population	Incidence
	B	C	Y	Unknown	Total			
Total	6 (23)	7 (27)	9 (35)	4 (15)	26			
Year of illness								
2006	0	4	0	2	6	23	20	0.440
2007	2	1	1	1	5	19	20	0.367
2008	3	0	4	1	8	31	20	0.581
2009	1	0	1	0	2	8	20	0.142
2010	0	2	3	0	5	19	20	0.354
Death								
Yes	2	2	1	0	5	19		
No	4	5	8	4	21	81		
Age range, y								
17–19	2	3	4	2	11	42	7	**2.343**
20–24	1	3	5	1	10	38	33	0.434
25–29	1	1	0	1	3	12	23	0.188
30–34	0	0	0	0	0	0	14	0.000
35–39	2	0	0	0	2	8	12	0.250
>40	0	0	0	0	0	0	10	0.000
Clinical syndrome								
Meningitis alone	2	4	1	4	11	42		
Sepsis alone	3	1	4	0	8	31		
Both	1	2	4	0	7	27		
Vaccine type								
Conjugate	3	2	4	1	10	38		
Polysaccharide	3	4	4	2	13	50		
Unknown	0	1	1	1	3	12		
Years from vaccination to illness								
<1	NA	6	7	0	13	81		
1–2	NA	1	1	0	2	13		
2–3	NA	0	0	0	0	0		
>3	NA	0	1	0	1	6		
Military branch								
Air Force	1	1	0	0	2	8	23	0.120
Army	2	3	0	0	5	19	37	0.189
Coast Guard	1	0	0	0	1	4	3	0.509
Marine Corps	2	3	5	3	13	50	14	**1.353**
Navy	0	0	4	1	5	19	23	0.302
Location								
Continental United States	4	5	8	3	20	77	71	0.418
Outside continental United States	2	2	1	1	6	23	29	0.310
Race/ethnicity								
Asian	1	0	0	0	1	4	4	0.328
Black	0	3	3	0	6	23	18	0.472
Hispanic/Latino	0	0	1	0	1	4	12	0.123
White	3	4	5	4	16	62	61	0.367
Native American/Alaskan	0	0	0	0	0	0	2	0.000
Unknown	2	0	0	0	2	8	3	
Sex								
F	2	1	1	0	4	15	14	0.401
M	4	6	8	4	22	85	86	0.359
Military rank								
Recruit	2	1	4	2	9	35	12	**1.057**
Enlisted	4	6	5	2	17	65	73	0.330
Officer	0	0	0	0	0	0	15	**0.000**

*Exact denominators are not available in all categories. Percentage values may not equal 100 because of rounding. **Boldface** indicates that incidence for the category is significantly different from the incidence in the remaining population (Poisson p<0.05). NA, not applicable (no serogroup B vaccine).

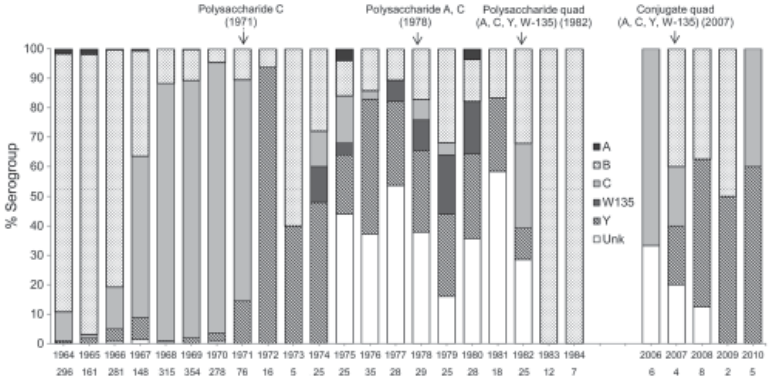

Figure 2. Proportion of each meningococcal serogroup among all isolates tested (1964–1984) or all cases (2006–2010), United States. Years of introduction of vaccine types are indicated by arrows. Unknown during 1964–1980 indicates isolates from a serogroup other than A/B/C/W135/Y or an unknown serogroup; during 1981–1984 indicates isolates that were not B, C, or Y; and during 2006–2010 indicates that no specimen is available and group is unknown. No data were available for 1985–2005. Data for 1964–1984 are from Brundage et al. (*1*) and Brundage and Zollinger (*2*). C, *Neisseria meningitidis* serogroup C; A, *N. meningitidis* serogroup A; quad, quadravalent (*N. meningitidis* serogroups A, C, W-135, and Y). A color version of this figure is available online (wwwnc.cdc.gov/EID/article/18/9/12-0257-F1.htm).

28% Y (CDC, unpub. data). Serogroup B was not evident after 2008, and serogroups A and W-135 did not appear during 2006–2010.

Fifteen of the meningococcal disease cases (6 serogroup B) that occurred during 2006–2010 were in persons who had been vaccinated. The mean time from vaccination to illness was 77 months (SD 97) for serogroup B infection. When the polysaccharide vaccine was used, the mean time to illness was 12 months (SD 14) for serogroups C and Y combined; when the conjugate vaccine was used, it was 4 months (SD 2). The apparent difference in mean time to illness for the 2 vaccine groups may be misleading because among the group of 8 persons who received the polysaccharide vaccine was 1 whose time to illness was 43 months.

Seven infections were serogroup C and 8 were serogroup Y; all of these cases occurred in persons vaccinated >21 days before onset of disease. Of those 15 cases, 13 occurred <1 year after vaccination, one <2 years after vaccination, and one 3–4 years after vaccination. Of the 13 cases in which the time between patient vaccination and illness was <1 year, 6 patients had been vaccinated with MPSV-4 and 7 with MCV-4.

Of the 8 cases during 2006–2007 for which the *N. meningitidis* serogroup was determined, 1 was caused by serogroup Y. During 2008–2010, this serogroup was associated with 8 of the 14 cases with known serogroup, 4 of which occurred in persons who had been previously vaccinated with MPSV-4. Nevertheless, this did not represent a significant increase in the prevalence of serogroup Y infection (p = 0.052).

Fatal Cases

The overall case-fatality rate (CFR) among military personnel with meningococcal disease during 2006–2010 was 19% (5/26 cases), which was not significantly different from the CFR of 13% among the age-matched general population (exact p = 0.24; general population data from CDC, unpub. data). Of the 5 fatal cases, 4 were in persons who had been vaccinated with MPSV-4 (4 deaths among 12 known MPSV-4–vaccinated persons) and 1 in a person vaccinated with MCV-4 (1 death among 12 MCV-4–vaccinated persons).

The Army, Air Force, Navy, and Marine Corps had 3, 1, 1, and 0 fatal meningcococcal disease cases, respectively. Along with a review of data made available by the AFHSC (A. Eick, pers. comm.) and the US Department of Defense Mortality Surveillance Division (L. Pearse, pers. comm.), a review of Army data from various sources published before the current surveillance program began gave us a reliable count of fatal cases during 1997–2005. This, with the Army data cited above (*1*,*2*), enabled us to compare CFRs between historical periods. During 1964–1981, which was the last year of recording until 1997, the CFR in the Army was 6.9%. During 1997–2010, the CFR was 16%. This difference was not significant (p = 0.14).

Discussion

Although there have been vaccine failures (disease occurring 1–48 months after vaccination), universal vaccination continues to have a protective effect against meningococcal disease in the US military. Analyses indicate that during 2006–2010, even as incidence in the general population was declining, *N. meningitidis* serogroup, clinical syndrome, mortality rates, and overall disease incidence among members of the military were not statistically different from those for the US general population except in the 17- to 19-year-old age group, for which the military rate was higher.

A composite of 100 years of meningococcal disease incidence data for the US Army and general population demonstrates the stark difference in incidences until 1971, when the first polysaccharide *N. meningitidis* serogroup C vaccine was introduced into the military (Figure 1). The introduction of the serogroup A/C vaccine in 1978 further reduced the number of infections caused by 2 of

the 3 most prevalent *N. meningitidis* serogroups (the third being serogroup B). From 1990 on, <0.60 cases per 100,000 person-years have been reported. Vaccination against serogroup C may be seen as particularly critical given the history of outbreaks associated with serogroup C in the general population (*6*). Possible reasons for the drop in cases since 1990 are changes in hygiene policies and an increase in routine use of benzathine G penicillin as prophylaxis against group A streptococcal infections in military recruit camps starting in 1991 (J. Brundage, pers. comm.). Previous studies have demonstrated a relationship between meningococcal infection rates and respiratory disease rates, which have also fallen in the military population over the same period, although the association is not entirely consistent (*9*–*12*).

During 2000–2010, the differences in overall incidence between the Army, the military as a whole, and the general US population were small (incidences of 0.40, 0.49, and 0.44, respectively). Although lower incidence may be expected within the universally vaccinated military population, several factors put military members at higher risk, such as a high percentage of relatively young personnel originating from disparate regions and populations with differing carriage rates, as well as challenging operating environments and crowded living conditions. The risk is evident in the military's elevated rates of other infectious diseases, such as respiratory infections (*9*–*12*); however, Artenstein et al. (*13*) suggested that elevated respiratory disease rates were not likely to be relevant. The current infection rates among the military may represent a limit of vaccine effectiveness in this highly vulnerable population.

The year-to-year proportion of *N. meningitidis* serogroups among meningococcal infections has varied in the US general population (review of 2002–2009 annual Active Bacterial Core Surveillance reports; *5*) and the military population (Figure 2). As expected, the percentage of serogroup C infections was reduced dramatically after the introduction of the serogroup C vaccine, and consequently, so was the total number of meningococcal disease cases. Data from 1981–2005 are not available, but it appears that the quadrivalent polysaccharide vaccine was successful in reducing infection rates for all serogroups even further, perhaps by as much as 80% (Figures 1, 2).

During 2007–2008, the military gradually switched from the polysaccharide vaccine (MPSV-4) to the conjugate vaccine (MCV-4). For the cases in our study from 2006–2007 for which vaccine information was available, all but 1 person was vaccinated with the MPSV-4. A spike in cases occurred in 2008, the first year that MCV-4 was universally adopted, but only half of those infected had been vaccinated with MCV-4. It is too soon to determine whether the change to MCV-4 has had an effect on the overall incidence of meningococcal disease in the military population.

Of the 15 vaccine failures (disease occurrence 1–48 months after vaccination), 12 occurred <12 months after vaccination and 2 occurred 1–4 years after vaccination. The unique conditions of the first year of the military-training environment may lead to relatively high rates of observed vaccine failures. The number of recruits entering the military each year has been relatively constant; thus, so has the number of vaccinations. No trend was seen for vaccine failures (4, 1, 3, 1, 4 for the years 2006–2010, respectively), and no data suggest a different rate of vaccine failure for the 2 vaccines. The expectation with the newer MCV-4 was that disease incidence would diminish, but so far, that does not appear to be the case.

Although studies have demonstrated the waning of correlates of protection over time after initial meningococcal vaccination (*14*,*15*), most military personnel do not receive a booster during their military careers (*4*). Boosters are effective (*16*) and are given 5 years after vaccination to personnel traveling to high-risk areas. A study to gather serologic data to address the question of long-term vaccination coverage is underway.

During 2006–2010, *N. meningitidis* serogroup distribution in the military was similar to that for the age-matched general population (general population data from CDC, unpub. data), although the military has seen more serogroup Y infections during the past 3 years when compared with the historical predominance of serogroup C. Serogroup distribution varies year-to-year in the general population; a large increase in the percentage of serogroup Y infections was seen during 1989–1991 (*17*). The increase in serogroup Y infections in military personnel is contemporaneous with the changeover to the MCV-4 vaccine, but half of those cases were in persons who had been vaccinated with MPSV-4. Our data cover only the 2–3 years after the adoption of MCV-4 in the military and are insufficient to support claims about the effect of vaccine change on serogroup Y incidence; thus, we were unable to identify any vaccine-related trends in cases related to particular serogroups. All of the serogroup Y infections have occurred in persons <25 years of age (Table 2).

N. meningitidis serogroup B is not included in the vaccines, but this absence has not led to its dominance over other circulating strains that are included in the vaccine. In fact, serogroup B accounts for fewer infections than serogroups C and Y, which suggests an unknown factor is preventing an increase in the proportion of serogroup B infections in the military.

As in the general US population, the highest incidence of meningococcal disease in the military was among young adults, but the incidence among 17- to 19-year-old military personnel exceeded that among the same age group in the general population (general-population rate from CDC, unpub. data). However, rates for the military should be

compared with those in similar general settings, such as college dormitories, where rates as high as 5.1 have been reported (*18*).

In the Army, the 1997–2010 CFR of 16% for meningococcal disease appears strikingly higher than the CFR of 7% for 1964–1981, a period spanning the use of 3 different vaccines (*1*). Given that the Army had only 1, nonfatal case during 2008–2010, it is reasonable to suggest that the high CFR during 1997–2010 is an anomaly.

In this study, while constituting only 14% of the total military population, the Marine Corps accounted for half the total cases of meningococcal disease in the military. In particular, Marine Corps recruits contributed a disproportionate 7 of 11 cases among the 17- to 19-year-old age group. Given that most cases across all military branches were among basic trainees (mainly the 17- to 19-year-old age group), among whom the rate of 1.18 is significantly higher than for enlisted and officer personnel, and that most of the basic trainee cases were from the Marine Corps, the exceptional rate of meningococcal disease in the Marine Corps is thus a function of the Marine Corps basic training rate. One possible reason for the high rate of infection among Marine Corps basic trainees is that the basic training period is 4 weeks longer than that of other branches, although only 2 of 8 Marine Corps cases occurred during the last 4 weeks of training. In addition, data from Brundage et al. (*1*) and AFHSC (Angelia Eick, pers. comm.) show that the Marine Corps rates over previous 5- and 10-year periods were 0.34 and 0.47, respectively. Thus, the current 5-year rate may be a short-term anomaly.

In summary, while meningococcal disease has historically been associated with substantial illness among members of the US military, disease incidence during the past 5 years is at a historic low and is comparable to the rate among the age-adjusted US population. The current rates in the military have been maintained since the 1990s and can be attributed to effective meningococcal vaccines. While some observed trends suggest covariation between length of basic training, age, and service branch, surveillance has not shown consistent trends by which military rates meaningfully differ from those in the general population. Covariation was seen, however, between the higher rate among basic trainees, age group, and Marine Corps versus the rest of the military.

In the military setting, and especially in the basic training setting, epidemic meningococcal disease remains controlled due to a robust immunization program. To date, no evidence of a change in overall disease rates has been associated with the switch to MCV-4 vaccine. However, the increase of serogroup Y infections among the military population warrants further observation.

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Prevention and Control of Fish-borne Zoonotic Trematodes in Fish Nurseries, Vietnam

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Worldwide, >18 million persons were infected with fish-borne zoonotic trematodes in 2002. To evaluate the effectiveness of interventions for reducing prevalence and intensity of fish-borne zoonotic trematode infections in juvenile fish, we compared transmission rates at nurseries in the Red River Delta, northern Vietnam. Rates were significantly lower for nurseries that reduced snail populations and trematode egg contamination in ponds than for nurseries that did not. These interventions can be used in the development of programs for sustained control of zoonotic trematodes in farmed fish.

Liver and intestinal trematodes are major fish-borne zoonotic parasites of humans. The World Health Organization and the Food and Agriculture Organization of the United Nations estimate that >18 million persons were infected with fish-borne zoonotic trematodes (FZTs) in 2002 (*1*,*2*), and the World Health Organization recently added FZT infections to its list of emerging infectious diseases. FZTs are especially problematic in Asian countries, where fish are a main source of protein (*3*). They are highly prevalent in Vietnam, in cultured and wild-caught fish (*4*–*7*), and cause major concern for food safety (*8*,*9*).

In aquaculture systems, the main risk factors for FZT infection and transmission include contamination of pond environments with FZT eggs from infected hosts, i.e., humans, cats, dogs, pigs, and fish-eating birds. Factors that promote the diversity and population growth of snail intermediate hosts (families Thiaridae and Bithynidae) also increase risk (*10*–*14*). In Vietnam, a large proportion of aquaculture production, mainly of cyprinid fishes, originates from small family farms that integrate other agricultural activities. These integrated fish–livestock systems are called VAC systems (in Vietnamese, the word *vuon* means garden, *ao* means pond, and *chuong* means pigpen). Prevalence studies have demonstrated that in Vietnam, these integrated systems pose the highest risk for FZT transmission (*12*,*15*). The ponds become contaminated through use of animal, and occasionally human, fecal waste as pond fertilizer and through runoff water from pond banks and adjoining fields. Fish fry (newly hatched fish) are introduced into these ponds at about 2–3 days of age, and when they become juveniles (after 6 weeks), they are transferred to grow-out ponds. Efforts to control snail invasion and their population growth in fish ponds are usually minimal. FZT egg contamination of pond environments and FZT transmission are increased by the practice of feeding fish waste to domestic animals and by the cultural preference for eating raw or inadequately prepared fish dishes (*16*).

Recent findings from an investigation in northern Vietnam (*4*,*15*,*17*) revealed high rates of FZT transmission, especially in fish nurseries, where FZT prevalence among stocked FZT-free fish fry increased to 14.1%, 48.6%, and 57.8% after 1 week, 4 weeks, and when overwintered in ponds, respectively. The juvenile fish raised in nurseries are eventually transferred to grow-out farms, thereby potentially seeding a large number of grow-out farms with FZTs.

Our study objective was to evaluate the effectiveness of interventions to prevent FZT transmission in nurseries

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in northern Vietnam. Intervention goals were to reduce FZT egg contamination and to control snail populations in nursery ponds. The study is the first stage in developing a practical and sustainable program for prevention of FZT infections in aquaculture.

The project objective, planned interventions, and sampling procedures were approved by all participating institutions and their research committees in Vietnam. Risks, rights, and benefits were explained to all participating intervention farmers at the beginning of the project. Farmers were informed that they were free to withdraw from the study at any time. Persons from households associated with nonintervention nurseries were examined at the end of the study, and treatment was provided for free to those with egg counts indicating infection with trematodes. Thus, all household members received the same health benefits.

Materials and Methods

Study Area Design

The intervention study was conducted in 4 provinces in the Red River Delta of northern Vietnam (Thai Binh, Nam Dinh, Ninh Binh, and Thanh Hoa Provinces) (Figure 1). These provinces were selected because of their known high prevalence of FZT infections among humans, animals, and cultured fish (*8*,*9*). The study design was a parallel group design of nurseries: 1 intervention group and 1 nonintervention group. Nurseries were randomly selected from a list provided by the Department of Agriculture and Rural Development of each province. The selection criteria were as follows: small farms (<1,500 m^2 of ponds), farms managed by household members, and nursery production of rohu (*Labeo rohita*) in an integrated fish–livestock system. Only 1 pond (study unit) per farm was selected for each intervention and nonintervention nursery. The 2 groups of nurseries were matched as closely as possible by type of production system, location, and size. The fry supplied from hatcheries to the nurseries were confirmed free of FZT metacercariae. Rohu, the fish species produced in all selected nurseries is a well-documented host for FZTs (*15*).

In 2009, a total of 18 intervention nurseries and 18 nonintervention nurseries from the 4 provinces were included in the study. The intervention trial period was May–September 2009 and involved 1 production cycle of juvenile fish. Studies have shown this to be a period of high FZT transmission (*15*). In 2010, different groups of 6 intervention and 6 nonintervention nursery ponds from Nam Dinh and Ninh Binh Provinces were added to the trial (for a total of 24 in each group), which was conducted during May–September 2010.

Figure 1. The 4 River Delta provinces of Vietnam (light gray borders).

Interventions

The interventions were based on previously identified risk factors for FZT transmission in aquaculture (Figure 2) (*13*,*17*). In 2009 and 2010, we evaluated the combined effects of the interventions aimed at reducing environmental contamination with FZT eggs and reducing snail populations in ponds. All farmers for the nurseries selected for intervention participated in a training course on FZT biology and epidemiology. The intervention study objectives and design were explained, and the farmers were given specific instructions on the planned interventions for their individual nursery, determined on the basis of evaluations of risk factors associated with their nurseries. All farmers in the intervention group provided detailed information about the management practices they used to suppress snail populations and their habitat when preparing the nursery ponds before stocking and about the continuing management of the pond after stocking of fry.

At the start of the trial, the ponds at the intervention nurseries were emptied of water and dried for 5 days, during which time the top 3–5 cm of mud from the pond floor was removed to reduce the number of snails. Farmers were also instructed to remove all vegetation from the pond to reduce snail habitat. The banks of the ponds were lined with concrete to help control vegetation. Furthermore, 2 days before the ponds were refilled with water, aquatic vegetation within 3 m of each side of the water intake outside the pond was removed. A filter sock with a 5-mm mesh was fitted around the water inlet pipe to help reduce snail immigration into the pond.

To assess the effects of snail-control interventions, snail density was estimated before fish were stocked and during the nursery cycle. Samples were obtained in a scoop net, 0.25 m wide, cast 2 m off shore, and dragged perpendicularly back to the pond bank. Thus, the area sampled was 0.25 m × 2 m. A total of 5 such samples per pond were obtained. The samples were washed, and all live snails were brought to a field laboratory, where they were examined for cercarial infection according to procedures described by Dung et al. (*14*). Snail sampling was conducted before the intervention cycle and at 3, 6, and 9 weeks after stocking of fry during May–September of 2009 and 2010. Snails were identified by using taxonomic keys (*18*,*19*). Only snails that were potential hosts of FZTs were included in the analysis, i.e., species of the families Thiaridae (*Melanoides tuberculata*, *Sermyla tornatella*, *Thiara scabra*, and *Tarebia granifera*) and Bithynidae (mainly *Bithynia fuchsiana* and *Parafossarulus striatulus*).

Before the trials, all residents, workers, and immediate neighbors of the intervention nurseries were examined for trematode infections by medically trained staff. The Kato–Katz fecal egg count method was used (*20*). Two Kato–Katz smears of 41.7-mg fecal samples were independently examined under a microscope at 400× magnification. FZT eggs were counted and recorded as eggs per gram. All persons in whom trematode eggs were found were treated with praziquantel at 25 mg/kg, administered 3×/d for 1 day (total 75 mg/kg). During the 2009 and 2010 intervention trials, fecal examinations were repeated after 30 and 60 days. Residents and workers of nonintervention nurseries were examined for FZT infection at the end of the trials and offered free treatment with praziquantel if found to be infected with FZTs.

Domestic animals (dogs, cats, and pigs) from the households of the intervention nursery workers and the neighboring households were examined for FZT eggs according to the fecal examination procedure described by Lan Anh et al. (*21*). Animals in which eggs were found were given 1 dose of praziquantel at 40 mg/kg (*22*,*23*). Fecal examinations were repeated every 30 days during the production cycle, and animals with positive results were again treated.

Prevention of FZT egg contamination of the nursery ponds consisted of constructing 10–15 cm elevated cement barrier walls along the pond banks to keep surface water runoff from entering the pond. A 50-cm-high fine mesh net was placed around the ponds to keep dogs and cats out. In all intervention nurseries, deliberate discharge of liquid wastes from pigpens and human latrines into the pond as fertilizer was terminated by blocking drains with cement and bricks. During training sessions, farmers were instructed to deposit pig manure and human excreta fertilizer away from the pond to prevent fecal contamination from surface runoff water.

In addition to providing treatment for FZT- infected humans and animals, we also cautioned farmers and members of intervention households to not eat raw or inadequately cooked fish and to not feed uncooked fish to dogs, cats, or pigs. For intervention nurseries, a project team had regular contact at least weekly with the farmers during pond preparation and during the nursery production cycle. During these visits, they discussed experiences and answered questions about the interventions.

Farmers of nonintervention nurseries were informed that a survey was being conducted on nursery management practices and that examination of their juvenile fish was needed for the survey. These farmers were not asked to alter any of their routine practices during the study periods. At the end of the project, all household members from nonintervention nurseries were informed about FZTs and the interventions to reduce FZT transmission.

Fish Sampling and Examinations

Before stocking of fry, 100 fry from the supplying hatchery were analyzed for metacercariae by the compression method (*24*). At 3, 6, and 9 weeks after stocking of the fry, 100 juvenile fish from each pond were randomly collected in a seine net. The fish were placed on ice and transported within 36 h to the laboratory, where they were maintained at 4°C for a maximum of 5 days until processed. Each whole fish was then ground with a small handheld homogenizer and transferred to a beaker for digestion in a pepsin solution (8 mL concentrated HCl and 6 g pepsin in 1,000 mL water) to release metacercariae (*17*). After digestion, the metacercariae were isolated by sediment washing and identified and counted by examination with a microscope. For each juvenile fish, the number of metacercariae was determined and expressed as intensity (total metacercariae/fish). Up to 10 randomly selected metacercariae from each infected fish were identified to the species level, and the rest of the metacercariae were sorted into >1 type groups: liver flukes (Opisthorchiidae), intestinal flukes (Heterophyidae), and nonzoonotic or dead metacercariae. Metacercariae

were identified by using morphologic criteria (*11*,*17*). All metacercaria species recovered were pooled for statistical analyses.

Data Analyses

Data were double-entered into an Access database (Microsoft Corporation, Redmond, WA, USA) and analyzed by using statistical analysis system software (STATA 11; StataCorp LP, College Station, TX, USA). In 2009, we excluded 1 intervention and 2 nonintervention nursery ponds from the analysis because of high numbers of juvenile fish. In 2010, we excluded another 2 intervention nurseries because the farmers failed to follow intervention protocols.

The number of juvenile fish examined for metacercariae and the area (m^2) searched for snails were used as offsets for the statistical analysis. The analysis used was negative binomial regression (*25*) in a generalized linear model (log link function) among sampling times; interventions and the interaction between sampling times and interventions were entered as factors adjusting for clustering within ponds. The ancillary parameter (α) of the variance function ($var = \mu + \alpha \mu^2$) was estimated in a full maximum-likelihood estimation (*25*). The total number of infected juvenile fish was analyzed by using logistic regression with the same predictor as for metacercaria counts and adjusting for clustering within ponds. Furthermore, the numbers of host snails, infected host snails, and relative egg contamination potential were tested as predictors for infections in fish and snails. The relative egg contamination potential for the animals studied was estimated; i.e., actual fecal egg counts in humans, dogs, cats, and pigs were multiplied by the estimated daily feces production, which was 160, 99, 20, and 1,516 g, respectively (*21*,*26*). The egg numbers were summed for all humans and animals within a given household.

Results

Metacercariae in Fish

A total of 9,266 juvenile fish in 2009 and 5,911 in 2010 from intervention and nonintervention nurseries were examined for FZT metacercariae. The following FZT species were identified: *Clonorchis sinensis* (Opisthorchiidae), *Centrocestus formosanus*, *Haplorchis pumilio* (Heterophyidae), and *Haplorchis yokogawi* (Heterophyidae). Among the recovered metacercariae, 94.00% belonged to the family Heterophyidae, 0.05% were *C. sinensis*, and 5.95% could not be identified.

During 2009, prevalence of FZT infection increased over time among juvenile fish from intervention and nonintervention ponds (Figure 3); however, the increase was significantly less for intervention farms ($p<0.001$).

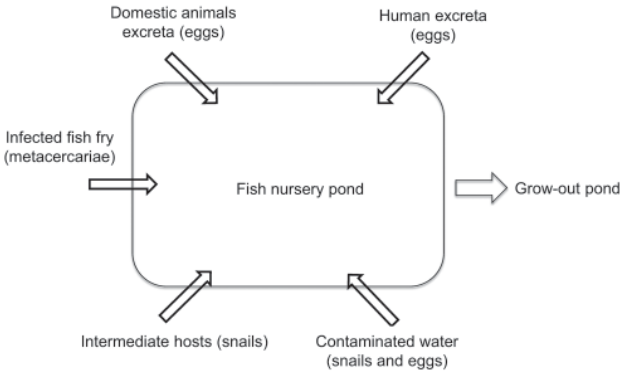

Figure 2. Main risk factors for transmission of fish-borne zoonotic trematodes in fish nurseries, Vietnam. Each risk factor (arrow pointing into pond) is also an intervention point.

The difference in prevalence between intervention and nonintervention nursery ponds increased over time as shown by the significant interaction term ($p<0.01$) between intervention and time of sampling (Figure 3). At 3 weeks after stocking, the odds of infection in the intervention group were 0.77 (95% CI 0.19–3.12) of that in the nonintervention group; after 6 weeks, odds were 0.47 (95% CI 0.08–2.72); after 9 weeks, odds were only 0.22 (95% CI 0.04–1.14). The intensity of metacercariae infection increased over time ($p<0.001$) and was significantly lower among juvenile fish from the intervention nurseries ($p<0.05$) (Figure 4). The difference in intensity of metacercariae between intervention and nonintervention nurseries increased over time, and the interaction between the 2 types of nursery was significant ($p<0.01$). Thus, at 3 weeks after stocking, the intensity of infection in the intervention group was 0.75 (95% CI 0.14–3.82; $p>0.05$) of that in the nonintervention

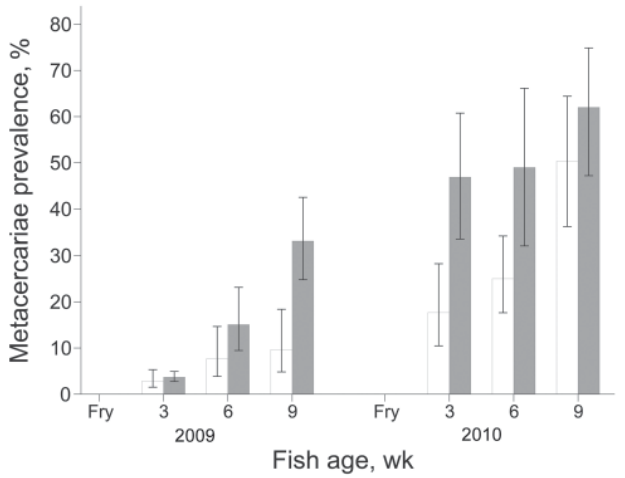

Figure 3. Mean prevalence of fish-borne zoonotic trematode metacercariae in juvenile fish from intervention (white bars) and nonintervention (gray bars) nurseries. Error bars indicate SEM.

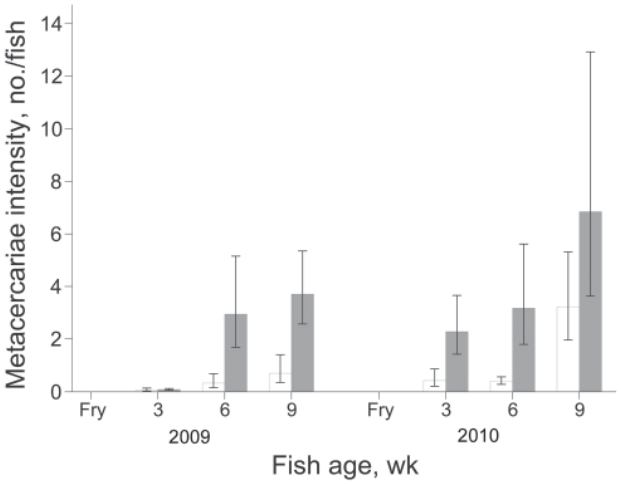

Figure 4. Mean intensity of fish-borne zoonotic trematode metacercariae/fish for juvenile fish from intervention (white bars) and nonintervention (gray bars) nurseries. Error bars indicate SEM.

group; and after 6 and 9 weeks, the intensity was 0.11 (95% CI 0.02–0.68; p<0.05) and 0.19 (0.04–0.87; p<0.05), respectively.

In 2010, a similar pattern developed during the production cycle. At 3 weeks after stocking, the odds of infection in the intervention group were 0.24 (95% CI 0.05–1.23) of that in the nonintervention group, and after 6 and 9 weeks these odds were 0.35 (95% CI 0.07–1.79) and 0.62 (95% CI 0.12–3.19), respectively. Prevalence of infection increased considerably between 6 weeks and 9 weeks at intervention and nonintervention nurseries (Figure 3), consequently, the interaction between intervention and time of sampling was not significant.

In 2010, intensity of infection also increased over time (p<0.001) and the interaction term between intervention and time was significant (p<0.01). Thus, at 3 weeks after stocking, intensity of infection in the intervention group was 0.19 (95% CI 0.03–1.01; p>0.05) of that in the nonintervention group, and at 6 weeks it was 0.13 (95% CI 0.03–0.47; p<0.05). However, at 9 weeks, the intensity in juveniles from intervention nurseries increased to 0.47 of that in the nonintervention nurseries (95% CI 0.10–2.29; p<0.05) (Figure 4).

Effect of Interventions on Snail Populations

During 2009–2010, a total of 30,590 snails were collected. The potential intermediate host of FZTs, i.e., snails of the families Thiaridae and Bithynidae, accounted for 57% (n = 17,478) and 0.04% (n = 133), respectively, of total collected snails. During these years, density of potential host snails (snails/m^2) did not differ between intervention and nonintervention nurseries (Figure 5). The density of host snails was higher in the intervention group than in the nonintervention group when we adjusted for preintervention counts; differences were not significant when this adjustment was not made. However, for 2009, the odds of finding infection in host snails in the intervention group were 0.14 (95% CI 0.07–0.27; p<0.001) of that in the nonintervention group, and the interaction between time and treatment was not significant. For 2010, a significant interaction between time and treatment was found (p<0.05), but odds ratios and 95% CIs between intervention and nonintervention nurseries were <0.01 at all 3 sampling times (p<0.001).

Excretion of Eggs by Humans and Domestic Animals

In 2009, providing treatment for humans in the farm households before and during the interventions reduced the prevalence of FZT eggs from 14.8% to 3.2% (Table); prevalence among comparable members of nonintervention households, who were tested at the end of the production cycle, was 14.2%. In 2010, FZT infections in humans were low or absent among members of intervention households and 12.5% among members of nonintervention households (Table).

In 2009, FZT prevalence and density among pigs from intervention and nonintervention nurseries were low (0.8% and <0.5 eggs/g, respectively), and no significant differences were found between intervention nurseries at the start and nonintervention nurseries at the end of the production cycle (Table). Because of the low prevalence among pigs, these data were not included in the 2010 analyses. During 2009, prevalence of FZT eggs among cats decreased from 50.0% to 15.4% and among dogs from 18.8% to 15.6%. In 2010, prevalence among cats decreased from 57.1% to 0 and among dogs, from 11.8% to 6.7% (Table).

Calculations of mean relative contamination index (no. eggs/household/day) for humans, cats, dogs, and pigs

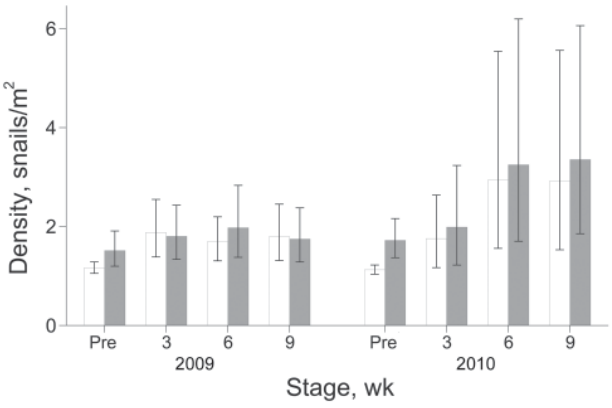

Figure 5. Geometric mean density of potential intermediate snail hosts per square meter in intervention (white bars) and nonintervention (gray bars) fish nursery ponds. Error bars indicate SEM. Pre, preintervention sampling of snails before stocking of fry.

Table. Prevalence and intensity of FZT infections, Vietnam*

Year and animal tested	Egg prevalence in nurseries†			No. eggs/g			Maximum no. eggs/g			Mean potential egg contamination index‡		
	Intervention§			Intervention§			Intervention§			Intervention§		
	None	Before	End	None	Before	End	None	Before	End	None	Before	End
2009												
Man	71 (14.1)	169 (14.8)	188 (3.2)	233.4	55.8	15.9	7,026.4	2,434.1	2,290.2	165,707	40,799	2,174
Cat	6 (50.0)	14 (50.0)	13 (15.4)	3.5	34.6	0.2	9.4	461.5	2.0	70	786	3
Dog	13 (30.8)	48 (18.8)	45 (15.6)	2.3	0.1	1	25.3	2.4	16.3	213	34	252
Pig	85 (1.2)	259 (0.8)	290 (0.3)	0	0	0	0.8	1.8	1.2	101	0	130
2010¶												
Man	32 (12.5)	70 (1.4)	66 (0)	24.7	0.2	0	431.7	12.0	0	25,324	0	0
Cat	0	7 (57.1)	2 (0)	0	12.9	0	0	60.0	0	0	80	0
Dog	2 (0)	17 (11.8)	15 (6.7)	0	3.8	0.1	0	49.0	1.0	0	1.073	0

*FZT, fish-borne zoonotic trematode; man, human (male or female); before, before intervention; end, end of production cycle.
†No. hosts examined (% egg prevalence)
‡Number of eggs excreted by total numbers of final hosts (humans, cats, dogs, and pigs) to pond or pond surroundings.
§FZT egg examinations at nonintervention farms were performed only at the end of the production cycle. The FZT infection status at nonintervention nurseries at the end of the production cycle is comparable with the infection status at intervention nurseries before the intervention was implemented.
¶Because of low FZT prevalence among pigs, pig data were not included in 2010 analyses.

were based on total volumes of excreted feces and the actual egg intensities estimated (Table). Total potential egg contamination before the intervention did not differ significantly between intervention and nonintervention households. Total egg contamination potential did not correlate with prevalence and intensity of metacercaria infection in juvenile fish or with prevalence of infection in host snails.

Discussion

Although prevalence and intensity of FZT infection in juvenile fish increased over time in intervention and nonintervention nurseries, the transmission rate was significantly reduced at intervention nurseries. Because of the need to ensure preharvest food safety in aquaculture products (*27*), the results of introducing risk-reduction measures into fish nurseries are encouraging. For the few previous attempts to prevent FZT infections in aquaculture in Thailand and Vietnam (*28*,*29*), success was limited.

The effect of interventions was similar in 2009 and 2010, except for week 9 in the 2010 cycle, when prevalence and intensity of infection in fish increased dramatically at intervention and nonintervention nurseries. We believe that flooding of the nurseries, which occurred in Nam Dinh in August 2010 after an unusually high tide, caused mixing of water from surrounding areas and contamination of the ponds with potentially infected snails. Sizes of snail populations show large variations among farms. In addition to local ecologic factors, this variation could also be influenced by flood-induced increases in snail distributions and migrations, leading to sudden rises in snail numbers. Because the increase in snail numbers occurred in intervention and nonintervention ponds, the increase was not associated with our control measures. Higher dikes around the ponds could have prevented the flooding of ponds, but the farmers cannot afford to build higher dykes. Flood surface water could also have increased contamination of ponds with FZT eggs from surrounding households and farms. Control of snails is the most challenging part of the interventions; the number of collected snails did not differ significantly between intervention and nonintervention nurseries. Future interventions should focus more on snail control, including new methods like biological and chemical control.

The treatment of FZT infections in humans, cats, dogs, and pigs clearly reduced fecal egg prevalence and density. Although humans had the highest potential for egg contamination, this potential might not reflect their role in sustaining FZT infections on the farm. Because cats and dogs shed most of the eggs into the environment, future interventions for reducing fecal egg contamination of the pond environment should focus on these hosts. In the nursery communities involved in this project, dog meat was often consumed; and at the time of slaughter, intestines are typically washed in the ponds. This practice could be a major source of egg contamination.

In addition to treatment to rid these hosts of infection, the prevention of latrine drainage into the ponds and more emphasis on training farmers about risk factors related to egg contamination should be beneficial. Training should emphasize the risks associated with free-ranging dogs and cats and their indiscriminate defecation near the ponds.

The lack of effect on density of potential intermediate host snails between intervention and nonintervention nurseries suggests that the lower prevalence and intensity of FZTs in intervention nursery snails resulted from treatment of FZT infections in people and animals.

An integrated approach for controlling FZTs in aquaculture would include measures to prevent and control fecal pollution of ponds from animals and humans and reduction of infected snail hosts through better aquaculture management practices; however, the element of practicality cannot be ignored. Although pond management, snail control, and reduction of fecal pollution from latrine drainage can be incorporated reasonably well in a good management program, the treatment of FZT infections in humans and animals is more costly and difficult for the farm owner, family, and employees. It should be noted that the full effect of interventions will probably increase over repeated production cycles as a result of improved management skills of farmers and lower snail and egg concentrations in ponds and surrounding environments. Future efforts to develop a sustainable prevention and control strategy for FZTs should compare the effectiveness of interventions involving only pond management and snail control with and without treatment of FZT infection of household humans and animals.

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Surveillance for Influenza Viruses in Poultry and Swine, West Africa, 2006–2008

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To determine the extent of animal influenza virus circulation in Côte d'Ivoire, Benin, and Togo, we initiated systematic year-round active influenza surveillance in backyard birds (predominantly chickens, guinea fowl, and ducks) and pigs. A total of 26,746 swab specimens were screened by using reverse transcription PCR. Animal influenza prevalence was estimated at 0 (95% CIs for each of the 2 study years 0–0.04% to 0–1.48% [birds] and 0–0.28% to 0–5% [pigs]). In addition, 2,276 serum samples from the same populations were negative for influenza-specific antibodies. These data indicate that the environments and host populations previously identified as harboring high levels of influenza virus in Southeast Asia do not do so in these 3 countries. The combination of climate and animal density factors might be responsible for what appears to be the absence of influenza virus in the backyard sector of the 3 countries.

Relatively little is known about the emergence, prevalence, and circulation of animal influenza viruses in Africa. There is no recent evidence of influenza infection in pigs in West Africa. In 2007, Gaidet et al. (*1*) found a 3.5% prevalence of avian influenza virus in wild birds in Africa; the highest prevalence in Mauritania and Senegal, and the most frequently infected species were Eurasian and African ducks. In addition, low-pathogenic avian influenza viruses of subtypes H1N8, H3N8, H4N2, H4N6, H4N8, H5N1, H5N2, H5N8, H6N2, H7N7, H9N1, and H11N9 have now been detected in wild birds in Nigeria, Egypt, Zambia, and South Africa (*2–7*).

Even less is known about avian influenza in domestic poultry in Africa. South Africa has had numerous outbreaks of many distinct influenza subtypes in chickens and ostriches, including H5N2, H5N3, H6N2, H9N2, H10N7, and H6N8 (*3*,*8–11*). Egypt is still facing recurrent highly pathogenic avian influenza (HPAI) (H5N1) outbreaks (*12*). In contrast, none of the other affected African countries have reported the pathogen since July 2008 (*13*).

We performed a systematic active surveillance study of animal influenza in Côte d'Ivoire, Benin, and Togo. These 3 West African countries reported cases of HPAI (H5N1) only in 2006, 2007, or 2008 (*13*,*14*). We aimed to confirm the current absence of HPAI (H5N1) from the region and determine whether any other influenza virus strains might circulate in domestic birds and pigs.

Materials and Methods

Sampling Sites

Samples were collected exclusively in live-bird markets and backyard farms. The latter were preferred to commercial farms because the outbreaks of HPAI (H5N1) reported during 2006–2008 occurred most often in backyard flocks (for 11/12, 4/5, and 2/3 outbreaks in Côte d'Ivoire, Benin, and Togo, respectively) or on small farms (308–7,771 birds per farm) (*13*,*14*). Sampling sites were selected in the 3 countries for 1) their density of poultry farms (backyard and commercial, even though we focused

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on the backyard sector, as in the district of Abidjan and in the Middle-Comoé region in Côte d'Ivoire; Lokossa in Benin; Lomé and the Maritime Province in Togo), 2) the presence of water bodies and the possible contact of domestic birds with wild waterfowl (South-Comoé in Côte d'Ivoire; Malanville in Benin); and 3) their past outbreaks of HPAI (H5N1) (district of Abidjan in Côte d'Ivoire; Lomé and the Maritime Province in Togo).

In Côte d'Ivoire, samples were collected during January 2009–December 2010. Three regions were selected (Figure 1). Specimens were collected in the district of Abidjan (i.e., Bingerville, Marcory, Treichville, Port-Bouet, Koumassi, Yopougon), the Middle-Comoé region (i.e., Agnibilékro, Takikro, Abengourou, Niablé), and the South-Comoé region (i.e., Aboisso, Adiaké). In Benin, samples were collected during November 2008–September 2010 in live-poultry markets in Malanville, Gogounou, and Dérasi in the provinces of Borgou and Alibori in the north of the country (Figure 1). A total of 200 swab samples were collected from birds in Lokossa (Mono Province) in 2009. The specimens from pigs were collected from animals in slaughterhouses in Parakou (Borgou Province).

In Togo, swab specimens from birds were collected in January and March 2009 and during February–December 2010. Locations were live-poultry markets in Adidogomé, Aklakou, Tabligbo, Vogan, Agoé, Akodesséwa, Aného, Tsévié, Adawlato, and Gbossimé, in Lomé and in the Maritime Province (Figure 1).

In each region in Côte d'Ivoire, a minimum of 5 villages were randomly selected among those willing to participate. Birds from live-bird markets were randomly selected before sampling (5 randomly selected birds per vendor, number of vendors randomly selected depending on the total number of specimens to collect in a given market).

Sample Collection

At each sampling site, >25 birds were clinically examined, and tracheal and cloacal swab samples were collected at least monthly. In Côte d'Ivoire, nasal swab samples from pigs were collected monthly in 2009 and every 3 months in 2010. In backyard flocks in Côte d'Ivoire, serum was collected every 3 months. Each selected market was visited 1×/month in Togo and 2×/month in Benin.

The samples were collected in viral transport media as described (*15*) and then stored in liquid nitrogen or on ice during sampling and transportation to the laboratory, which never exceeded 1 day. Swabs were then immediately stored either at –80°C in Côte d'Ivoire and Benin or in liquid nitrogen in Togo before further processing. Serum was stored at –20°C before further processing.

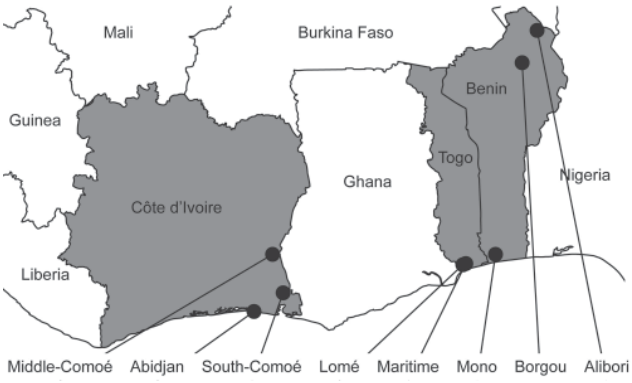

Figure 1. Collection sites of bird and pig samples, West Africa, 2006–2008. Côte d'Ivoire, Benin, and Togo are in gray. Sampling provinces are indicated by black circles.

Serologic Testing

Serum was screened for influenza antibodies by performing ELISAs and/or hemagglutination inhibition (HI) assays. ELISAs were performed by using the FlockChek AI MultiS-Screen Ab Test Kit (Idexx, Westbrook, ME, USA) according to the manufacturer's instructions. HI assays to detect influenza virus were performed as described (*15*,*16*) by using inactivated H5 (A/whooper swan/Mongolia/244/05), H6 (A/turkey/Massachussetts/65), H7 (A/ruddy turnstone/New Jersey/65/85), and H9 (A/duck/Hong Kong Special Administrative Region, People's Republic of China/Y280/97) antigens and positive- and negative-control serum. Serum samples were screened for Newcastle disease virus (NDV)–specific antibodies by performing HI assays with inactivated reference antigen and positive- and negative-control serum.

Molecular Testing

Tracheal and cloacal swabs were processed as described (*17*,*18*). The samples were screened either individually or in pools of 2 or 5 swabs. RNA was isolated by using the RNeasy mini kit (QIAGEN, Valencia, CA, USA), the QIAmp viral RNA minikit (QIAGEN), or the MagMAXTM-96 AI/ND viral RNA isolation kit (Applied Biosystems/Ambion, Austin, TX, USA) with a Kingfisher Flex magnetic particle processor (Thermo Scientific, Rockford, IL, USA). RNA was eluted in 50 μL of nuclease-free water.

The swab samples from Côte d'Ivoire were tested by using 2-step reverse transcription PCRs (RT-PCRs). The RT step was performed by using random hexamers (Invitrogen, Carlsbad, CA, USA) with 10 μL of extracted RNA and the First-Strand cDNA Synthesis kit (GE Healthcare Europe GmBH, Orsay, France,) according to the manufacturer's protocol. Next, 5 μL of the cDNA obtained was used as the template for the PCR step. The PCR was performed

by using the Gene Amp PCR System 2400 (Perkin-Elmer, Applied Biosystem, Paris, France) as described (*14*).

The swabs from Benin and Togo were tested by using 1-step RT-PCRs performed with the Qiagen 1-step RT-PCR kit (QIAGEN) with either an ABI 9700, ABI 2720 (Applied Biosystems, Vienna, Austria) or ABI 7500 (Stratagene; Applied Biosystems, Carlsbad, CA, USA) thermocycler. For conventional RT-PCR screenings, we used primers that target the influenza A matrix gene (*19*) and the following cycling conditions: 1 cycle of 50°C for 30 min; 1 cycle of 95°C for 15 min; 40 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min; and 1 cycle of 72°C for 10 min. For real-time RT-PCR screenings, we used influenza A primers (*18*) with the following cycling conditions: 1 cycle of 50°C for 30 min; 1 cycle of 95°C for 15 min; and 40 cycles of 95°C for 10 sec and 60°C for 30 sec.

Swab samples from birds were screened for other avian pathogens. We used PCR to screen for DNA viruses (infectious laryngotracheitis [ILTV], Marek's disease virus [MDV], and chicken anemia virus [CAV]) and 2-step RT-PCR to screen for RNA viruses (NDV, infectious bronchitis virus [IBV], avian metapneumovirus [aMPV], and infectious bursal disease virus [IBDV]) as described (primers available on request).

Results

Sample Collection

We collected 25,136 swab and 1,819 serum samples from birds and 1,610 swab and 457 serum samples from pigs during the 2-year survey in the 3 countries. Of the bird samples, 70% were from live-poultry markets and 30% from backyard flocks (Tables 1, 2). Specimens were collected year-round, and monthly samples ranged from 20 to 160 and from 218 to 1,778 per month, from swine and poultry respectively.

RT-PCR Screening

The 26,746 total swab samples collected from birds and pigs in Côte d'Ivoire, Benin, and Togo all tested negative for influenza A genome by RT-PCR, irrespective of collection month or host, and the annual prevalence per country was null (95% CI 0.04–4.79%) (Tables 1, 3). To verify that cold-chain or storage problems had not simply degraded our samples' nucleotides, we screened a subset of 2,427 swab samples collected from birds during early 2009 and 2010 from Benin and Togo for other RNA avian viruses (NDV, IBV, IBDV, or aMPV) and DNA viruses (CAV, ILTV, or MDV). Of the 2,427 samples collected in Benin and Togo, the prevalence of the other viral pathogens ranged from 0 for MDV and IBDV to 4.9% for NDV (119 positive samples), 2.8% for ILTV (68), 2.1% for CAV (51), 1.4% for IBV (34), and 0.3% for aMPV (7) (Figure 2). In addition, 3,330 swab samples collected from birds in Côte d'Ivoire in 2010 were screened for NDV; NDV prevalence ranged from 0.3% to 1.4% depending on time (data not shown). Taken together, these results show the fair quality of our specimens. Cold-chain and sample quality were unlikely to account for the absence of detected influenza virus RNA.

Serologic Testing

Because influenza virus infection might last only a few days in birds and pigs, we could have missed the virus in the animals sampled. Therefore, we conducted serologic screening, which provides insight into the infection history of an animal's entire life. None of the serum samples collected in birds in Côte d'Ivoire, Benin, and Togo were positive for influenza antibodies by ELISA or HI assay. Although 16 of 457 pig serum samples from Côte d'Ivoire were weakly positive by ELISA, none were confirmed positive by HI; they most likely were all negative for influenza antibodies. NDV antibodies were detected in 20% of serum samples from birds in Côte d'Ivoire and in 32% of bird serum samples from Togo (data not shown).

Influenza Seasonality and Environmental Factors

Determining the factors contributing to the seasonality of influenza has been difficult because some countries report having influenza activity year-round and others report having 2 peaks of activity or a combination of these patterns. To follow up on recent results (*20*) seemingly confirming the year-round activity hypothesis that states that influenza spread in the tropics is due to contact rather than aerosol transmission, we compared the average livestock production, temperature, and relative humidity (RH) levels in Côte d'Ivoire, Benin, and Togo with those of Nigeria, Egypt, Vietnam, and Indonesia. Côte d'Ivoire, Benin, and Togo produce significantly less bird and pig meat and fewer bird eggs than do Nigeria, Egypt, Vietnam, or Indonesia (Table 4). West Africa is hot and humid all year, with temperatures ranging from 22°C to 32°C and RH ranging from 63% to 82% (Table 4).

Table 1. Active surveillance for animal influenza, West Africa, November 2008–December 2010

	Bird samples tested, no			Pig samples tested, no.	
Country	Oropharyngeal swab	Cloacal swab	Serum	Nasal swab	Serum
Benin	5,230	4,959	100	62	0
Côte d'Ivoire	6,240	6,253	1,283	1,548	457
Togo	1,149	1,305	436	0	0

Table 2. Bird species and collection sites in surveillance for animal influenza, West Africa, November 2008–December 2010

Country	Bird species, %				Type of collection site (% of samples collected)*
	Chicken	Guinea fowl	Duck	Pigeon	
Togo	70	11	13	6	LBM (100)
Benin	88	11	0.001	0.002	LBM (100)
Côte d'Ivoire	92	1	7	0	LBM (34), BB (66)
Total	88	7	5	0.5	LBM (70), BB (30)

*LBM, live-bird markets; BB, backyard birds.

Discussion

HPAI (H5N1) was detected in West Africa during February 2006–July 2008 (*13*,*14*), and all the strains characterized belonged to clade 2.2 (*22*,*23*). Whether wild birds or trade brought the virus to Africa remains unclear (*24*–*26*). However, evidence suggests that the pathogen was first detected in People's Republic of China and might have transited through western Asia, Russia, or Europe (*27*), with wild birds probably playing a role in introducing HPAI (H5N1) to Africa (*27*–*31*). Incidentally, Côte d'Ivoire, Benin, and Togo are on the Black Sea–Mediterranean and East Atlantic flyways that these birds use for migration (*32*).

Within a year after the initial outbreaks, the clade 2.2 strains were thought to have become endemic in Nigeria, and intra–clade 2.2 reassortant viruses were characterized, highlighting the effects of the virus virus and its evolution within the country (*33*,*34*). Nigeria has not reported an HPAI (H5N1) outbreak since July 2008 and now seems free of the pathogen (*13*). The neighboring countries reported only isolated outbreaks in 2006, 2007, or 2008, but no sustained transmission has been reported (*13*).The subtype H5N1 outbreaks in backyard poultry in West Africa were associated with less severe symptoms and lower death rates than those usually described in such outbreaks.

That HPAI (H5N1) is endemic in several Southeast Asian countries and in Egypt but did not persist in West Africa, except for a couple of years in Nigeria, is intriguing. Despite the effect of viruses such as NDV, influenza virus was not detected in any of the swabs or serum samples collected during our active surveillance for animal influenza in Côte d'Ivoire, Benin, and Togo. Several factors, such as type of hosts available, animal density, and climate with its effect on virus persistence in the environment and on virus transmission, might have prevented continued circulation of the virus in the region.

Ducks in particular are a natural reservoir for influenza and play a major role in influenza transmission (*35*). Fewer ducks are raised in Africa than in Southeast Asia (Table 4), which might limit the virus pool and sustainability on the continent. Moreover, the few ducks in West African are backyard birds in low density flocks, not free-range birds in large flocks on lakes and rice paddies as they often are in Southeast Asia. Chickens have so far been the first host infected by subtype H5N1 in Egypt (*36*), but duck meat production is much higher in Egypt than in Côte d'Ivoire, Benin, Nigeria, or Togo (Table 4). The structure of live-bird markets in West Africa also differs substantially from that in Southeast Asia, with fewer birds, fewer species (large majority of chickens and guinea fowls), and often lower confinement (authors' observations).

We believe that temperature and humidity might be critical parameters for the survival of influenza virus in West Africa. Temperature and humidity affect the duration of virus persistence in the environment. In the guinea pig model, influenza transmission by the aerosol route depends on humidity and temperature, although contact transmission does not (*37*,*38*). Of the experimental conditions tested, only 5°C, 20°C, and 35% RH allowed 100% aerosol transmission; thus, warmer and more humid environments might have less aerosol transmission of influenza virus (*37*). Côte d'Ivoire, Benin, Nigeria, and Togo are hot and humid countries year-round. Therefore, one would expect shorter virus persistence in the environment, and, according to the data obtained by using the guinea pig model, one would expect contact transmission rather than aerosol transmission of influenza in West Africa. Still, aerosol transmission may occur in Egypt because the RH is

Table 3. Prevalence of animal influenza, West Africa, 2009–2010

Year	Country	Animal*	Specimens tested, no.	Prevalence, % (95% CI†)
2009	Côte d'Ivoire	Bird	3,895	0 (0–0.08)
	Côte d'Ivoire	Pig	62	0 (0–4.79)
	Benin	Bird	5,669	0 (0–0.06)
	Benin	Pig	1,112	0 (0–0.28)
	Togo	Bird	204	0 (0–1.48)
2010	Côte d'Ivoire	Bird	8,598	0 (0– 0.04)
	Côte d'Ivoire	Pig	436	0 (0–0.7)
	Benin	Bird	3,720	0 (0–0.09)
	Togo	Bird	2,250	0 (0–0.14)

*Backyard and live-bird market poultry.
†95% CIs were calculated with a sensitivity of 99% and a specificity of 100% for the reverse transcription PCR.

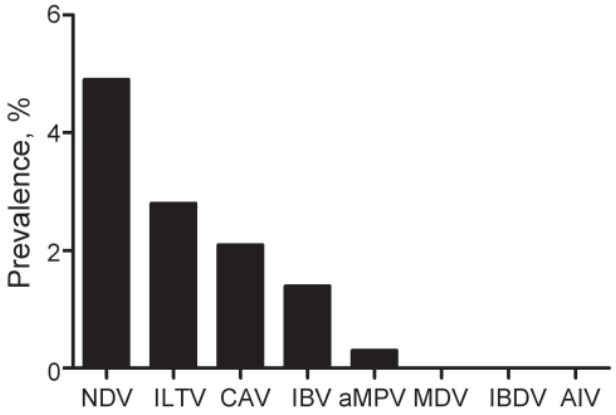

Figure 2. Prevalence of 8 avian viruses detected by reverse transcription PCRs of a subset of 2,427 tracheal and cloacal swab samples collected in live-bird markets, Benin and Togo, 2009. NDV, Newcastle disease virus; ILTV, infectious laryngotracheitis; CAV, chicken anemia virus; IBV, infectious bronchitis virus; aMPV, avian metapneumovirus; MDV, Marek's disease virus; IBDV, infectious bursal disease virus; AIV, avian influenza virus.

much lower than in West Africa and the temperature drops to 9°C in the winter. However, Vietnam and Indonesia (2 countries to which HPAI [H5N1] outbreaks are endemic) are just as hot and humid year-round as West Africa, yet subtype (H5N1) is maintained in birds. Thus, the climate cannot be the only factor limiting influenza in West Africa.

We considered an additional factor—animal density—while trying to determine why influenza might not be sustained in West Africa. The amount of eggs and bird meat and pork produced is considerably lower in Côte d'Ivoire, Benin, Nigeria, and Togo than in Egypt, Nigeria, Vietnam, and Indonesia. Thus, we hypothesized that a high animal density might be required for sustained transmission of the virus. This high density would explain why subtype H5N1 seems to have persisted in Nigeria, with its large avian commercial sector, for a couple of years while causing only sporadic outbreaks in neighboring countries. Our hypothesis is in agreement with the recent finding that influenza prevalence in Egypt is higher in commercial flocks than it is in backyard flocks (*36*).

If influenza virus transmission is limited by climate and animal density, then these limitations should apply to other similar pathogens. In that case, the high prevalence of NDV in Benin and Togo is surprising. However, we had a sampling period bias because all of the NDV (and noninfluenza virus) from screened specimens from Benin and Togo was from specimens collected during January–March when NDV is known to cause disease in the western African backyard sector. Moreover, Songer et al. showed that NDV aerosol transmission at 23°C is better at 10% RH than at 90% RH or 35% RH (*39*). Although we lack data on whether NDV transmission differs from influenza transmission, different viruses, even those with the same nucleic acid core, might have different sensitivities to aerosol generation, depending on the RH level (*39*). Further experiments are warranted to determine whether NDV and influenza A virus transmission patterns actually differ and whether temperature and humidity have any role in that process.

We cannot exclude the possibility that influenza might exist in areas that we did not check. Such influenza hot spots have been discovered for influenza, including 1 at Delaware Bay in the United States (*40*). The requirements for continued circulation of influenza virus in animals (and the role of domestic animals in maintenance and interspecies spread) are not well understood. Future surveillance in West Africa should include more collection sites and include the commercial sectors and wild bird population to survey putative faster transmission and new introductions.

Table 4. Role of influenza virus stability, as influenced by temperature and relative humidity, on hen egg and livestock production, Benin, Côte d'Ivoire, Egypt, Nigeria, Togo, Indonesia, and Vietnam*

Country	Livestock production					Temperature, °C (range)¶#	Relative humidity, % (range)#**
	Hen eggs†‡	Chicken meat‡§	Turkey meat‡§	Duck meat‡§	Pork‡§		
Benin	306	22	ND	ND	4	26.3 (23–30)	74.9 (70–81)
Côte d'Ivoire	610	23	ND	ND	7	26.7 (22–32)	75.3 (70–82)
Egypt	7,000	629	10.5	39	2	22 (9–35)	35.2 (35–46)
Nigeria	12,284	243	ND	ND	218	26.4 (21–33)	84.7 (80–88)
Togo	174	9	ND	ND	9	26.6 (22–32)	70.6 (63–78)
Indonesia	1,123	1,450	ND	31	637	27.7 (23–33)	80.6 (75–85)
Vietnam	247	448	ND	82	2,470	24.1 (13–33)	71.1 (67–76)

*Livestock production: Food and Agriculture Organization data for 2008 (*21*), temperature and relative humidity data: www.climatetemp.info/, March 28, 2012. ND, no data.
†Combination of official data, Food and Agriculture Organization estimates, and calculated data.
‡× 10^6 eggs.
§× 10^6 kg.
¶Annual mean value (coldest monthly average low temperature to warmest monthly average high temperature).
#Temperature and relative humidity were measured in the following cities: Cotonou, Benin; Abidjan, Côte d'Ivoire; Cairo, Egypt; Lagos, Nigeria; Lomé, Togo; Jakarta, Indonesia; and Hanoi, Vietnam.
**Annual mean value (driest monthly relative humidity average to most humid monthly relative humidity average).

Our systematic year-round active influenza surveillance program in the backyard sector in Côte d'Ivoire, Benin, and Togo showed a prevalence of 0 (95% CI 0–0.04% to 0–1.48% in birds and of 0–0.28% to 0–5% in pigs). We hypothesize that the combination of climate and animal density factors might be responsible for what appears to be the absence of influenza virus in the backyard sector of the 3 countries we studied.

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Control of Fluoroquinolone Resistance through Successful Regulation, Australia

Allen C. Cheng, John Turnidge, Peter Collignon, David Looke, Mary Barton, and Thomas Gottlieb

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Learning Objectives

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

- Describe restrictions in Australia regarding use of fluoroquinolones, based on a review
- Describe development of fluoroquinolone resistance in Australia compared to that in other countries, based on a review
- Describe potential harms of restricting fluoroquinolone use, and strategies to eliminate those harms, based on a review

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Fluoroquinolone antimicrobial drugs are highly bioavailable, broad-spectrum agents with activity against gram-negative pathogens, especially those resistant to other classes of antimicrobial drugs. Australia has restricted the use of quinolones in humans through its national pharmaceutical subsidy scheme; and, through regulation, has not permitted the use of quinolones in food-producing animals. As a consequence, resistance to fluoroquinolones in the community has been slow to emerge and has remained at low levels in key pathogens, such as *Escherichia coli*. In contrast to policies in most other countries, this policy has successfully preserved the utility of this class of antimicrobial drugs for treatment of most infections.

Nalidixic acid, the first quinolone introduced into clinical practice, was developed in the 1960s; its use was largely confined to the treatment of urinary tract infections. After the development of several fluoroquinolone antimicrobial drugs, including ciprofloxacin and norfloxacin in the 1980s, and then ofloxacin and levofloxacin, and more recently gatifloxacin and moxifloxacin, the use of this class of antimicrobial drugs increased greatly worldwide. Estimates from the late 1990s suggested that quinolones were the most prescribed antibacterial agent worldwide (*1*). Soon after these drugs were registered, the government of Australia developed policies to restrict use of quinolone antimicrobial drugs by humans and to prevent their use in food-producing animals. These policies have been associated with low rates of resistance to this valuable antimicrobial drug class in Australia.

Usefulness of Fluoroquinolones

Quinolone antimicrobial drugs are commonly used as first-line empiric therapy for urinary tract infections, upper and lower respiratory tract infections, enteric infections, and gonococcal infection. They are particularly useful against deep infections caused by gram-negative bacteria, including those, such as *Pseudomonas* spp., that are resistant to other orally administered antimicrobial drugs. Specific quinolone antimicrobial drugs administered to pets and food-producing animals are known to transmit cross-resistance to humans (*1*).

Contribution of Fluoroquinolone Use to Fluoroquinolone Resistance

Quinolones act by inhibiting bacterial DNA gyrase and/or topoisomerase IV (*2*). Target modification is a common mechanism for resistance, in which ≥1 point mutations in the *gyrA* or *parC* genes generate unequivocal resistance. This mutation can be induced in vitro by exposure to antimicrobial drug concentrations of >8× the MIC (*3*). Other mechanisms can also mediate resistance, including decreased expression of porins, leading to decreased membrane permeability, and overexpression of antimicrobial drug efflux pumps (*2*). The transfer of quinolone resistance by mobile genetic elements has the potential to rapidly disseminate resistance, and its contribution to the spread of resistance is being increasingly recognized (*4*). Under certain circumstances, resistance to fluoroquinolones can emerge during treatment. Some studies have reported that < 50% of all patients taking quinolones for prostatitis are colonized with quinolone-resistant *Escherichia coli* strains (*5*) and have described quinolone resistance after treatment courses of as few as 3 days (*6*). Whether resistance is caused by de novo resistance mutations or amplification of resistant strains already present in low numbers is not known. Furthermore, even parenteral fluoroquinolones are actively excreted into the intestine and may select for resistance in normal intestinal flora.

Although other factors are likely to contribute to resistance in persons, ecologic data show an association between fluoroquinolone use and resistance. This finding is supported by differences between the usual habitats of certain bacterial species and the effect fluoroquinolone use has on resistance development. Because some bacteria, such as *Streptococcus pneumoniae, Neisseria gonorrhoeae*, and *Salmonella enterica* serovar Typhi are transmitted from human to human, resistance in these organisms is likely to indicate human use of antimicrobial drugs and consequent antimicrobial drug selection pressure. Resistance in *N. gonorrhoeae* and *S.* Typhi are also influenced by variations in global epidemiology of disease and in ease of availability of quinolone antimicrobial drugs, including over-the-counter access in Asia, where much higher levels of resistance have been documented (*7*). Resistance in *Campylobacter* spp. and non-typhoidal salmonella is more likely to reflect antimicrobial drug administration to food-producing animals (*8*). *E. coli* is widely distributed among humans, animals, water, and some foods; thus, selection pressure is likely to be exerted by antimicrobial drug use in human and agricultural sectors. This likelihood is supported by molecular typing studies in which researchers examined *E. coli* strains resistant to trimethoprim-sulfamethoxazole, quinolones, and extended-spectrum cephalosporins in humans and in commercial poultry products in the United States, where these antimicrobial drugs are or have been used in poultry production (*9*). The authors found that resistant strains in humans were more closely aligned with resistant isolates in poultry than to susceptible human strains, suggesting that the resistant strains in humans were most likely to be of poultry origin.

Low Use of Quinolone by Humans and Prohibition of Its Use in Food-producing Animals in Australia

Three quinolones are available for systemic use in humans in Australia: norfloxacin, ciprofloxacin, and moxifloxacin. Other quinolones have been available in the past (nalidixic acid, enoxacin, trovafloxacin, and gatifloxacin) but have been withdrawn from the market for a variety of reasons. In Australia, national guidelines for antimicrobial drug use in humans have been published and expanded since 1976. Indications for antimicrobial use are reviewed by a panel of infectious diseases experts approximately every 3 years (*10*). These guidelines are widely promulgated and generally accepted as a standard for prescribing antimicrobial drugs in the community and in hospitals.

The use of quinolone antimicrobial drugs in Australia has been actively constrained by guidelines that recognize their status as a reserve antimicrobial drug. For example, in the current guidelines, ciprofloxacin is not listed as an option in the management of lower urinary tract infection, and it is listed as a treatment for acute pyelonephritis only when resistance to all other recommended drugs is proven or the causative organism is *Pseudomonas aeruginosa*. For treatment of foot infections in persons with diabetes, ciprofloxacin is only recommended as an alternative for patients with penicillin hypersensitivity; the drug is listed for water-related infections caused by *Aeromonas* spp., but is not listed for treatment of wounds caused by other organisms. For respiratory infections, moxifloxacin is not listed as an option for the empiric treatment of community-acquired pneumonia in outpatients, except in patients who have severe penicillin hypersensitivity; ciprofloxacin is listed as an option to treat Legionella infection and in directed therapy for infections in which a susceptible pathogen has been identified. In almost all other countries, quinolones have been freely available and used for a broad range of indications as first-line therapy and have been promoted in treatment guidelines for conditions such as community-acquired pneumonia and uncomplicated urinary tract infections (*11*,*12*)

Australia has a subsidized outpatient pharmaceutical plan, the Pharmaceutical Benefits Scheme (PBS). Relatively expensive drugs (more than AU$30, in 2010 dollars) are not used widely unless prescribed by doctors according to indications listed by PBS. After 1988, ciprofloxacin use was subsidized by the PBS for "serious infections for which no other oral antimicrobial agent is appropriate." In response to growing expenditures in the early 1990s, the Pharmaceutical Benefits Advisory Committee consulted with the National Health and Medical Research Council Working Party on Antibiotics, which suggested that specific indications would result in a more targeted use of quinolones. This suggestion was subsequently adopted by the PBS, and the list of indications underwent several modifications over the years, eventually leading to the PBS authority indications listed in Table 1.

The quinolones used in the treatment of respiratory infections, moxifloxacin and gatifloxacin, were approved for use in Australia in 2001. The Expert Advisory Group on Antimicrobial Resistance, the successor to the Working Party on Antibiotics, advised the Pharmaceutical Benefits Advisory Committee on the listing of moxifloxacin. For a few years, moxifloxacin was available only with authorization for treatment of *S. pneumoniae* pneumonia with proven penicillin resistance and was voluntarily

Table 1. Quinolone antimicrobial drugs available for use in humans in Australia under the authority of the PBS*

Drug/route of administration	PBS listed indication	Consumer cost in AU$			Generic available
		Private market†	PBS GB	PBS CB	
Ciprofloxacin					
Oral	Respiratory tract infection proven or suspected to be caused by *Pseudomonas aeruginosa* in severely immunocompromised patients Bacterial gastroenteritis in severely immunocompromised patients Infections proven to be caused by *P. aeruginosa* or other gram-negative bacteria resistant to all other oral antimicrobial drugs Joint and bone infections, epididymo-orchitis, prostatitis, or perichondritis of the pinna suspected or proven to be caused by gram-negative or -positive bacteria resistant to all other appropriate antimicrobial drugs Gonorrhea	25.20	34.20	5.60	Yes
Topical, ear	Treatment of chronic suppurative otitis media in Aborigines or Torres Strait Islanders >1 mo of age Treatment of chronic suppurative otitis media in a patient <18 y of age with perforation of the tympanic membrane Treatment of chronic suppurative otitis media in a patient <18 y of age with a grommet in situ	24.51	19.38	5.60	No
Topical, eye	Bacterial keratitis	33.71	28.58	5.60	No
Ofloxacin					
Topical, eye	Bacterial keratitis	35.40	32.24	5.60	No
Norfloxacin					
Oral	Acute bacterial enterocolitis; complicated urinary tract infection	31.68	17.16	5.60	Yes
Moxifloxacin‡					
Oral	No longer listed	70.65	NA	NA	No

*PBS, Pharmaceutical Benefits Scheme; GB, general beneficiaries; CB, concessional beneficiaries, including pensioners; NA, no longer available in PBS.
†Price to consumer varies between retail outlets.
‡Data from 2011.

withdrawn from the PBS by the manufacturer for marketing reasons. Moxifloxacin continues to be promoted by industry for use in hospitals and on the private (non-PBS) market, but estimated community use remains low (Figure 1). No applications for use in Australia have been made for other quinolones used widely elsewhere in the world, including oral ofloxacin and levofloxacin.

Although some quinolones have been approved for use in companion animals (Table 2), they have not been approved for use in food-producing animals in Australia. The National Registration Authority for Agricultural and Veterinary Chemicals (now the Australian Pesticides and Veterinary Medicines Authority) sought advice on the administration of enrofloxacin to pigs in the mid-1990s. The Expert Advisory Group on Antimicrobial Resistance advised that quinolones were an antimicrobial drug class of high importance in humans and should never be licensed for food-producing animals because of the risk for drug resistance in enteric pathogens and their potential transmission to humans through the food chain. This was supported by similar international guidance published at the time (*13*); the application was subsequently withdrawn. A range of quinolones have been registered in Australia for dogs and cats, but typing studies in Australia have so far demonstrated that quinolone-resistant *E. coli* found in humans are generally different from those in companion animals (*14*).

In other countries where fluoroquinolones are used in food production, they are also often added to the drinking water provided for many food production animals, including poultry. In many middle-income countries, particularly those in Asia and Latin America, many quinolone antimicrobial drugs are licensed for use in cattle and poultry, and exposure in these animals is probably frequent and widespread (*8*,*13*). In the United States, quinolones were widely used in poultry until Food and Drug Administration officials withdrew approval for this use in 2005 because of the unacceptable resistance risk (*15*). In Europe, quinolone use remains a small proportion of antimicrobial drugs used in food animals (*16*).

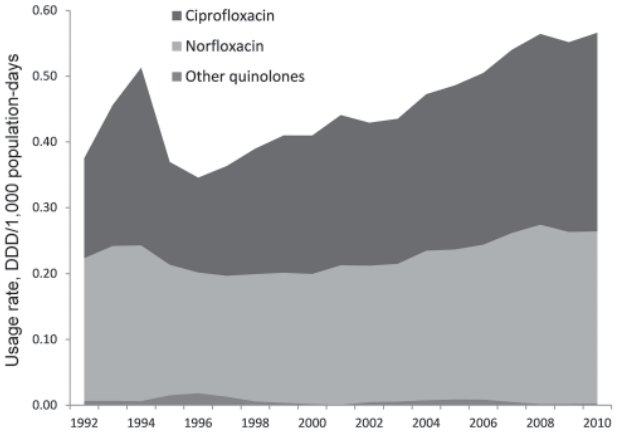

Figure 1. Data from Drug Utilization Sub-Committee Drug Utilization Database on Pharmaceutical Benefits Scheme and the Repatriation Pharmaceutical Benefits Scheme (RPBS) on subsidized medicines and estimates of non-subsidized medicines. RPBS data were calculated from continuous data on all prescriptions dispensed from a validated sample of community-based pharmacies. In-patient hospital prescribing is not included. Usage rate calculated on the basis of medication use of 1,000 persons per day. DDD, defined daily dose.

Low Resistance Rates in Australia Compared with Other Countries

Data for bacterial resistance are available from longitudinal studies performed by the Australian Group on Antimicrobial Resistance (www.agargroup.org/), a network of 30 participating laboratories representing all states and mainland territories that periodically perform nationally representative studies of bacterial isolates from hospitalized and nonhospitalized patients. Quinolone resistance rates in disease-causing isolates of *E. coli* have remained consistently low in Australia. Before 2006, studies included isolates from both community-acquired and hospital-acquired *E. coli* infections and showed a rise in the percentage of isolates from 1992 (0.4%) to 1998 (1.0%) to 2006 (4.9%) (*17*). Since then, surveillance of isolates from community-acquired infections has shown resistance in 89 (4.1%) of 2,155 strains tested in 2008 (*18*) and 108 (5.2%) of 2,092 strains tested in 2010 (Australian Group on Antimicrobial Resistance, unpub. data). This finding is in contrast to data published regarding the United States, where resistance in urinary isolates increased from 3% to 17% during 2000–2010 (*19*), and Europe, where resistance correlated with antimicrobial drug use was described in as high as 45% of isolates in 2008 (*20*) (Figure 2). Similar ecological correlations have been observed between the use of quinolones in pigs and poultry and quinolone resistance in E. coli from humans (*21*).

Quinolone resistance in locally acquired *Campylobacter* spp. is relatively uncommon in Australia compared with most other countries. Several case–control studies in Australia showed that the proportion of disease caused by resistant *Campylobacter* spp. was low (0% in 2003 and 2.6% in 2006) and mainly attributable to resistant strains in returned travelers (*22*). Quinolone resistance in isolates of *Campylobacter* spp., *Salmonella* spp., and *E. coli* from a variety of food-producing animals and products is rare in Australia (*23*,*24*). Notably, there is no importation of fresh meat into Australia. This finding is in contrast to the situation in other countries where bacterial resistance in humans, food-producing animals, and products emerged

Table 2. Quinolone antimicrobial drugs available for use in companion animals in Australia under the authority of the Pharmaceutical Benefits Scheme

Drug (date registered)	Indication
Difloxacin (2001)	Treatment of infections in dogs caused by difloxacin-sensitive organisms
Enrofloxacin (2004)	Treatment of urinary and respiratory tract infections, deep pyodermas, wounds, abscesses, and discharging sinuses in dogs caused by enrofloxacin-susceptible bacteria
Orbifloxacin (1999)	Treatment of diseases in dogs and cats caused by orbifloxacin-sensitive bacteria
Ibafloxacin (2007)	Treatment of urinary tract, respiratory tract, skin, and soft tissue infections in dogs and cats caused by ibafloxacin-sensitive bacteria

in the 1990s, coincident with the increase in quinolone use among humans and animals, and led to the US Food and Drug Administration's 2005 withdrawal of approval of quinolones for use in poultry production (*15*). Resistance rates >80% in *Campylobacter* spp. have been reported in some countries, such as Thailand (*25*). Quinolone resistance in *Campylobacter* spp. emerged in the United Kingdom after the licensing of enrofloxacin for veterinary use (*13*). This finding is consistent with those of the rapid emergence of resistance in *Campylobacter* spp. in chickens treated with quinolone (*26*).

In North America, quinolone resistance in pneumococci increased after levofloxacin was added to respiratory infection guidelines and its use became widespread (*27*). Resistance remains relatively low (*28*), probably reflecting the minimal use of quinolone in children, among whom pneumococcal carriage is more common than among adults. However, trends in ciprofloxacin-resistant *S. pneumoniae* closely parallel increased quinolone use in Canada, demonstrated by an increase in resistance from 0.6% in 1997 to 7.3% in 2006, and higher rates in elderly persons (*29*). In a similar manner, in Spain, ciprofloxacin resistance rose from 0.6% during 1991–1992 to 3.0% during 1997–1998, and was associated with an increase in quinolone use (*30*). By comparison, results from surveys in 2005 and 2007 by the Australian Group on Antimicrobial Resistance demonstrated low levels of antimicrobial drug resistance in Australia, demonstrated by moxifloxacin resistance detected in 0.1% of 1,775 isolates in 2005 and 0.2% of 1,814 isolates tested in 2007 (*31*).

An exception to the generally low rates of resistance in Australia is quinolone resistance in gonococci, which threatens to erode the effectiveness of treatment programs. In 2009, the Australian Gonococcal Surveillance Programme reported that 43% of tested gonococci were resistant to ciprofloxacin; however, ≈33% of those infections were acquired overseas (*32*). Resistance rates remain low in the Northern Territory, where acquisition is almost all local.

Conclusions

Causation is sometimes defined by the counterfactual, or in this case, asking, "what would have happened if restriction had not occurred?" This question cannot conclusively be answered by ecologic data, and other factors are also likely to contribute to the observed heterogeneity between and within countries (*33*). However, it is notable that Australia, among the few countries to have successfully limited the use of quinolones in humans and prohibited their use in food-producing animals by public policy, and moreover has a safe water supply and a lack of imported uncooked meats from countries with high levels of resistant bacteria, remains among countries where quinolone resistance is low among virulent pathogens, such as *E. coli*. The link between fluoroquinolone use and resistance is supported by in vitro studies (*34*), analogy with other evolutionary processes, experimental studies in

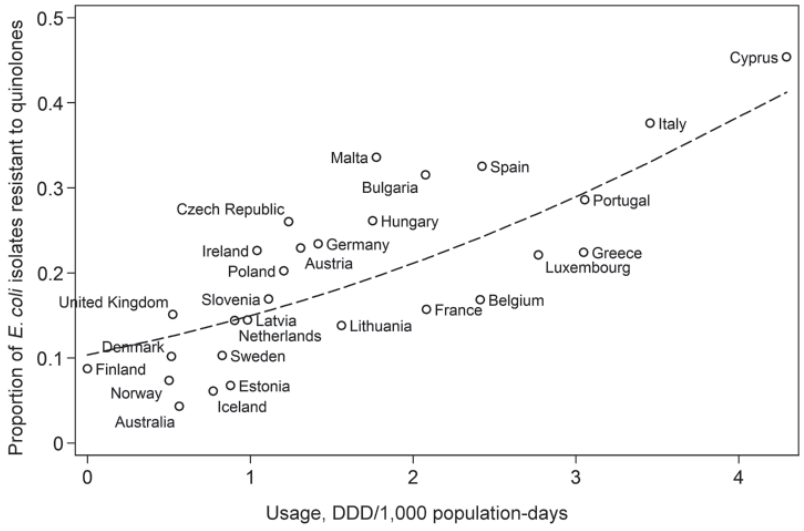

Figure 2. Quinolone use data for Europe from the European Surveillance of Antimicrobial Consumption initiative for antibiotic use in ambulatory care settings and European Antimicrobial Resistance Surveillance System. Use data for Australia from the Australian Group on Antibiotic Resistance (community isolates) and Drug Utilization Sub-Committee Drug Utilization Database (Commonwealth of Australia). Line represents logit-modeled relationship between resistance and usage, weighted by number of isolates tested. Usage rate calculated on the basis of medication use of 1,000 persons per day. DDD, defined daily dose; *E. coli*, *Escherichia coli.*

humans and animals (*5*,*6*), and ecologic studies showing a temporal association and a dose-response relationship between fluoroquinolone use and resistance (*20*).

Regulatory controls are probably the most effective, albeit crude, tool available to restrict antimicrobial drug use. Australia has had a long tradition of prescriber education through the National Prescribing Service and Therapeutic Guidelines. However, the effectiveness of educational initiatives is not clear because of the lack of published formal evaluations. Bulletins and letters about serious adverse events associated with flucloxacillin were sent to general practitioners by the Therapeutic Goods Administration, but they had minimal impact on prescribing volumes. Only subsequent restrictions imposed by the Therapeutic Goods Administration and PBS resulted in a 30% reduction in flucloxacillin use and 50% reduction in reported adverse hepatic events during 1994–1995 (*35*). However, the effectiveness of restriction of public subsidies as a tool to restrict human antimicrobial drug use is likely to be eroded as the price of antimicrobial drugs decreases (*36*). This measure is also not useful in influencing use of antimicrobial drugs in hospitals. To date, the relatively high cost of fluoroquinolones, such as ciprofloxacin, relative to other subsidized antimicrobial drugs, has meant that quinolone use has been restricted in Australia.

Data support the assertion that quinolone use in the community is low and use in hospitals in Australia is moderate. The Drug Utilization Sub-Committee Drug Utilization Database is used to estimate total outpatient antimicrobial drug use. This estimate was obtained by combining Medicare Australia data for government-subsidized medicines (PBS and Repatriation Pharmaceutical Benefits Scheme) with an estimate of nonsubsidized medicines, which is calculated from prescriptions dispensed from a validated sample of community-based pharmacies (*37*). Total use is converted to a rate of defined daily doses per person by using internationally standardized methods (*38*).

Although data from the early 1990s showed that retail sales for all antimicrobial drugs in Australia was higher than in countries in Europe, recent data show that quinolone use in Australia has remained lower than in countries in Europe (*37*) (Figure 2). The National Antimicrobial Utilization Surveillance Project collects data on broad-spectrum antimicrobial drug use in 32 hospitals. Usage data is based on purchasing data and suggests that although overall hospital use of these drugs in Australia is higher than that in Denmark, the Netherlands, and Sweden, quinolone use in Australia is similar to or lower than use in hospitals in these countries (*39*).

Have the limitations on prescribing quinolone in Australia had deleterious effects on human and animal health? Quinolones can still be prescribed when they are necessary or the indicated preferred treatment. Difficulties could be theorized where appropriate empirical therapy is delayed. However, in most of circumstances, empirical regimens in national prescribing guidelines indicate use of β-lactam agents, aminoglycosides, or macrolides and recommend quinolones only when microbiological confirmation of etiology and susceptibility testing demonstrates they are appropriate. The few circumstances where quinolones would be preferred empiric therapy (severe community-acquired pneumonia in patients with immediate hypersensitivity to penicillin) are usually accounted for in local stewardship protocols for severely ill patients in hospitals.

Regarding controls for food-producing animals, although the potential for antimicrobial resistance remains a criterion for Australian Pesticides and Veterinary Medicines Authority registration, external advice from the Expert Advisory Group on Antimicrobial Resistance ceased in 2004. Since then, other valuable classes of antimicrobial drugs used in humans have been registered for animal use, including third- and fourth-generation cephalosporins. Furthermore, there is no requirement for postmarketing surveillance of resistance for newly registered antimicrobial drugs in humans or animals. In an attempt to reestablish a coordinated and comprehensive approach to antimicrobial drug resistance in Australia, we recently proposed the formation of a national antimicrobial drug resistance management body to implement national surveillance, coordinate education and stewardship programs, implement infection-prevention and control policies, support research, and advise regulatory authorities (*40*). Our proposal is in concert with other recent international calls for urgent action on antimicrobial drug resistance.

We believe that this single example of coordinated public policy points the way toward ensuring that drug prescribing is controlled and appropriate. Antimicrobial drugs are unique because their use leads toward their inevitable ineffectiveness as resistance develops. There are few new agents in development, so there is an imperative to preserve the effectiveness of the currently available antimicrobial drugs for as long as possible. We believe that these precious drugs should be regulated differently from all other drugs and that a single regulating body should be used to license and restrict their use to the most appropriate circumstances in both human and animal health and agriculture. The evidence that the control of quinolone prescribing in Australia has led to the continued usefulness of this class of drugs as valuable antimicrobial agents is compelling, and it serves as a call for countries to review the way that antimicrobial agents are regulated and subsidized so that we can continue to treat infections in the future.

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Multiple Synchronous Outbreaks of Puumala Virus, Germany, 2010

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To investigate 2,017 cases of hantavirus disease in Germany, we compared 38 new patient-derived Puumala virus RNA sequences identified in 2010 with bank vole–derived small segment RNA sequences. The epidemic process was driven by outbreaks of 6 Puumala virus clades comprising strains of human and vole origin. Each clade corresponded to a different outbreak region.

Human hantavirus disease is manifested as hemorrhagic fever with renal syndrome (HFRS) in Asia, Europe, and most probably Africa (*1*). Since 2001, laboratory-confirmed cases of HFRS in Germany, by law, must be reported by local health authorities to the Robert Koch Institute in Berlin, the central federal institution responsible for disease control and prevention. Approximately 200 HFRS cases/year are reported in nonepidemic years (incidence 0.25 cases/100,000 persons).

Local outbreaks of HFRS were reported in 2004–2005, a large outbreak with 1,688 cases was reported in 2007, and 2,017 cases were reported in 2010 (*2–4*). In 2010, the total HFRS incidence in Germany increased to 2.47 cases/100,000 persons. This increase was caused by outbreaks in specific regions; administrative districts in these regions reported incidences of ≤80 cases/100,000 persons (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1447-Techapp.pdf). In contrast to previous outbreaks (*5*), the 2010 outbreak did not lead to increased incidence of HFRS being reported in countries neighboring Germany, such as Belgium (www.wiv-isp.be/epidemio/labo) and France (www.invs.sante.fr/fr/Dossiers-thematiques/Maladies-infectieuses/Zoonoses), which indicated the distinctive features of this outbreak in Germany.

Although 3 hantaviruses, Puumala virus (PUUV), Dobrava-Belgrade virus, and Tula virus circulate in rodent hosts in Germany and can infect humans (*6–8*), most hantavirus infections in humans are caused by PUUV. The natural reservoir of PUUV, the bank vole (*Myodes glareolus*), is widely distributed in Germany and other countries in Europe. Bank vole abundance fluctuates every few years with ≈3–4 years between maximum peak densities of up to several hundred animals per hectare (*9*). Years with high vole densities and increased numbers of registered human hantavirus infections were preceded by years of intense coverage with beech mast (nuts of the European beech tree [*Fagus sylvatica*]) (*10*).

Diagnosis of hantavirus infection in humans is usually based on serologic testing. Since 1994, only 22 human-derived PUUV sequences have been identified in Germany. We conducted molecular epidemiologic analysis of the 2010 outbreak in Germany by compiling 38 new PUUV sequences of human origin, which were compared with bank vole–derived small (S) segment RNA sequences from the different outbreak regions.

The Study

We established a countrywide alert network that included several physicians and diagnostic laboratories. This network enabled assessment of serum samples from patients during the early clinical phase of infection.

A total of 491 serum samples were tested for antibodies to hantavirus. Of these samples, 377 were positive for IgG against PUUV, of which 330 were positive for IgM against PUUV by an in-house ELISA (*11*) and immunoblot assay (*recom*Line Bunyavirus; Mikrogen, Neuried, Germany). These acute-phase antibody-positive samples were then screened by using a nested pan hantavirus reverse transcription PCR (*12*) specific for a conserved region within the polymerase gene (large genomic segment). Using this approach, we identified 102/330 IgM- and IgG-positive samples that were PCR positive. Sequencing of this large segment region confirmed infection by PUUV.

To differentiate virus strains within the PUUV species, a higher variable nucleotide sequence was targeted

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by amplification of a 504-nt fragment of the genomic S segment encoding the nucleocapsid protein (*13*). Using this approach, we found that 38 of 102 PUUV-positive samples were S segment positive. Nucleotide sequences of these 38 samples were determined and used for molecular phylogenetic analysis. The dataset also included sequences obtained from human and *M. glareolus* vole samples collected in previous years and from voles captured during the 2010 outbreak. Rodent trapping was performed after permission was obtained from Federal State authorities (permit no. NRW 20.09.210, BW 35-9185.82/0261).

Residences of patients in Germany from whom viral sequences were obtained and corresponding rodent trapping sites are shown in Figure 1. Results of molecular phylogenetic analysis of these strains are shown in Figure 2. In addition to 4 molecular clades (Swabian Jura, clade 1; Bavarian Forest, clade 2; Spessart Forest, clade 3; and Münsterland, clade 6) found during the 2007 outbreak, 2 novel clades (North East Hesse, clade 4; and Teutoburg Forest, clade 5) were defined. All 6 clades comprised human-derived and vole-derived sequences.

Identification of new PUUV clades in 2010 was associated with a higher incidence of human disease than in 2007. For example, in the Federal State of Hesse, incidence increased from 0.35 cases/100,000 persons in 2007 to 2.80 cases/100,000 persons in 2010. This increase in cases enabled us to collect blood specimens from patients shortly after clinical onset of disease, characterize these specimens, and define a new PUUV clade (North East Hesse, clade 4).

To analyze diversity within and between different clades, we aligned each clade and calculated mean pairwise amino acid and nucleotide identities. In addition, we identified the consensus sequence of each clade and compared each with those of other clades (Table). Sequences within 1 clade show pairwise amino acid identities >97% and nucleotide identities >96%. Identities between clades range from 90.3% (Swabian Jura vs. Spessart Forest) to 97.7% (Münsterland vs. Teutoburg Forest) on the amino acid level and 81.3% (Teutoburg Forest vs. Swabian Jura) to 89.0% (Münsterland vs. Teutoburg Forest) on the nucleotide sequence level.

Within a particular molecular clade, virus strains from localities near each other could be differentiated. For example, within PUUV strains from the Bavarian Forest, which has been known as an outbreak region since 2004 (*13*,*14*), newly characterized strains 10 Mu Eb51 and 10 Mu Eb14 (Bavarian Forest; Figures 1, 2) originated from localities only 25 km apart. Because migration distances >1 km for bank voles are efficiently prevented by natural and artificial barriers (*15*), local vole populations are likely associated with specific PUUV strains.

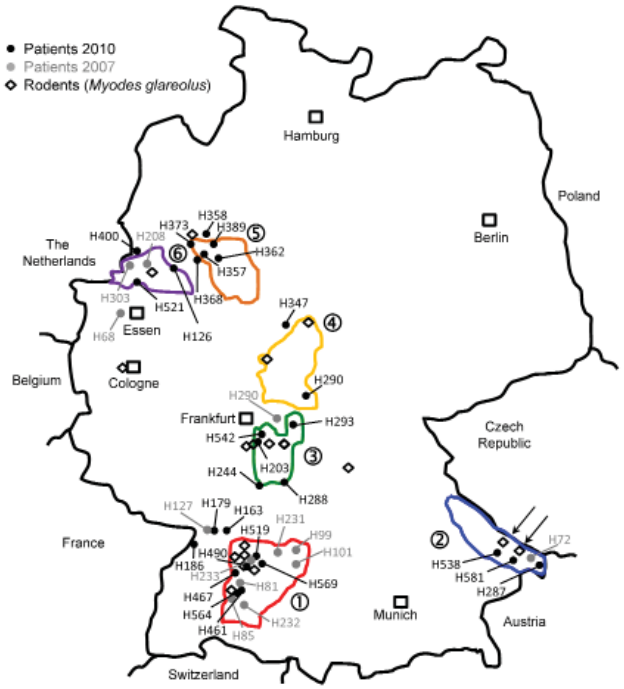

Figure 1. Distribution of investigated Puumala virus infections in Germany. Black dots indicate sequences obtained from patient samples in 2010; gray dots indicate sequences obtained from patient samples in 2007; diamonds indicate sequences obtained from rodent (*Myodes glareolus*) samples. Areas surrounded by lines indicate outbreak regions (numbered 1–6) where Puumala virus nucleotide sequences of human and vole origin have been analyzed. Numbers of the outbreak regions/virus clades and designations of local virus strains are also used in Figure 2. Arrows in outbreak region no. 2 indicate trapping sites of rodents from which strains 10 MuEb14v and 10 MuEb51 were obtained, which originated from localities 25 km apart.

Conclusions

Three years after a large 2007 PUUV outbreak, a subsequent epidemic with >2,000 human cases occurred in Germany. On the basis of beech mast coverage in 2009, growth of the reservoir rodent population in 2010 was expected. Model monitoring studies in July 2010 found mean ± SD bank vole densities of ≤141 ± 13 voles/hectare in investigated outbreak regions. PUUV seroprevalence in these bank vole populations was >40%. Bank vole densities in the same regions decreased to <15 voles/hectare in April 2011 (D. Reil, U.M. Rosenfeld, unpub. data).

Phylogenetic analysis of the involved PUUV strains identified 6 clades comprising virus strains of human and vole origin. Each clade clearly corresponds to a different outbreak region. High molecular similarities of human- and rodent-derived PUUV sequences from the same geographic origin and their clear molecular distinction from viruses of neighboring regions indicate spatial evolution of each virus clade. Thus, we conclude that the 2010 epidemic

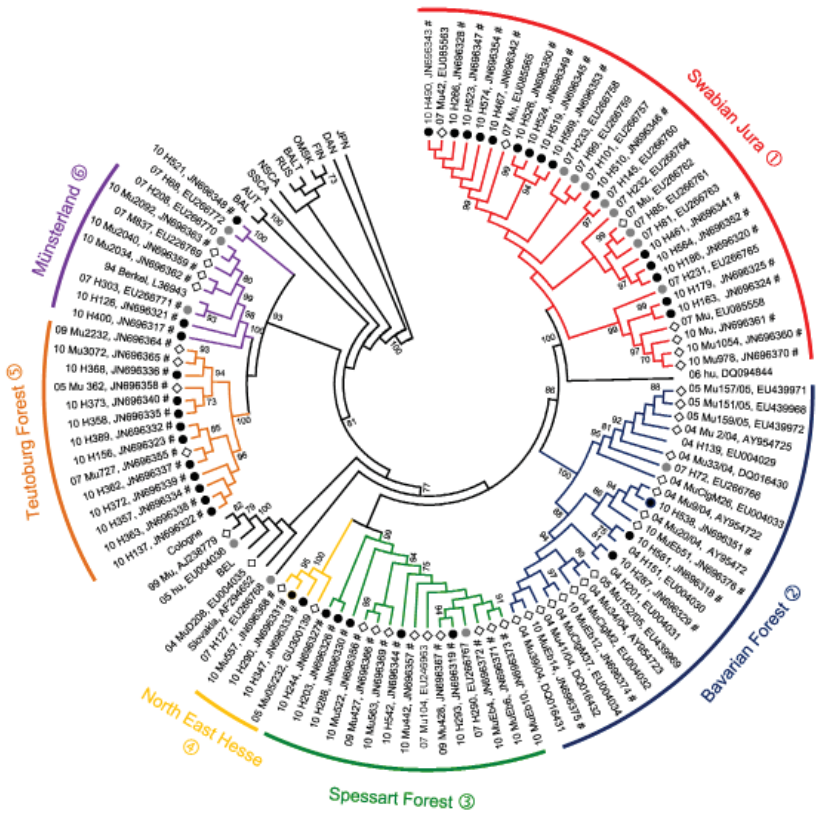

Figure 2. Neighbor-joining phylogenetic tree (TN93 evolutionary model) of Puumala virus (PUUV) strains constructed on the basis of partial sequences of the small segment (504-nt sequence, nt positions 392–894). Bootstrap values >70%, calculated from 10,000 replicates, are shown at the tree branches. Analysis was performed by using MEGA5 software (www.megasoftware.net). PUUV-like sequences from Japan (JPN) were used as outgroup. Numbers from 04 to 11 in front of the sample names indicate the year (2004–2011) when the sample was collected. Black dots indicate human samples from 2010, gray dots indicate human samples from 2007, and diamonds indicate rodent samples. Novel sequences from this study are indicated by the symbol #. For numbers (1–6) of PUUV clades that correspond to the 6 defined outbreak regions, see Figure 1. Phylogenetic clades are shown in parentheses followed by names and numbers. For clarity, previously characterized PUUV clades from other parts of Europe are shown in simplified form. BEL, Belgium; BAL, Balkan; AUT, Austrian; SSCA, South Scandinavian; NSCA, North Scandinavian; RUS, Russian; BALT, Baltic; OMSK, Russian from Omsk region; FIN, Finnish; DAN, Danish.

was not caused by countrywide spread of the same virus but resulted from multiple local outbreaks associated with simultaneous increases in densities and infection rates of bank voles in the different geographic regions.

At least for the investigated 504-nt S segment region, no evidence was found that viruses had undergone changes over time or as a result of rodent-to-human transmission. Lack of human-to-human transmission, local distribution of bank voles, and a high degree of identity between viruses of human and rodent origin enabled us to allocate certain virus strains to defined geographic regions. Future investigations may enable generation of risk maps with higher resolution and establishment of more sophisticated preventive measures in high-risk areas.

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Table. Amino acid and nucleotide sequence identity rates within and between different Puumala virus clades, Germany*

Clade	No. samples	% Amino acid identity†	% Nucleotide identity†	% Identity					
				SJ (1)	BF (2)	SF (3)	NEH (4)	TF (5)	ML (6)
SJ (1)	30	97.9	96.2		91.5	90.3	91.5	90.9	92.6
BF (2)	24	99.3	97.9	85.7		95.2	96.3	95.2	96.8
SF (3	15	99.3	97.0	84.0	87.2		96.0	93.7	95.4
NEH (4)	4	99.3	96.9	83.9	85.8	86.7		95.4	97.1
TF (5)	13	99.4	98.2	81.3	84.4	83.3	84.8		97.7
ML (6)	9	100	98.7	83.1	84.7	83.1	84.4	89.0	

*Sequences of the 504-bp small segment were compared. Values above the diagonal indicate amino acid identity, and values below the diagonal indicate nucleotide identity. Numbers in parentheses indicate outbreak regions as shown in Figures 1 and 2. SJ, Swabian Jura; BF, Bavarian Forest; SF, Spessart Forest; NEH, North East Hesse; TF, Teutoburg Forest; ML, Münsterland.
†Within each clade.

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MRSA Harboring *mecA* Variant Gene *mecC*, France

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We describe human cases and clustered animal cases of *mecA*$_{LGA251}$–positive methicillin-resistant *Staphylococcus aureus* in France. Our report confirms that this new variant has a large distribution in Europe. It may represent a public health threat because phenotypic and genotypic tests seem unable to detect this new resistance mechanism.

Since its first description in the early 1960s, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major public health issue because of worldwide spread of several clones. More than 20 years later, the specific genetic mechanism of its resistance has been identified as a mobile genetic element (staphylococcal cassette chromosome *mec*) integrated into the *S. aureus* chromosome, within which the *mec*A gene encodes a specific methicillin-resistant transpeptidase (penicillin-binding protein 2a) [PBP2a] (*1*). This protein has a low affinity for β-lactam antimicrobial drugs. Thus, bacteria expressing this protein are resistant to all types of these drugs.

A new divergent *mec*A homolog (*mec*C or *mecA*$_{LGA251}$, in reference to LGA251 isolates from which it was characterized) (*2*,*3*) was recently described in a novel staphylococcal cassette chromosome *mec* named type XI (*2*). This newly identified protein has <63% aa identity with PBP2a encoded by *mec*A and was described in *S. aureus* or coagulase-negative staphylococci. This new *mec*A homolog has been detected in bacteria from dairy cattle in England and humans in England, Scotland, and Denmark (*3*). We report the emergence of human and animal cases of *mec*A-variant MRSA identified in France.

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The Study

The first case was detected in a 67-year-old man admitted to Aix-en-Provence Hospital in southern France on November 8, 2007, because of suspected joint infection of his left knee 3 years after total knee joint replacement. He reported gonalgia that had been present for 6 months. The patient was apyretic but asthenic and reported a 10-kg weight loss. He had voluminous knee effusion. The synovial fluid leukocyte count was >1,000 cells/mm^3 with 86% neutrophils, findings consistent with a prosthetic knee infection (*4*). The patient had a deep and hyperkeratosic lesion on his left heel that was considered to be the source of infection.

Direct microscopic examination of the fluid identified gram-positive cocci in grape-like clusters. *S. aureus* was identified in pure culture. Susceptibility tests performed by using the disk diffusion method as recommended by the Société Française de Microbiologie (Paris, France) (www.sfm-microbiologie.fr) showed methicillin resistance. The presence of the *mec*A gene was investigated by using 2 methods, an-in house PCR (*5*) and the GenoType *Staphylococcus* test (Hain Lifescience GmbH, Nehren, Germany), but results were negative.

A 2-stage revision of arthroplasty of the infected knee was performed. Initial treatment was rifampin (1,800 mg/day) and ofloxacin (400 mg/day) for 5 months. Ofloxacin was then replaced with clindamycin (1,800 mg/day) for 2 months because of quinolone-induced tendinitis. The unusual length of treatment was required because of an abnormal delay in healing, persistence of knee inflammation, and subclinical inflammatory syndrome. The clinical outcome was favorable at a 3-year follow-up.

The second case was detected in 2 cows with clinical mastitis on the same farm in the Meurthe-et-Moselle District of northeastern France in December 2008. Two *S. aureus* strains were isolated from milk samples from these 2 cows. Drug susceptibility profiles determined by using the disk diffusion method were identical; both isolates showed methicillin resistance and susceptibility to other antimicrobial drugs, an unusual profile in veterinary microbiology. Only 1 strain was stored and sent to the French Agency for Food Environmental and Occupational Health and Safety (Lyon, France) where a *mec*A PCR (*6*) result was negative.

After failure of empiric treatment with cefalonium, a first-generation cephalosporin, the 2 cows were successfully treated with neomycin/spiramycin. Because these mastitis cases occurred in a context of recurrent bacterial infections (clinical and subclinical mastitis caused by *S. aureus* in dairy cows, diarrhea caused by *Escherichia coli* in veal calves), hygienic measures were instituted, including decontamination of milking machines, disinfection of teats before milking, and application of standard practices for

infection control. These measures were successful, and MRSA was not detected again on this farm.

Using primers and the protocol reported by García-Álvarez et al. (*3*), we detected a *mec*A variant (*mec*A_{LGA251}) in both isolates. Sequencing of a PCR-amplified fragment confirmed >99% homology with the sequence obtained for the LGA251 strain. Molecular typing of both isolates showed that they harbored an *agr* allele 3, were *spa* type t843, and belonged to clonal complex 130 (sequence type [ST] 130 for the cow isolate and ST1945, a single-locus variant of ST130 that differs by only 1 nucleotide within the *pta* gene, for the human isolate). These characteristics matched those of the most prevalent clones described by García-Álvarez et al. (*3*).

These isolates were also subjected to DNA microarray analysis by using the StaphyType Kit (Alere Technologies GmbH, Jena, Germany). Results confirmed assignment of the isolates to clonal complex 130, and showed that both isolates had hybridization profiles identical with those of 2 *mec*A variant isolates described by Shore et al. (*2*), except that the isolates were negative for *ccr*-A3 and *ccr*-B3. Both isolates were misidentified as methicillin-susceptible *S. aureus* by use of this microarray genotyping approach and the real-time PCR (GeneOhm Staph SR; BD Diagnostics, San Diego, CA, USA) because of *mec*A was not amplified.

The human isolate was determined to be methicillin sensitive by using the BD Phoenix PMIC/ID60 panel (BD Diagnostics) (oxacillin MIC 0.5 mg/L, cefoxitin MIC 4 mg/L, moxalactam MIC 16 mg/L). In addition to the inability of the Xpert MRSA/SA SSTI assay (Cepheid, Sunnyvale, CA, USA) reported by Shore et al. (*2*) to detect *mec*A variant isolates, these data confirm the inability of commercial molecular methods and some phenotypic panels currently available to identify all *mec*A variant isolates. These findings raise fears that such MRSA isolates are misidentified and that their prevalence is underestimated.

Conclusions

Our results provide information on global distribution of the *mec*A variant gene. Geographic and temporal diversity of the isolates suggest that such strains are widely distributed and were not recently introduced in France. Moreover, animal cases were described only in the United Kingdom, and no cluster of clinical cases (2 cases of cow mastitis) was reported. Our data demonstrate the ability of such strains to cause clustered cases.

This new methicillin-resistance mechanism in *S. aureus* may be a new public health threat. Global dissemination of *mec*A_{LGA251} *S. aureus* should be investigated and controlled in humans and animals. Control measures should include rational use of antimicrobial drugs, accurate and rapid microbiological laboratory services, and specific infection-control measures.

In addition, the epidemiologic situation should be carefully monitored. However, such monitoring is made difficult by a combination of 3 issues. The first issue is the inability to detect *mec*A variant MRSA by using commercial (used for screening) molecular approaches or in-house (used for confirmation) amplification tests. The second issue is lack of sensitivity of some commercial phenotypic panels used for routine drug susceptibility testing. The third issue is the need for specific and easy-to-read phenotypic tests to confirm methicillin resistance caused by additional PBP (i.e., absence of synergy between oxacillin and amoxicillin/clavulanic acid disks to exclude borderline oxacillin-resistant *S. aureus* without being able to exclude a modified PBP-resistant *S. aureus* phenotype). The first step in overcoming this difficulty would be inclusion in the surveillance system systematic characterization of clinical strains harboring methicillin-resistance genes associated with susceptibility to all other antimicrobial drugs, a profile typical of *mec*A-variant MRSA isolates.

This report of new *mec*A variants in France confirms their wide geographic range, but many questions remain. The prevalence of *mec*A_{LGA251}–positive isolates in France and other countries should be evaluated in livestock and humans. The origin, evolutionary mechanisms, potential animal reservoirs, and mode of dissemination of *mec*A-variant clones over large areas remain unknown. The clinical effect of expression of the PBP2a variant has not been definitively established in patients and should be explored in animal models.

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Prevalence of Oral Human Papillomavirus Infection among Youth, Sweden

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Human papillomavirus (HPV) causes cervical, head, and neck cancers. We studied 483 patients at a youth clinic in Stockholm, Sweden, and found oral HPV prevalence was 9.3% and significantly higher for female youth with than without cervical HPV infection (p = 0.043). Most oral HPV types matched the co-occurring cervical types.

Human papillomavirus (HPV) causes cervical, head, and neck cancers (*1*). Recent reports show that oropharyngeal cancer, the head/neck cancer for which HPV infection is most common, is increasing (*2*). HPV vaccination with Gardasil (Merck, Whitehouse Station, NJ, USA) and Cervarix (GlaxoSmithKline, Brentford, UK) prevents cervical infection with HPV types 16 and 18, but less is known about oral HPV infection (*3*,*4*). To evaluate oral HPV prevalence before HPV vaccination of the public, we performed a study at a youth clinic in Stockholm, Sweden, where we previously reported high cervical HPV prevalence (70%) among female youth (*5*). We compared oral HPV prevalence in male and female youth visiting the clinic and studied oral HPV prevalence and type concordance in relation to cervical HPV infection.

The Study

The study, performed during 2009–2011 with permission from the Stockholm Regional Ethical Committee, enrolled 408 female and 82 male youth, 15–23 years of age, who visited a large youth clinic in Stockholm (*5*). None had been vaccinated for HPV. In brief, ≈4,000 female and ≈800 male youth visit the clinic each year for birth control advice and treatment for sexually transmitted diseases (*5*). The low participation rate in our study was the result of periods of high workload with no enrollment, but when asked, most persons participated.

Oral samples from enrollees were obtained after 30 s of mouthwashing with 15 mL of 50% Listerine (Johnson & Johnson, New Brunswick, NJ, USA). Samples were stored at 4°C for a maximum of 3 days and then centrifuged at 6,000 × *g* for 10 min; the resulting pellet was stored at –20°C. DNA was extracted by using the Gentra Puregene Buccal Cell Kit (QIAGEN AB, Stockholm, Sweden) and dissolved in a 100-μL DNA hydration solution (provided with the kit).

Cervical samples (n = 180) were collected from female youth and prepared as described (*5*). A 10-μL aliquot for each sample was analyzed for 24 mucosal HPV types by using a Luminex-based multiplex assay, as described by Schmitt et al. (*6*), using a MAGPIX instrument (Luminex Corporation, Austin, TX, USA). Of the 180 cervical samples, 107 had been analyzed previously by using a Luminex 100 instrument (*5*), but there were no differences in sensitivity between the MAGPIX and Luminex 100 instruments. For comparison with the previous study (*5*), HPV types were classified as described by Muñoz et al. (*7*). Samples with values <30 for β-globin were excluded. Oral and cervical HPV prevalence was compared by using a 2-tailed Fisher exact probability test, and HPV16 concordance in oral and genital samples was measured by k.

Of the 490 oral samples, 7 were excluded because of insufficient material; of the remaining samples, 9.3% (45/483) were positive for HPV, 9.2% (37/401) in female and 9.8% (8/82) in male youth. Most HPV types detected in oral samples from both sexes were high-risk HPV (Table 1); >2 HPV types were found in 7 oral samples. Figure 1 shows the prevalence and 95% CIs for each HPV type. HPV16 was the most prevalent high-risk type detected (2.9%, 95% CI 1.7%–4.8%), and HPV42 was the only low-risk type detected (1.0%, 95% CI 0.4%–2.4%).

The prevalence of oral and cervical HPV was compared for 174 female youth from whom oral and cervical samples were obtained together and contained sufficient material; cervical HPV was detected in 129 (74.1%). Most HPV types detected in the cervix were high-risk types. Figure 2 shows the prevalence and 95% CI for each HPV

Table 1. HPV prevalence in oral and cervical samples from 24 female youth with oral HPV infection, Stockholm, Sweden*

	No. (%) samples	
HPV-positive categories	Oral	Cervical
All HPV types	45 (9.3)	129 (74.1)
All high-risk HPV types	35 (7.2)	113 (64.9)
HPV 16	14 (2.9)	66 (37.9)
HPV 18	1 (0.2)	25 (14.4)
Total no. samples	483	174

*HPV, human papillomavirus.

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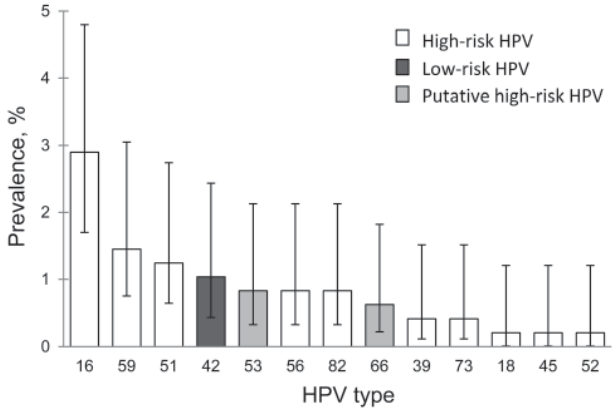

Figure 1. Prevalence of human papillomavirus (HPV) types in oral samples from 24 female youth with oral HPV infection, Stockholm, Sweden. The 4 most common HPV types were high-risk types HPV16 (2.9%, 95% CI 1.7%–4.8%), HPV59 (1.4%, 95% CI 0.7%–3.0%), and HPV51 (1.2%, 95% CI 0.6%–2.7%) and low-risk type HPV42 (1.0%, 95% CI 0.4%–2.4%).

type. HPV16 was the most prevalent high-risk type detected (37.9%, 95% CI 31.0%–45.3%), followed by low-risk type HPV42 (17.8%, 95% CI 12.9%–24.2%).

Oral HPV infection was more frequent in female youth with (22/129, 17.1%) than without (2/45, 4.4%) cervical HPV infection (p = 0.043). HPV types commonly detected in the cervical tract were observed in the oral tract (Figures 1, 2), but fewer HPV types were detected in the oral compared with the genital tract. In addition, median fluorescent intensity for HPV was lower in oral compared with genital samples (data not shown).

Among 24 female youth with oral HPV infection, 22 (91.7%) also had a cervical HPV infection (Table 2). Furthermore, for 20/22 (90.9%) of those who had concomitant oral and cervical HPV infection, oral HPV types were completely concordant with cervical HPV types, but the opposite was not true because there were more HPV types in general per cervical site (Table 2). For example, for female youth nos. 10 and 13, both of whom had several oral HPV types, all types in the oral tract were detected in the cervical tract but not vice versa (Table 2). Calculating k 0.4400 for HPV16 in oral and cervical sites in the 24 persons with HPV-positive oral samples resulted in moderate agreement; slight agreement was obtained when calculating k 0.1032 for the 129 female youth with HPV-positive cervical samples and 0.1345 for all 174 female youth.

Conclusions

In this study, oral HPV prevalence was similar among male and female youth (9.3% vs. 9.8%) but higher for female youth with (17.1%) than without (4.4%) cervical HPV infection. Moreover, most female youth with oral HPV infection had cervical HPV infection with type concordance and dominance of HPV16.

Oral HPV prevalence in our cohort was comparable to that in other reports (*8*,*9*). Likewise, the higher oral HPV prevalence in female youth who had concurrent genital infection and the finding that most female youth with oral HPV also had genital HPV infection was similar to findings in the study by Giraldo et al. (*10*). Nevertheless, some differences in prevalence of HPV in cervical samples were seen between the study of Giraldo et al. (*10*) and our study; these differences could be attributed to the different populations, biologic sampling methods, and the assays used. We found a higher HPV type concordance between oral and cervical infections than that found in other studies (*11*). This difference may partly be because of differences between the populations included and the techniques used for detection of different HPV types (*11*).

We also found that most HPV types commonly found in cervical samples were detected in oral samples, which suggests no major differences between HPV types in the cervical and oral tracts. This finding is similar to some reports, but not all (*10–14*). The lower prevalence of oral than cervical HPV was consistent with previous findings (*10–14*); however, these findings may be underestimates because the continuous production of saliva causes viral DNA to be swallowed and disappear from the oral cavity. This may also partly, but not completely, explain the lack of or weaker HPV type concordance between cervical and oral locations.

Our study has several limitations. The sample size of our cohort is relatively small, particularly with regard to those with available concurrent cervical and oral samples. In addition, our cohort represents a sexually active group seeking assistance for sexually transmitted diseases or preventive measures, which means their overall HPV prevalence may be higher compared with that of other persons of the same age in Stockholm. We also do not have demo-

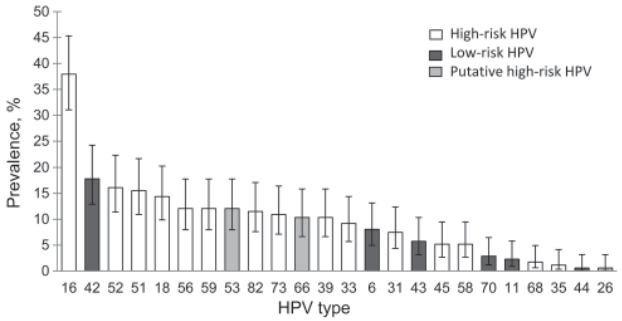

Figure 2. Prevalence of human papillomavirus (HPV) types in cervical samples from 24 female youth with oral HPV infection, Stockholm, Sweden. The 4 most common types were high-risk types HPV16 (37.9%, 95% CI 31.0%–45.3%); HPV52 (16.1%, 95% CI 11.4%–22.3%), and HPV51 (15.5%, 95% CI 10.9%–21.6%) and low-risk type HPV42 (17.8%, 95% CI 12.9%–24.2%).

Table 2. HPV types detected in oral and cervical samples from 24 female youth with oral HPV infection, Stockholm, Sweden*

	Types detected from oral samples			Types detected from cervical samples		
Patient no.	High risk	Putative high risk	Low risk	High risk	Putative high risk	Low risk
1	**16**			**16**, 18, 51, 82	53	
2	**16**			**16**, 33, 45	66	43
3	**16**			**16**, 18, 39		6, 42
4	**16**			**16**, 18, 31, 45, 73		42, 43
5	**16**			**16**, 31, 51, 52, 59	53	42
6	**16**			**16**, 51		
7	**16**			**16**		42
8	**16**			**16**		
9	**18**			**18**		6
10	**39, 51, 56, 59**			16, **39**, **51**, 52, **56**, **59**, 82	66	42
11	**51**			**51**		42, 43
12	**51**			**51**, 82		
13	**56, 59**			16, **56**, **59**		
14	**82**			18, 51, 52, 58, 68, **82**	53	42
15	**82**			16, 18, 51, 58, **82**	53	
16		**53**		33, 45, 51, 82	**53**	
17		**53**			**53**	42, 43
18			**42**			**42**
19			**42**	45, 56, 59	66	**42**
20			**42**	16, 33, 52, 56, 59	53	6, **42**
21	82			16, 33, 45, 73		11
22	73			16, 18, 52, 56	53, 66	
23	16					
24			42			

*__Boldface__ indicates HPV types found in both oral and cervical samples from the same person. HPV, human papillomavirus.

graphic and behavioral data for the participating youth, and these data could affect the calculation of risk for infection.

In conclusion, we found the prevalence of oral HPV infection, with dominance of HPV16, was similar for male and female youth, but among female youth, infection was more common for those who had co-occuring genital HPV infection, and most oral HPV types were also found in the genital tract. These data emphasize the importance of investigation to determine if the current HPV vaccines also prevent oral HPV infection.

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Demographic Shift of Influenza A(H1N1)pdm09 during and after Pandemic, Rural India

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Population-based active surveillance in India showed higher incidence rates for influenza A(H1N1)pdm09 among children during pandemic versus postpandemic periods (345 vs. 199/1,000 person-years), whereas adults had higher rates during postpandemic versus pandemic periods (131 vs. 69/1,000 person-years). Demographic shifts as pandemics evolve should be considered in public health response planning.

Influenza epidemics and pandemics has been recognized for centuries (*1*,*2*), and the effects that influenza can have on public health infrastructure were demonstrated globally during the 2009–2010 pandemic (*3*). The dynamics of influenza transmission are dependent on many factors, including probability of infection, susceptible populations within age groups, and close contacts between susceptible and infected persons (*4*,*5*). Data from 3 recent influenza pandemics show that school-aged children have the highest disease rates and may serve as a key source of transmission to adults (*2*,*6*–*8*).

A recent mathematical modeling study suggested that initial exposure to a novel influenza virus among a highly susceptible population (school-aged children) results in a shift in transmission patterns as infection spreads, with adults more affected during later phases (*9*). To investigate if transmission of influenza A(H1N1) pdm09 (pH1N1) followed this demographic shift pattern, we examined comprehensive weekly active community surveillance for febrile acute respiratory illness (ARI) in 3 rural villages in northern India, as well as available extensive epidemiologic data.

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The Study

All residents of 3 villages in Ballabgarh (n = 16,861) in Haryana, India, have been under weekly household surveillance for febrile ARI since November 2009 as part of a clinical trial of seasonal inactivated trivalent influenza vaccine in children 6 months–10 years of age (NCT00934245; www.clinicaltrials.gov); 95% of eligible children were recruited for this trial (Table 1). Information on febrile ARI, which consists of reported fever plus any respiratory complaint (e.g., cough, sore throat, nasal congestion, runny nose, earache, or difficulty breathing), was collected for all household members either by self-report or by proxy by trained field workers. Consent was obtained from all participants.

During November 2009–October 2010, of the 12,896 eligible persons with febrile ARI, samples were collected from 10,002 (78%); missing samples were because those persons were not available for testing at the time of home visit. Throat and nasal swab specimens were collected from all available febrile ARI patients and tested by using real-time reverse transcription PCR (*10*). Incidence rates (IRs; reported as per 1,000 person-years) and corresponding 95% CIs were calculated for the peak periods of influenza circulation. The pandemic period was defined as November 2009–January 2010 and the postpandemic period as August–October 2010 (first postpandemic period). The Institutional Review Boards of All India Institute of Medical Sciences, University of Alabama, and Centers for Disease Control and Prevention approved the study. Informed consent was obtained for all persons included in the study.

Two distinct peaks of pH1N1 activity were identified during the pandemic and postpandemic periods (Figure 1), with some circulation during the intervening period (February–July 2010, <0.6%). Rates of positive test results for pH1N1 were higher during peak pandemic (21%) compared with peak postpandemic (13%) periods, whereas influenza B positivity was higher during the postpandemic period (Table 1). The median age of persons with pH1N1 illness during the postpandemic period was significantly higher than during the pandemic period (18 vs. 9 years of age; $p<0.001$).

IRs for pH1N1 were higher for children 0–5 and 6–18 years of age (IR 375 and 331, respectively) than for adults (IR 8–86) during the pandemic period (Table 2). The differences in IRs of pH1N1 across age groups disappeared during the postpandemic period, however, this occurred primarily because of a decrease in IRs among the 0- to 5- and 6- to 18-year-old age groups (incidence rate ratio [IRR] 0.6) and concurrent increases among older age groups (IRR 1.6–8.7). These changes were statistically

Table 1. Demographic data for persons under surveillance and incidence of febrile ARI and influenza A(H1N1)pdm09 during pandemic and postpandemic periods, Ballabgarh, India*

Demographics and test results	Pandemic period, November 2009–January 2010	Postpandemic period, August–October 2010
Mean no. persons under surveillance (person-years)	7,340 (1,835)	16,396 (4,134)
No. febrile ARI episodes (incidence rate/1,000 person-years)	1,515 (826)	4,933 (1,203)
No. (%) persons tested for influenza	1,094 (72)	3,907 (79)
No. (%) positive for influenza	265 (24)	902 (23)†
No. (%) positive for influenza A(H1N1)pdm09	231 (21)	506 (13)
No. (%) positive for influenza B	34 (3)	377 (10)
Influenza incidence rate/1,000 person-years	205‡	278
Median age, y (interquartile range)		
All persons with influenza	9 (4–17)	15 (6–30)§
Persons with influenza A(H1N1)pdm09	9 (5–18)	18 (7–32)§
Persons with Influenza B	7.5 (4–16)	13 (5–27)

*ARI, acute respiratory infection.
†Total no. positive during postpandemic period included 18 persons with influenza A (H3N2) infection and 1 person co-infected with influenza B and A(H1N1)pdm09.
‡The rate of influenza positivity of sampled febrile ARI case-patients was adjusted to unsampled case-patients assuming similar characteristics for the 2 groups.
§Wilcoxon rank-sum test, p<0.001.

significant (p<0.0001; Figure 2, panel A). The overall IR for influenza B was higher during the postpandemic period; IR for influenza B remained higher for children <18 years of age regardless of pandemic period (Figure 2, panel B).

The overall IR for pH1N1 was higher for children <18 years of age (345) than for adults >18 years of age (69) during the pandemic period, whereas IRs were similar among children (199) and adults (131) during the postpandemic period. However, the IR of pH1N1 was significantly higher (p<0.0001) among children during the pandemic period compared with the postpandemic period (IRR 0.6), whereas the rate for adults was higher during the postpandemic period (IRR 1.8) (Figure 2, insets). In contrast, the IR for influenza B remained 2.5× higher for children (IR 184) than adults (IR 72) during the postpandemic period.

Conclusions

Data from this large-scale, community-based, prospective surveillance program demonstrated that the introduction of the pH1N1 strain into a naive population in northern India initially affected preschool- and school-aged children during the first phase of the pandemic, with a demographic shift to adults during the postpandemic phase. The analysis has several unique characteristics. By chance, the study began soon after the emergence of pandemic influenza in northern India, which enabled the analysis of pandemic and postpandemic periods in the same study population; we were able to measure incidence of pandemic and influenza B cocirculating in the community. Because we used active surveillance, we likely captured most febrile ARI cases among all age groups, and therefore our results likely are robust and unbiased. If similar patterns occurred during future pandemics in other (e.g., urban) populations, interventions should be redirected from children to adults during the postpandemic phases.

School-aged children often are at the leading edge of a pandemic, and they remained the top-priority group for vaccination during the 2009 influenza pandemic (*11*,*12*). Our findings suggest that the high IR of pH1N1 among schoolchildren led to naturally acquired immunity, which lowered the susceptibility of this population to illness during the postpandemic phase. Conversely, an increase in IR among adults during the postpandemic phase supports previous observations that pandemic influenza transmission shifts from highly susceptible children during a pandemic period to adults during the postpandemic phase (*9*). These age-specific demographic shifts in IRs were also observed for the major pandemics of 1918, 1957, and 1968 (*5*–*7*).

Our study has several limitations. First, the population under surveillance during the pandemic was relatively small because of phased enrollment during the initial study implementation and not all febrile ARI case-patients could

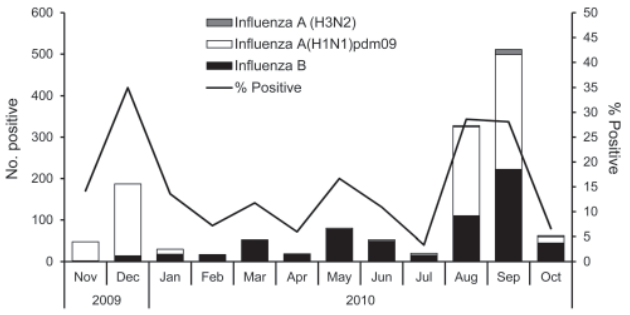

Figure 1. Monthly trends of positive influenza test results during active surveillance in a community-based study, rural India, November 2009–October 2010. Of 1,409 positive test results, 748 (53.1%) were for influenza A(H1N1)pdm09, 642 (45.6%) for influenza B, 18 (1.3%) for influenza A (H3N2), and 1 for co-infection with influenza B and A(H1N1)pdm09. Children 6 months–10 years of age received trivalent seasonal influenza vaccine (intervention) or inactivated polio vaccine (control) during November–December 2009; coverage was 92%.

Table 2. Incidence rates for influenza A(H1N1)pdm09 and influenza B among persons with febrile ARI during pandemic and postpandemic periods, by age group, Ballabgarh, India*

Age group, y	Pandemic period, November 2009–January 2010					Postpandemic period, August–October 2010					Incidence rate ratio (95% CI)
	Person-years†	Febrile ARI, no. cases	No. tested‡	No. (%) positive‡	Incidence (95% CI)§	Person-years†	Febrile ARI, no. cases	No. tested‡	No. (%) positive‡	Incidence (95% CI)§	
Influenza A(H1N1)pdm09											
0–5	230	499	411	71 (17.3)	375 (300–463)	498	1,174	1,036	103 (10.0)	235 (194–281)	0.6 (0.5–0.8) ¶
6–18	486	435	281	104 (37.0)	331 (282–386)	1,061	1,245	1,012	158 (15.6)	183 (158–210)	0.6 (0.4–0.7)¶
19–44	777	362	250	46 (18.4)	86 (67–110)	1,754	1,596	1,171	174 (14.9)	135 (118–153)	1.6 (1.2–2.1)#
45–59	215	135	93	9 (9.7)	61 (32–104)	493	556	413	56 (13.6)	152 (120–191)	2.5 (1.3–4.9)#
>60	128	84	59	1 (1.7)	8 (0.2–44.0)	294	362	275	15 (5.5)	68 (42–105)	8.7 (1.4–360.0)#
Influenza B											
0–5	230	499	411	12 (2.9)	65 (37–108)	498	1,174	1,036	103 (10.0)	235 (194–281)	3.6 (2.1–6.6)
6–18	486	435	281	15 (5.3)	47 (30–71)	1,061	1,245	1,012	138 (13.6)	160 (137–186)	3.4 (2.2–5.5)
19–44	777	362	250	7 (2.8)	13 (6–24)	1,754	1,596	1,171	100 (8.5)	77 (65–92)	6.0 (3.2–12.8)
45–59	215	135	93	0	0 (0–17)	493	556	413	19 (4.6)	53 (34–77)	0 (0–2.9)**
>60	128	84	59	0	0 (0–29)	294	362	275	17 (6.2)	75 (47–113)	0 (0–2.4)**

*ARI, acute respiratory infection.
†For surveillance.
‡For influenza.
§Per 1,000 person-years.
¶p<0.0001 (incidence lower in postpandemic than in pandemic period).
#p<0.02 (incidence higher in postpandemic than in pandemic period).
**No influenza B–positive results for these age groups in pandemic period.

be sampled; these effects were corrected by using the person-time method for calculating IRs. However, we recognize that febrile ARI case-patients who were not sampled may have had milder disease, and, therefore, influenza rates may vary. Second, the IRs of pH1N1 reported during the pandemic period may be underestimates because the initial pandemic peak in nearby areas was observed during August 2009, with highest positivity rates for those 6–18 years old (*11*). Third, while no routine influenza vaccination program exists in this community, the incidence rates for pH1N1 among children may have been skewed because of protection afforded by seasonal influenza trivalent vaccination, administered during November 2009 (*13–15*). However, the effect, if any, of this vaccination on influenza B incidence has not been determined.

Despite these limitations, we believe that the age-specific demographic shift we observed for the 2009 influenza pandemic will be useful for future modeling

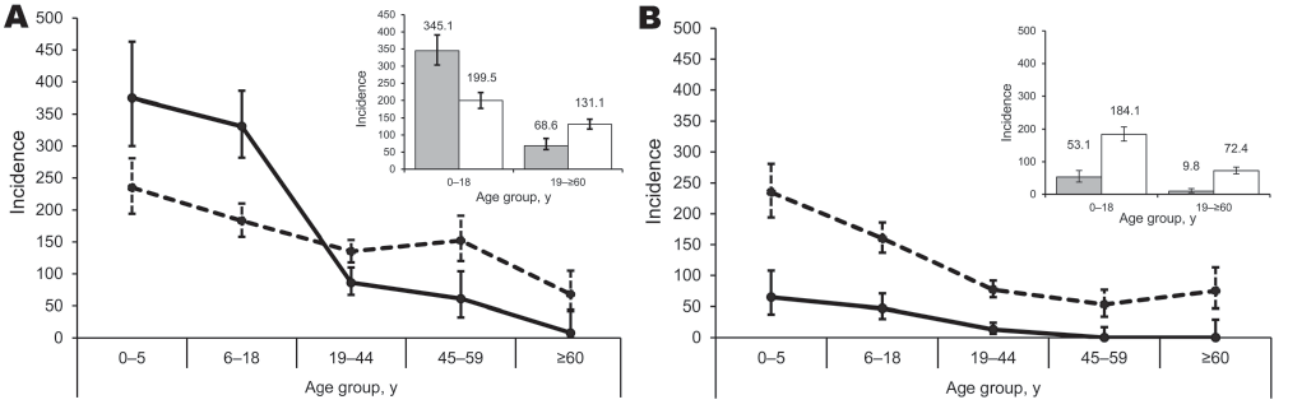

Figure 2. Incidence rates (per 1,000 person-years) for influenza A(H1N1)pdm09 (A) and influenza B (B) during pandemic (November 2009–January 2010; solid lines) and postpandemic (August–October 2010; dashed lines) periods in a rural community in northern India. Cumulative incidence rates for A(H1N1)pdm09 (A, inset) and influenza B (B, inset) during pandemic (gray bars) and postpandemic (white bars) periods are also shown, with incidence rates given on top of the bars. Error bars indicate 95% CIs.

projects addressing this issue. Future pandemic preparedness activities should focus on targeted interventions for different age groups as the pandemic evolves, as well as on the severity of disease in different age groups.

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Hospitalizations Associated with Disseminated Coccidioidomycosis, Arizona and California, USA

Amy E. Seitz, D. Rebecca Prevots, and Steven M. Holland

We analyzed hospitalization databases from Arizona and California for disseminated coccidioidomycosis–associated hospitalizations among immunocompetent persons. Racial/ethnic disease ratios were characterized by a higher incidence of hospitalization among blacks compared with other groups. This finding suggests that HIV infection, AIDS, and primary immune conditions are not a major factor in this disparity.

Coccidioidomycosis is a fungal infection caused by inhalation of *Coccidioides immitis* or *C. posadasii* spores (*1*,*2*). Most (≈60%) persons infected with *Coccidioides* spp. are asymptomatic (*3*), but symptomatic primary pulmonary coccidioidomycosis develops in ≈40% (*4*). An estimated 1% of all infections, asymptomatic and symptomatic, progress to extrathoracic disseminated coccidioidomycosis infection (*3*,*4*), which is more common among persons with underlying immune conditions, such as advanced HIV/AIDS. Although disseminated coccidioidomycosis is most often found in immunocompromised persons, it can also occur among persons without known predisposing conditions (*5*). The epidemiologic association between ethnicity and disseminated coccidioidomycosis within disease-endemic regions has been described (*6*,*7*) but not specifically among persons with disseminated coccidioidomycosis without HIV/AIDS or primary immune deficiencies. In the United States, coccidioidomycosis is predominantly localized to specific areas of southern California and Arizona (*8*). We focused our analyses of racial/ethnicy disease ratios on these regions.

The Study

We used the Arizona and California State Inpatient Databases (SID) from the US Agency for Healthcare Research and Quality (AHRQ) to describe clinical and demographic characteristics of persons hospitalized for disseminated coccidioidomycosis who did not have known primary immune conditions, HIV infection, or AIDS. The SID contains state-level data, including information from 90% of community hospital inpatient stays, and is maintained as part of the Healthcare Utilization Project at AHRQ. We extracted records from the available annual datasets for Arizona (2000–2009) and California (2003–2008) that listed any diagnosis of disseminated coccidioidomycosis (code 114.3 from the International Classification of Diseases, 9th Revision, Clinical Modification [ICD-9-CM]). We excluded records that listed any diagnosis of HIV infection or AIDS (ICD-9-CM code 042) or primary immune deficiency (ICD-9-CM code 279.xx). This study was not considered human subject research by the National Institutes of Health Office of Human Subjects Protection.

We calculated the average annual incidence of hospitalizations as the average annual number of disseminated coccidioidomycosis hospitalizations divided by the midyear population, as determined by US Census estimates (*9*). By using the revisit files available from AHRQ, we assessed differences in readmissions by race by comparing the proportion of persons with only 1 disseminated coccidioidomycosis infection among race groups. We also calculated all-cause hospitalization rates for blacks and whites in our dataset by using total hospitalizations from HCUPnet (http://hcupnet.ahrq.gov). In years for which patient state of residency was available, we assessed the potential bias from hospitalizations of persons with out-of-state residency by determining the percentage of total admissions from out-of-state patients. We used the Mann-Whitney test for nonparametric comparisons; the significance level used for all statistical tests was $\alpha = 0.05$. All analyses were completed in SAS version 9.2 software (SAS Institute, Cary, NC, USA).

We identified 4,719 disseminated coccidioidomycosis–associated hospitalizations. The average annual incidence of hospitalizations/100,000 persons/year was 4.8 (95% CI 4.2–5.3) for Arizona and 0.89 (95% CI 0.79–0.99) for California. Overall, the rate of hospitalization for blacks in Arizona was 12.0-fold higher than the rate of hospitalization for whites and 20.8-fold higher than for Hispanics (Figure 1). The rate of hospitalization in California was 8.8-fold higher for blacks than for whites and 5.6-fold higher for blacks than for Hispanics (Figure 2).

The median age of patients with disseminated coccidioidomycosis–associated hospitalizations was lower than the median age of all hospitalizations for both blacks and whites in Arizona and California ($p<0.001$ for all comparisons). The median age at hospitalization for Hispanics was higher than the median age for all

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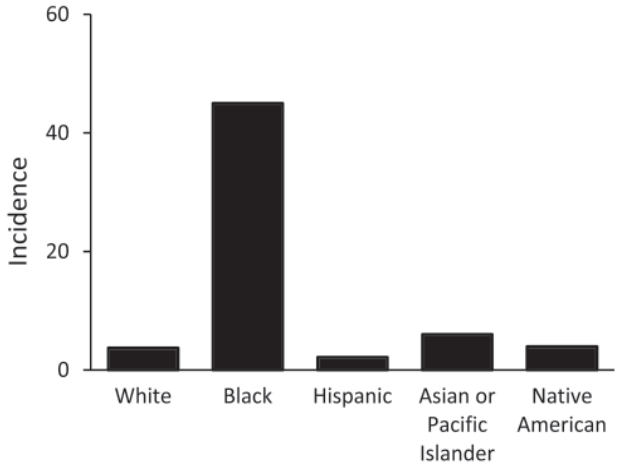

Figure 1. Average annual incidence (per 100,000 population) of disseminated coccidioidomycosis–associated hospitalizations, by race/ethnicity, Arizona, USA.

hospitalizations in Arizona ($p<0.001$) and California ($p = 0.01$) (Table).

The proportion of persons with >1 hospital admission for disseminated coccidioidomycosis among blacks was higher than for whites in California in 2008 (29.8% vs. 17.3%) and in Arizona in 2007 (19.3% vs. 17.0%). Among all disseminated coccidioidomycosis–associated hospitalizations, 98% of hospitalizations in Arizona and 99% of hospitalizations in California were from in-state residents, indicating minimal bias from nonresidents. All-cause hospitalization rates did not differ greatly between whites and blacks: 121 hospitalizations/1,000 persons for whites and 104 hospitalizations/1,000 persons for blacks in Arizona; 125 hospitalizations/1,000 persons for whites and 143 hospitalizations/1,000 persons for blacks in California.

Conclusions

We identified a higher incidence and lower patient age at hospitalization for disseminated coccidioidomycosis–associated hospitalizations among blacks in California and Arizona. We were unable to determine if the higher incidence of hospitalization was a result of environmental, host, or behavioral factors. However, this study suggests that HIV infection, AIDS, and primary immune conditions are not the main reason for the racial/ethnic disparity for hospitalizations associated with disseminated coccidioidomycosis.

Because most coccidioidomycosis cases in California occur in the San Joaquin Valley region (*6*) and 90% of the population of California lives outside this region (*10*), the California-specific incidence rate is likely to be an underrepresentation of the extent of the disease in the San Joaquin Valley region. In addition, because of this disparity, we are unable to make comparisons between rates for California and Arizona. The state-specific incidence is a better description of the extent of disease in Arizona because the 3 counties with 93% of the coccidioidomycosis cases in Arizona represent 79% of the state's population (*11*).

The classification of race/ethnicity in this dataset may not completely describe the true distribution of disease among the diverse groups comprising these populations. These categories include a wide range of racial/ethnic backgrounds, representing multiple potential environmental, social, cultural, behavioral, or genetic susceptibilities. However, the higher incidence among blacks suggests that unknown factors uniquely affect a high proportion of this population.

Our analysis is likely to be specific for accurately detecting the number of cases of disseminated coccidioidomycosis because most cases require hospitalization of the patient. Furthermore, in Arizona and southern California, where the infection is common, awareness of coccidioidomycosis and disseminated coccidioidomycosis is high, and disseminated coccidioidomycosis cases are likely to be recognized. This analysis method is also likely to be specific because results of cultures, biopsies, histologic testing, and serologic testing provide strong evidence of infection. However, our use of administrative data, such as the SID, is limited by the use of ICD-9-CM codes, and the sensitivity and specificity of those codes for disseminated coccidioidomycosis has not been evaluated.

An additional limitation of our study was that we could not determine if a single person was hospitalized multiple times during the study years. Although we identified higher rates of readmission among blacks, consistent with previous studies for coccidioidomycosis (*12*), this does not

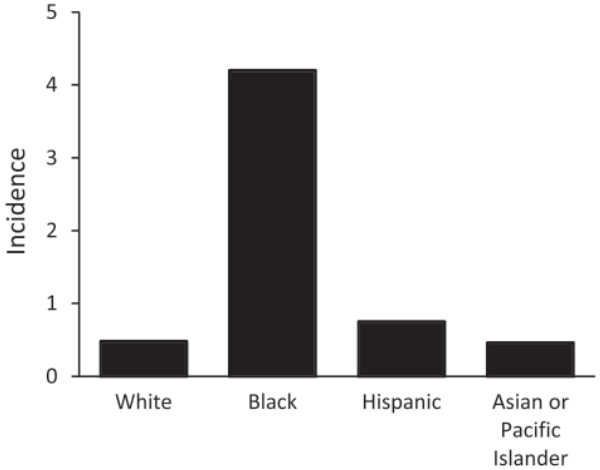

Figure 2. Average annual incidence (per 100,000 population) of disseminated coccidioidomycosis hospitalizations, by race/ethnicity, California, USA. (Note: Average annual incidence not reported for Native Americans because of low numbers.)

Table. Characteristics of patients hospitalized for all causes and for disseminated coccidioidomycosis, by patient race/ethnicity and age, Arizona and California, USA*

Characteristic	Arizona hospitalizations		California hospitalizations	
	Disseminated coccidioidomycosis	All causes	Disseminated coccidioidomycosis	All causes
Total, no. patients	2,770	6,180,404	1,949	19,979,016
Race/ethnicity, no. (%) patients				
White	1,316 (47.5)	4,207,269 (68.1)	451 (23.1)	10,541,469 (52.8)
Black	771 (27.8)	219,265 (3.5)	561 (28.8)	1,520,896 (7.6)
Hispanic	360 (13.0)	1,240,707 (20.1)	587 (30.1)	4,957,476 (24.8)
Asian/Pacific Islander	80 (2.9)	76,345 (1.2)	125 (6.4)	1,269,150 (6.4)
Native American	102 (3.7)	197,181 (3.2)	NR	13,819 (0.07)
Other	65 (2.3)	98,975 (1.6)	18 (0.9)	342,786 (1.7)
Information missing	76 (2.7)	140,662 (2.3)	NR	1,333,420 (6.7)
Median patient age, y				
Overall†	**47**	54	**43**	54
White	**56**	60	**51**	62
Black	**37**	43	**40**	51
Hispanic	**41**	33	**41**	35
Asian	39	39	**50**	57
Native American	**46**	39	NR	47

*__Boldface__ indicates a significant difference (p<0.05) compared with all-cause hospitalizations. NR, information not reported because cell size is <10 or to prevent calculation of a cell size <10.
†Excludes hospitalized patients with missing race/ethnic information.

completely explain the large relative rates that we identified. This finding could indicate different disease pathology with more serious or long-term infection.

Potential bias from out-of-state residents was minimal because most disseminated coccidioidomycosis hospitalizations occurred within the state of residence. Differences in all-cause hospitalization rates were not likely to account for the differences in rates observed for our study condition. A better understanding of the progression of disease, including the number of previous hospitalizations, information on all coexisting conditions, and the severity of disease, could help explain the differences in incidence of hospitalization.

Overall, we found a higher incidence of disseminated coccidioidomycosis–associated hospitalizations for blacks compared with whites and other racial/ethnic groups living in these coccidioidomycosis-endemic areas, a finding that is consistent with previous studies (*6*,*7*). However, our study identifies this difference specifically in the absence of HIV/AIDS and primary immune conditions among a large cohort, which suggests other, unknown reasons for this disparity among races/ethnicities.

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Reemerging Sudan Ebola Virus Disease in Uganda, 2011

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Two large outbreaks of Ebola hemorrhagic fever occurred in Uganda in 2000 and 2007. In May 2011, we identified a single case of Sudan Ebola virus disease in Luwero District. The establishment of a permanent in-country laboratory and cooperation between international public health entities facilitated rapid outbreak response and control activities.

The Patient

On May 6, 2011, a 12-year-old girl from Nakisamata village, Luwero District, Uganda, was admitted to Bombo Military Hospital. She exhibited fever, jaundice, and hemorrhagic signs: epistaxis, hematemesis, hematuria, and conjunctival, gingival, and vaginal bleeding. The attending physician made a preliminary diagnosis of disseminated intravascular coagulopathy with a functional platelet disorder, along with viral hemorrhagic fever (VHF) as a possible cause. The patient was isolated from the general ward because of a high clinical suspicion of VHF. Hospital staff involved in her care and treatment implemented isolation precautions, including the use of personal protective equipment, such as gowns, gloves, and masks.

The patient's condition worsened and despite tracheal intubation and supplemental oxygen, she died 3 hours after admission. Because the cause of death was unknown but suspicion of VHF was high, the body was disinfected by using a chlorine solution and then wrapped in plastic, taken to the hospital mortuary facility, and placed in a coffin, which was then sealed. The coffin was released to the girl's relatives for burial, with instructions not to open the coffin or touch the body before burial.

A blood sample collected at the hospital before the patient's death was transported to the US Centers for Disease Control/Uganda Virus Research Institute (CDC/UVRI) laboratory in Entebbe for diagnostic testing by reverse transcription PCR (RT-PCR), antigen-detection ELISA, and IgM for filoviruses as described (*1*–*5*). Evidence of infection with an Ebola virus of the genus and species *Ebolavirus Sudan ebolavirus* (SEBOV) was detected by RT-PCR and confirmed by antigen-detection ELISA. Results of ELISA IgM against Ebola viruses and all tests for Marburg virus were negative. SEBOV was also isolated from blood on Vero E6 cells at the Viral Special Pathogens Branch, CDC, Atlanta, GA, USA.

Overlapping PCR fragment copies of the complete virus genome were amplified, and the nucleotide sequence was obtained as described (*6*). Maximum-likelihood phylogenetic analysis confirmed SEBOV and demonstrated that the isolate (Nakisamata isolate, JN638998) was closely related (99.3% identical) to the Gulu SEBOV strain obtained from northern Uganda in 2000 (Figure 1). A postmortem diagnosis indicated Ebola hemorrhagic fever (EHF) caused by SEBOV as the cause of the patient's death.

An investigation team from the Uganda Ministry of Health, CDC Uganda, and UVRI traveled to Bombo Military Hospital and Nakisamata village, the home of the case-patient, on May 13, 2011. The village is located in Luwero District, ≈50 km north of Kampala. The investigation team established that the case-patient reported feeling ill on May 1. She had a mild headache and was given an over-the-counter analgesic. She had a fever with chills on May 4 and began vomiting on May 5. On May 6, she experienced intense fatigue and epistaxis. The patient's grandmother then took her to a local health clinic where she received adrenaline nasal packs for her epistaxis and injections of quinine and vitamin K. The patient's condition continued to worsen, and she experienced hematemesis and vaginal bleeding. She was then transported by motorcycle taxi to Bombo Military Hospital, ≈35 km north of Kampala, by her grandmother and father.

The investigation team identified 25 close contacts of the patient, comprising 13 persons who had physical contact after illness onset at her home and 12 hospital staff members. Four of the hospital contacts were classified as having a high risk for exposure to SEBOV because of possible exposure to the patient's body fluids: 2 persons who performed tracheal intubation and 2 who handled the body after death. On May 15, a team from CDC Atlanta arrived in Uganda to provide assistance in laboratory diagnostics and epidemiological response. Also on this date, Médecins Sans Frontières, a nonprofit medical humanitarian

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organization, began establishment of an isolation ward at Bombo Military Hospital. During outbreak response and follow-up surveillance 21 days after the death of the case-patient, 24 sick persons (18 from Luwero District and 6 from other locations in Uganda) were identified. Testing at the CDC/UVRI laboratory ruled out EHF in this group.

Relatives reported that the girl did not travel outside Nakisamata village in the 3 months preceding her illness and did not attend any funerals or have contact with anyone visiting from another town or village before her illness. They recalled no unusual deaths in the area in recent months. They also reported that she had not been exposed to any sick or dead animals in the village or nearby forested area.

During follow-up investigation in Nakisamata village, several species of bats (tentatively identified as belonging to the genera *Epomophorus*, *Hipposideros*, *Pipistrellus*, and *Chaerophon*) were found roosting in unoccupied houses and several classrooms of the village schoolhouse where the girl attended classes, ≈400 meters from her home. Sixty-four bats were collected, and testing of these bats for Ebola virus (EBOV) is ongoing.

Samples from 4 family members, none of whom reported illness, were obtained and tested for EBOV by RT-PCR, antigen-detection ELISA, and IgM and IgG ELISAs. Test results for 3 of the family members were negative. One juvenile relative had positive IgG test results at a titer of 1,600 but was IgM negative, indicating past infection with EBOV. Since IgM antibodies can persist for as long as 2 months after infection (*1*,*7*), this person's infection appears temporally unrelated to the case-patient, who had EHF attributed to SEBOV. No clinical information was available to determine whether the relative's infection was symptomatic. Contact studies and serosurveys suggest that some EBOV infections can go unrecognized (*1*,*8*–*10*,*11*).

Conclusions

This case represents the second documented occurrence of an identified single-case EHF outbreak (*12*). We were unable to identify an epidemiologic link to any suspected EHF cases before the girl's illness onset, or to conclusively identify a suspected environmental source of infection in and around the village in which she lived. This suggests that her exposure was zoonotic in nature and must have occurred in the vicinity of her residence, since her relatives reported that she did not travel. The fact that an additional family member had serologic evidence of an epidemiologically unrelated EBOV infection further supports the notion that zoonotic exposures have occurred in the vicinity of the case-patient's village.

Rapid laboratory identification in this outbreak supported mobilization of an investigation team 1 day after initial laboratory detection and the rapid establishment of an isolation facility at Bombo Military Hospital. In this instance, the initial high suspicion of EHF by clinical staff, the appropriate use of personal protective equipment and barrier protection by hospital staff, and the rapid laboratory confirmation of EHF in-country likely contributed to limiting the size of this outbreak.

The timeliness of diagnostic confirmation and outbreak response was much improved over that during

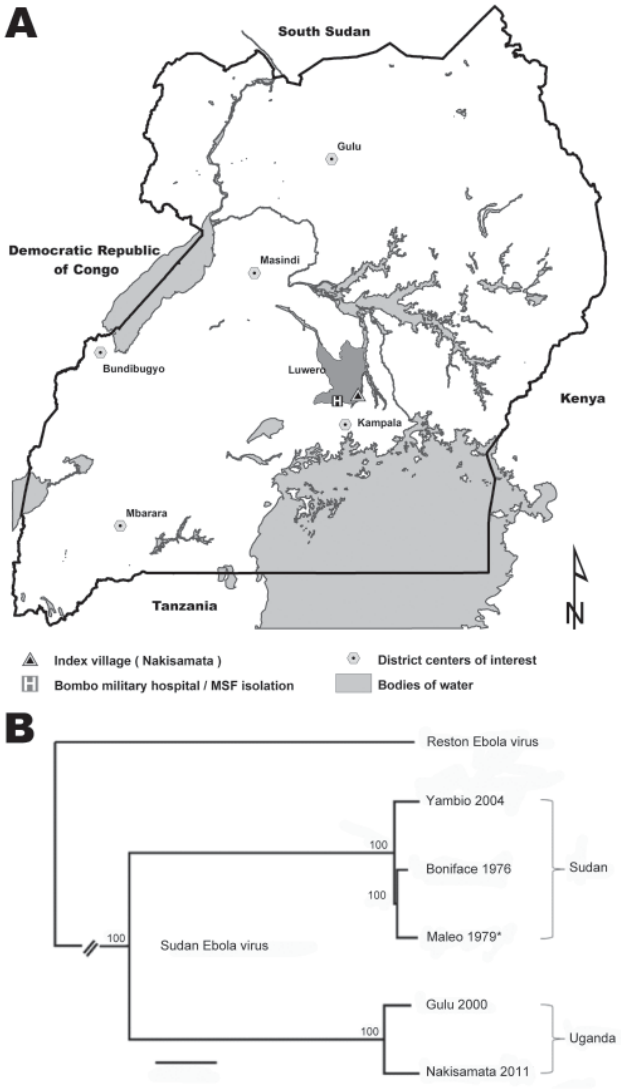

Figure 1. Sudan Ebola virus in Uganda, 2011. A) Geographic locations of Nakisimata village and Bombo Military Hospital with the isolation facility established by Médecins Sans Frontières (MSF) relative to locations where Sudan Ebola virus (SEBOV) was isolated during the current and previous outbreaks in Uganda. Scale bar indicates kilometers. B) Maximum likelihood tree obtained from full length sequences of SEBOV strains Nakisamata (JN638998), Boniface (FJ968794), Gulu (AY729654), and Yambio (EU338380) and the polymerase gene (*) of Maleo (U23458); full-length Reston Ebola virus (REBOV) (AY769362) is included as an outgroup. Bootstrap values listed at the nodes provide statistical support for 1,000 replicates. Scale bar indicates 0.006 substitutions per site.

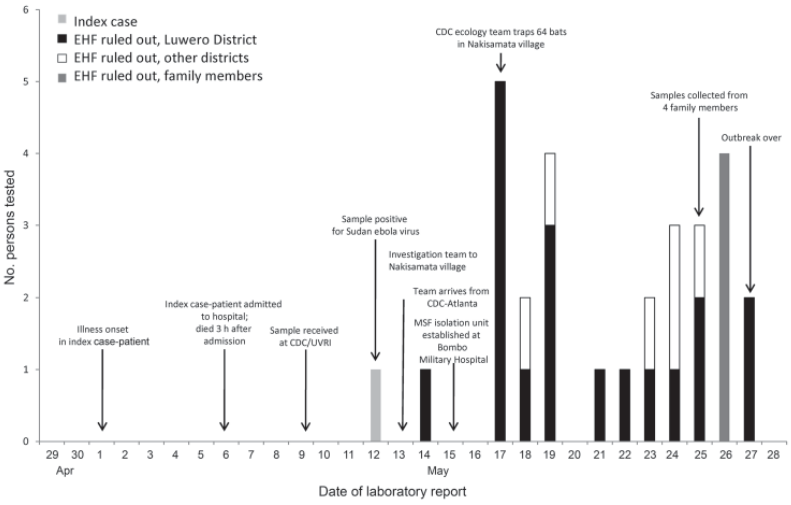

Figure 2. Timeline of Sudan Ebola virus outbreak, Uganda 2011, showing key events in the investigation and response. Also shown are the dates on which EHF was ruled out in other suspected cases by laboratory testing at the CDC/UVRI laboratory in Entebbe. EHF, Ebola hemorrhagic fever; CDC/UVRI, US Centers for Disease Control and Prevention, Uganda/Uganda Virus Research Institute Collaborative Laboratory; CDC, US Centers for Disease Control and Prevention; MSF, Médecins Sans Frontières.

previous EHF outbreaks in Uganda (timeline shown in Figure 2), during which transmission of the virus occurred for multiple months before the outbreaks were detected (*13–15*). This improvement was possible mainly because of collaboration by the CDC Viral Special Pathogens Branch and the Uganda Virus Research Institute to establish a permanent high-containment laboratory that is capable of performing diagnostic testing for filoviruses and other causes of VHF in Uganda. The limited extent of this outbreak also demonstrates the powerful utility of a national VHF surveillance system, coupled with the ability to rapidly diagnose and respond to limit the spread of such high-hazard infections in the community and health care facilities. Continued efforts are needed to build and sustain VHF surveillance networks across Africa.

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Francisella tularensis Subspecies *holarctica*, Tasmania, Australia, 2011

Justin Jackson, Alistair McGregor, Louise Cooley, Jimmy Ng, Mitchell Brown, Chong Wei Ong, Catharine Darcy, and Vitali Sintchenko

We report a case of ulceroglandular tularemia that developed in a woman after she was bitten by a ringtail possum (*Pseudocheirus peregrinus*) in a forest in Tasmania, Australia. *Francisella tularensis* subspecies *holarctica* was identified. This case indicates the emergence of *F. tularensis* type B in the Southern Hemisphere.

Tularemia is a zoonosis affecting a wide range of wildlife species, including mammals, birds, amphibians, and arthropods (*1*,*2*). Three subspecies of *Francisella tularensis* have been recognized as causes of disease in humans: ssp. *tularensis* (type A tularemia), ssp. *holarctica* (type B tularemia), and ssp. *novicida*. Type B tularemia is endemic to the Northern Hemisphere, and cases predominately occur in latitudes 30°N–71°N (*1*,*2*). We report a case of ulceroglandular tularemia in a human in Tasmania, Australia (latitude 42°S) who was bitten by a ringtail possum (*Pseudocheirus peregrinus*, Figure 1).

The Case

In February 2011, a 44-year-old woman was bitten on her right index finger by a wild ringtail possum near the Henty River on the western coast of Tasmania. The incident occurred in daylight, and the possum, normally nocturnal, appeared lethargic and unwell. The patient was immunocompetent and had never traveled outside of Australia. Three days later, an ulcer developed at the site of the bite, followed by the development of swollen and tender epitrochlear lymph nodes, fever, rigors, myalgias, and drenching night sweats. On day 4, the patient was prescribed oral β-lactam antimicrobial drugs by her local doctor. She took these for 2 weeks without clinical improvement. Axillary lymphadenopathy was palpable on day 14, and by day 17 the epitrochlear nodes had formed a spontaneously discharging sinus. A swab sample was collected from the primary ulcer site on day 17. No organisms grew after a 48-h incubation at 36°C in the following culture media: blood agar in ambient air, MacConkey agar in ambient air, and chocolate agar in 5% CO_2. The patient's antimicrobial drug regimen was altered empirically to ciprofloxacin 500 mg 2×/day and amoxicillin/clavulanic acid 875 mg/125 mg 2×/day for the next 4 weeks. Despite mild improvement in symptoms, epitrochlear and axillary lymphadenitis with suppuration persisted. Swab samples collected for fungal and mycobacterial culture from the epitrochlear sinus on day 30 were culture negative. On day 47, the patient was referred to the Royal Hobart Hospital in Tasmania. Histology from an excisional biopsy of the epitrochlear sinus on day 50 revealed acute inflammation and a few non-necrotizing epithelioid granulomas. This tissue and an axillary lymph node aspirate were sent to a reference laboratory for molecular studies.

In the reference laboratory, the 16S rRNA gene was amplified from the axillary aspirate by using primers targeting the U1, U3, and U5 regions. The resulting 1,331-bp amplicon (GenBank accession no. JQ277265) demonstrated 100% homology with 16S rRNA gene sequences from *F. tularensis* ssp. *tularensis* and *holarctica* stored in GenBank. PCR and sequencing of the *recA* gene (*3*) confirmed *F. tularensis* (GenBank accession no. JQ277266). Amplicons of 16S rRNA and *recA* genes were aligned with reference sequences from the GenBank/European Molecular Biology Laboratory/DNA DataBank of Japan databases and with sequences from 2 previous *Francisella* spp. reported from Australia (*4*). The 16S rRNA and *recA* gene sequences showed 100% query

Figure 1. Ringtail possum (*Pseudocheirus peregrinus*) as photographed by its bite victim, Tasmania, Australia, 2011.

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coverage and an Expect value of 0.00 in BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with 36 submissions of *F. tularensis* genomes (Figure 2). The unrooted bootstrap consensus tree generated from sequence alignment of the *recA* gene sequence, coupled with the neighbor-joining method, suggested homology between *F. tularensis* ssp. *holarctica* and the organism responsible for this patient's illness. Sequencing of the region of difference 1 (*5*) by using previously validated primers (*3*) indicated the region of difference 1 size was 835 bp, closely approximating the 840-bp size associated with *F. tularensis* ssp. *holarctica* biovar *japonica*.

Lymph node aspirate from the patient cultured in horse blood and in blood cysteine and chocolate media with and without supplement (BBL IsoVitaleX Enrichment; Becton, Dickinson and Co., Sparks, MD, USA) produced no growth after 2 weeks of incubation (37°C, 5% CO_2); the control strain of *F. tularensis* live vaccine strain grew within 3 days. We used reagents and protocols from the Laboratory Response Network, US Centers for Disease Control and Prevention, to perform real-time PCR for *F. tularensis*. The procedure amplified 2 of 3 targets (FT2 and FT3). IgG and IgM against *F. tularensis* antigens were detected by use of ELISA (Virion-Serion, Würzburg, Germany) in serum obtained 2 months after the onset of symptoms in concentrations of 147.13 and 222.74 U/mL, respectively (positive cutoff >15 U/mL). Confirmatory serology for *F. tularensis* was performed by the Centers for Disease Control and Prevention vector-borne diseases laboratory (Fort Collins, CO, USA) on serum samples collected 56 and 75 days after symptom onset. Both specimens were positive for *F. tularensis*, evidenced by a microagglutination titer of 128 (positive cutoff >128). On the basis of these results, the patient was treated with a 14-day course of intravenous gentamicin. Her symptoms resolved promptly, and the woman remained well at a 6-month follow-up visit.

This case demonstrates the well-recognized features of *F. tularensis* ssp. *holarctica* infection as described from the Northern Hemisphere. The mode of transmission, incubation period, clinical syndrome, lack of response to β-lactam antimicrobial drugs, and response to aminoglycoside therapy are all characteristic of type B tularemia (*6*). Although a clinical response to ciprofloxacin could have been expected, treatment failures have been reported when initiation of therapy is delayed (*7*). The lack of growth on blood and chocolate agar is also consistent with ssp. *holarctica* and contrasts with ssp. *novicida* and *F. philomiragia* (*4*,*6*). The inability to culture an organism on specialized media probably was caused by prolonged ciprofloxacin treatment before the specific culture attempt. This case met the definition for probable tularemia according to the 2007 World Health Organization guidelines (*8*); however, the combination of clinical, serologic, and molecular evidence strongly supports the diagnosis of infection with *F. tularensis* ssp. *holarctica*. This conclusion is consistent with recent reports of tularemia in which case definitions included a compatible clinical syndrome and positive *F. tularensis* real-time PCR or DNA sequencing (*9*,*10*).

Cases of human infection with other *Francisella* species have been reported from Australia (*4*). *F. philomiragia* was isolated from a lymph node in a child in Victoria with chronic granulomatous disease, and a *novicida*-like organism was cultured from the toe of a 53-year-old man after a cut received in brackish water in the Northern Territory. In contrast to type A and B tularemia, these organisms are rare human pathogens and are not associated with arthropod vectors or animal hosts.

More than 300 species of mammals, birds, invertebrates, and amphibians are currently recognized as being susceptible to type B tularemia (*1*). This report adds the ringtail possum, found widely throughout Australia, to the list of species that are susceptible to tularemia and have transmitted this pathogen to humans. Although transmission of tularemia is most often associated with species from the orders Lagomorpha and Rodentia, human

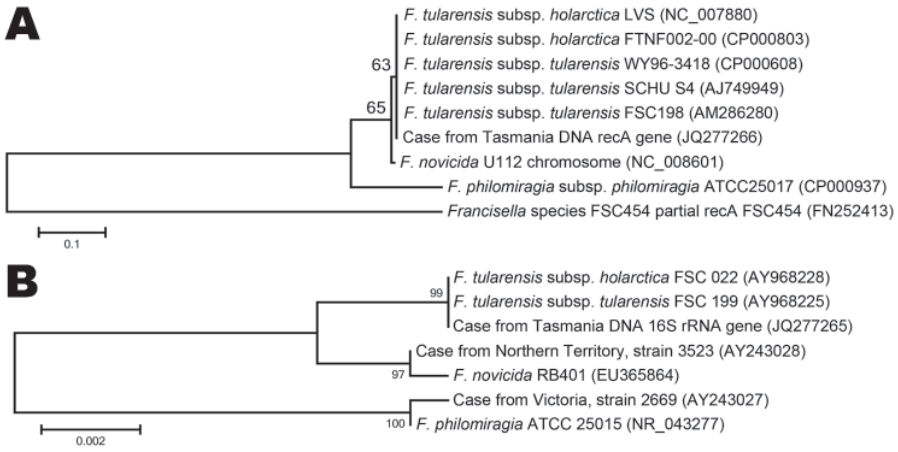

Figure 2. Nucleotide sequence comparison of the *recA* (A) and 16S rRNA (B) genes of *Francisella tularensis* subspecies *holarctica*, Tasmania, Australia, 2011. Reference sequences from the GenBank/European Molecular Biology Laboratory/DNA DataBank of Japan databases and 16S rRNA gene sequences from an *F. novicida* and an *F. philomiragia* infection reported from Australia (*4*) were aligned with amplicons of 16S rRNA and *recA* genes from samples with PCR results positive for *F. tularensis* from a 44-year-old woman. Scale bars indicate nucleotide substitutions per site.

infection following contact with marsupials has long been recognized. Within the United States, opossum-to-human transmission was described as early as 1929 (*11*).

The discovery of *F. tularensis* in this remote location of Australia raises questions about possible routes of spread and natural reservoirs of tularemia. Tularemia has emerged several times in nonendemic areas after the importation of infected wildlife (*12*) or changes in ecologic and climate conditions (*13*), or in settings of postwar social disruption (*14*); but no such recent events have occurred in Tasmania. An alternate possibility is introduction of tularemia to Australia by water birds migrating from Southeast Asia that, if infected, have the potential to contaminate surface waters with *F. tularensis* (*15*).

Conclusions

This case provides evidence of type B tularemia in Australia and should alert physicians and veterinarians working within the region to the possibility of infection with this organism. The transmission of *F. tularensis* to a human host after the bite of an animal native to Australia suggests an ecologic niche for this bacterium in the forests of western Tasmania. Further research is necessary to elucidate the role that ringtail possums, small mammals, ticks, tabanid flies, and mosquitoes prevalent in this unique location might play as natural reservoirs for and vectors of *F. tularensis*.

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Lack of Evidence for Chloroquine-Resistant *Plasmodium falciparum* Malaria, Leogane, Haiti

Ami Neuberger, Kathleen Zhong, Kevin C Kain, and Eli Schwartz

Plasmodium falciparum malaria in Haiti is considered chloroquine susceptible, although resistance transporter alleles associated with chloroquine resistance were recently detected. Among 49 patients with falciparum malaria, we found neither parasites carrying haplotypes associated with chloroquine resistance nor instances of chloroquine treatment failure. Continued vigilance to detect emergence of chloroquine resistance is needed.

Plasmodium falciparum malaria, which has been eliminated from most of the Caribbean islands, is endemic to the island of Hispaniola (*1*–*3*) and is especially prevalent in Haiti. Epidemiologic data from Haiti are scarce, but malaria is probably endemic to most areas situated at altitudes <300 m. The town of Leogane, situated in Ouest Province 30 km west of Port-au-Prince, is no exception, and we have reported a high incidence of malaria among febrile patients during the peak malaria transmission season of November 2010–March 2011 (*1*).

In sharp contrast to the situation in most other malarial regions in the world, *P. falciparum* parasites in Haiti remain susceptible to chloroquine (*4*,*5*). To the best of our knowledge, clinical failure of chloroquine for prophylaxis or treatment of *P. falciparum* malaria has not been reported, and the Centers for Disease Control and Prevention recommends chloroquine as a first-line option for the treatment and prophylaxis of malaria in Haiti (*6*).

All studies reporting universal chloroquine susceptibility of *P. falciparum* parasites in Haiti were performed before the 1990s (*4*,*5*). However, a recent report from the Artibonite Valley north of Port-au-Prince documented that the *P. falciparum* chloroquine resistance transporter (*pfcrt*) haplotypes were detected in 5 (6%) of 79 blood samples from Haitians with blood smears positive for *P. falciparum* parasites (*7*). Although this report did not include clinical data, did not document clinical chloroquine treatment failure, and reported the detection of haplotypes associated with chloroquine resistance in a minority of all tested samples, the emergence of chloroquine resistance would be of great concern. Because epidemiologic data from Haiti are scarce, at best, and because the malaria surveillance system operating in the country is inadequate, undetected cases of chloroquine-resistant malaria might exist. We aimed to search for chloroquine-resistant cases either clinically or by detecting *pfcrt* haplotypes in *P. falciparum* isolates in Leogane, Haiti.

The Study

The study was conducted during November 2010–May 2011 in a primary health care clinic in Ouest Province of Haiti. The clinic is situated in Leogane, a town of ≈200,000 residents, 30 km west of Port-au-Prince. The clinic is staffed by volunteer nurses and doctors from Israel and Canada and by local hired staff from Haiti. The clinic has been operating on a daily basis since November 2010 (*8*).

Malaria was diagnosed by using a rapid diagnostic test (RDT; Paracheck Rapid Test, Orchid Biomedical Systems, Goa, India) to detect histidine-rich protein II in patients with histories of compatible febrile illness. All patients visiting the clinic with undifferentiated fever were tested by using an RDT, and malaria was not diagnosed or treated in these patients without this laboratory confirmation. Microscopy was not available. Blood from patients with malaria was collected on filter paper blots, which were sent to the laboratory at the University of Toronto (Toronto, ON, Canada). A *P. falciparum* DNA sample was extracted from blotted filter paper blood samples and eluted in 100 μL of water by using QIAGEN columns (QIAGEN, Chatsworth, CA, USA) according to the manufacturer's instruction. A 10-μL aliquot of the DNA extract was used in real-time PCR, and results were confirmed by using a nested PCR and sequencing as described (*9*–*11*). Nested PCR was performed to amplify the *P. falciparum pfcrt* fragment around amino acid residue 72–76. DNA sequencing of the 134 base-pair PCR product was performed to determine the amino acid haplotype of residues 72–76 as described (*9*,*10*). The lysine to threonine mutation at residue 76 (K76T) in *pfcrt* was present in all chloroquine–resistance strains, and the amino acid haplotype of CVMNK was present in chloroquine-sensitive strains (Tables 1, 2).

Forty-nine patients with a febrile illness were positive by RDT for falciparum malaria. Their mean age (± SD) was 24.1 ± 14.4 years, and 28 were male. Forty-eight patients were Haitians with uncomplicated falciparum malaria; 1

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Table 1. Amino acid haplotypes of *pfcrt* K76T polymorphism, Haiti*

Chloroquine-resistant strains	Chloroquine-sensitive strains
CVIET	CVMNK
CVMNT	
CVMET	
SVMNT	

**pfcrt*, *Plasmodium falciparum* chloroquine resistance transporter.

was an expatriate with severe malaria. The patient with severe malaria was transferred to the United States for care.

All patients initially received chloroquine. One patient developed an allergic reaction to chloroquine and was subsequently treated with artemether–lumefantrine. No patients returned to the clinic after receiving chloroquine, even though they were encouraged to do so if fever persisted; all medical services were provided without charge. Attempts to contact patients by telephone 1 month after completion of treatment had limited success. Only 8 were contacted successfully; all were asymptomatic after treatment with chloroquine alone.

P. falciparum DNA was detected in all positive RDT samples. We did not observe any false-positive RDT results. Further evaluation was conducted for haplotypes associated with the chloroquine resistance gene. A total of 49 samples were amplified and sequenced. All falciparum isolates carried *pfcrt* haplotypes encoding the amino acid chloroquine-susceptible haplotype CVMNK (residues 72–76). The prevalence of chloroquine-sensitive strains was 100%.

Conclusions

Haiti has traditionally been considered a unique region of endemic chloroquine-sensitive falciparum malaria. However this situation was challenged by a recent report of circulating *P. falciparum* parasites possessing haplotypes associated with chloroquine resistance in ≈6% of blood samples obtained by passive and active case detection (*7*).

We did not detect any cases of chloroquine treatment failure; in many cases, however, complete follow-up 1 month after treatment was not possible. Nor did we detect any falciparum isolates with *pfcrt* haplotypes associated with chloroquine resistance.

Table 2. PCR primer sequences for *cytochrome b* of *Plasmodium falciparum* gene fragment *pfcrt*, Haiti*

Primer sequence	Product size
5′-CCGTTAATAATAAATACACGCAG-3′ (sense)	537 bp
5′-CGGATGTTACAAAACTATAGTTACC-3′ (antisense)	134 bp
5′-TGTGCTCATGTGTTTAAACTT-3′ (sense)	
5′-CAAAACTATAGTTACCAATTTTG-3′ (antisense)	

**pfcrt*, *Plasmodium falciparum* chloroquine resistance transporter.

In addition, as previously reported, we did not detect any cases of malaria in expatriates who were using chloroquine prophylaxis (*1*). These observations strengthen the current opinion that *P. falciparum* remains chloroquine sensitive in Haiti.

Our conclusions are subject to several limitations. Our study was conducted in Leogane, situated 30 km west of Port-au-Prince. The haplotypes associated with chloroquine resistance were detected previously in the Artibonite Valley (*7*) ≈100 km north of Port-au-Prince; thus, chloroquine susceptibility might have varied by geographical region. In addition, cases of malaria caused by chloroquine-resistant strains of *P. falciparum* could have been missed because of the small size of our cohort. Another limitation of our study is the incomplete follow-up of most patients. Conditions in Haiti after the 2010 earthquake make adequate follow-up difficult. However, because medical services and medications were provided free of charge and because patients were instructed to return if fever persisted or recurred, we find it unlikely that cases of chloroquine treatment failure were missed.

The issue of possible chloroquine resistance has implications for Haitians and for the large number of volunteers and expatriates employed by the various aid organizations living in Haiti since the earthquake. Our results support the current recommendation of chloroquine as a first-line choice for treatment of falciparum malaria and for malaria chemoprophylaxis in Haiti. However, continued vigilance to detect the emergence of chloroquine resistance is warranted.

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Infectious Diseases in Children and Body Mass Index in Young Adults

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In a cohort of 1,863 Filipinos, diarrhea, fever, and unsanitary conditions in infancy were associated with a decreased body mass index in adulthood; upper respiratory tract infection was associated with an increased body mass index. These finding support the hypothesis that infections early in life play a role in body habitus in adulthood.

Obesity rates are increasing globally, particularly in countries transitioning from a state in which mortality rates are driven primarily by infectious disease to a state in which mortality rates are driven by chronic diseases. In the Philippines, a shift in obesity rates occurred between 1985 and 2005, with weight increasing in 40-year-old women by 9.6 kg (*1*). By 2011, 24.6% of Filipino men and 28.4% of women were overweight (*2*).

Rapid changes in population health historically have been caused by the elimination or introduction of infectious pathogens. In the last 3 decades, infectious diseases among humans have been disappearing from society because of vaccines and improved vaccination practices (*3*), widespread antimicrobial drug use, expanded medical care access, and improved sanitation (*4*). Infections cause alterations in inflammatory cytokines and adipocytokines and raise metabolic rates, and they may influence the colonizing microbiota, which vary with the colonized person's weight (*5*). Childhood infections may thus affect a person's long-term weight. We sought to determine whether infectious disease prevalence in infancy predicted body mass index (BMI) in adulthood.

The Study

We analyzed data from the Cebu Longitudinal Health and Nutrition Survey, an ongoing study of a cohort of Filipino women who gave birth between May 1, 1983, and April 30, 1984, in Cebu, the Philippines (www.cpc.unc.edu/projects/cebu) (*6*). Our study included data from children who were born and reached young adulthood during 1983–2005. Anthropometric, dietary, and infectious disease symptom data were collected bimonthly during the first 24 months of life for children born during that time. Weight and height measurements were again obtained when participants were 22 years of age.

Childhood infectious disease prevalence was derived from symptoms reported at the 12 bimonthly visits. The prevalence for each child was calculated as follows: the proportion of visits when the child had a current fever, the proportion of visits when the child had a cough or nasal congestion within the prior week (upper respiratory infection [URI]), the proportion of visits when the child had diarrhea within the prior week, and the total number of days with diarrhea within the prior week. The child's highest educational level attained as an adult was used as a marker for adult socioeconomic status (SES). The mother's highest number of years of schooling or vocational training was used as a partial surrogate for childhood SES; other childhood SES markers included household crowding, toilet type, and presence of animal or human excrement around the home. For inclusion in models, we additionally selected a priori environmental factors likely to be correlated with infectious illness: water source and the proportion of visits in which the child was observed crawling in unsanitary conditions (i.e., in a yard containing excrement).

Outcomes included BMI and whether the person was considered "overweight" or "obese" (for Asian populations, BMI >23 kg/m^2 and >27 kg/m^2) (*7*). Median values for continuous variables and proportions for discrete variables were calculated overall and by overweight category; differences between groups were assessed by using a median or χ^2 test. Pearson coefficient was used to determine coefficient.

Linear regression was used to determine whether childhood infectious disease prevalence independently predicted increased BMI in adulthood. Logistic regression was used to examine the association between infectious disease in a child and an outcome of the person being overweight as an adult. We also explored the effect of infectious disease on BMI at age 24 months. All SES and potential exposure variables were also included in all models, as were birth weight and sex (*8*,*9*). Variables other than those for infectious disease prevalence were retained only when $p<0.05$.

A total of 1,893 participants had a measurable BMI reported as adults; of these, 1,863, who had at least 1 visit recorded during their first 24 months of life, were included in analyses (Table 1, Appendix, wwwnc.cdc.gov/EID/article/18/9/11-1821-T1.htm). Few of the adults were obese (3.1%), and 21.2% had >12 years of schooling (data not shown). Conditions during infancy reflected high levels of poverty.

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Subjects participated in ≈11.5 visits during the first 24 months of life (Table 1, Appendix). Cough and nasal discharge or congestion were common (reported at a mean of 6.85 and 7.09 visits, respectively); ear discharge and sore throat were rare (mean 0.36 and 2.37 visits, respectively). Reported fever during the visit was unusual (mean 0.82 of 12 visits, respectively); fever reported before the visit or ongoing diarrhea was slightly more common (mean 2.19 of 12 visits, respectively). The mean reported number of days of diarrhea in the 12 sampling weeks was equivalent to 30 days over a 2-year period (Table 1, Appendix).

Using multivariate regression modeling, we examined BMI when the participants were 24 months of age and again when participants were 22 years of age (Table 2). We identified reported diarrhea, crawling in unsanitary conditions, and URI in infancy as predictors of BMI at age 22 years. The individual effects of these factors, however, were opposing: diarrhea and crawling in unsanitary conditions were associated with decreased adult BMI ($p = 0.06$ and $p = 0.02$, respectively), and URI was associated with increased adult BMI ($p = 0.01$). Point estimates for the proportion of visits with fever, although not statistically significant, showed a trend toward decreased BMI. Significant predictors of BMI at 24 months included only proportion of visits with fever, which was associated with a decreased BMI. No interactions between infectious disease variables and other predictors were identified. Logistic regression modeling of overweight adults (BMI >23 kg/m^2) identified similar findings (data not shown). Again, reported URI in children was associated with higher prevalence of overweight adults, whereas crawling in unsanitary conditions in infancy was linked to decreased prevalence of overweight adults. The point estimates for both the number of visits with current fever and with reported diarrhea, although not statistically significant, showed that these factors were protective against being overweight in adulthood (Table 2).

Conclusions

In an economically disadvantaged Filipino population, we observed that frequency of diarrhea in infancy was associated with a lower BMI in adulthood. Time spent crawling in unsanitary conditions—a surrogate for SES and exposure to pathogenic fecal bacteria carried by humans—was also linked to BMI in adulthood. Cumulative infectious diarrheal diseases in children are known to affect their growth and development (*10*). Although this effect on growth may be because of decreased nutrient absorption, a more enduring consequence may be the modification of the gut microbiome (*11*). For example, *Helicobacter pylori*, a common component of the microbiome in populations with decreased household sanitation, has been associated with decreased adult weight (*12*). Studies of the colonic microbiota are under way to determine whether their establishment in childhood predicts adult adioposity.

In contrast to the frequency of diarrheal diseases, URI frequency was associated with increased BMI or a score that indicates that the person is overweight. This association ran counter to our original hypothesis that infectious illness in early infancy is associated with decreased BMI in adults. Researchers have postulated that exposure to specific respiratory pathogens (e.g., adenovirus 36) may increase weight (*13*). No consensus exists, however, regarding the importance of these individual pathogens, which may simply identify markers for other exposures. Another possible explanation for the opposite effects of respiratory and gastrointestinal infections may be that respiratory infections lead to antimicrobial drug use, (which has, itself, been linked to higher weight in animals and in humans [*14*]), whereas the gastrointestinal infections do not.

The Cebu study was largely a study of nutrition; infection was of only minor interest as a confounder. As such, infection relied on maternal recall without detailed temperature measurements or confirmatory diagnoses. Yet, despite the fact that the database was not designed with infectious disease exposures as a primary measure, we were able to identify a role of infection in BMI after adjusting for multiple markers of SES. Although childhood infections may be a surrogate for unmeasured characteristics that correlate with adult BMI levels, we were unable to identify these confounders despite the many covariates we assessed.

Table 2. Independent predictors of BMI in a cohort of persons during childhood and during adulthood, the Philippines, 1983–2005 *†

	BMI, age ≈24 mo		BMI, age ≈22 y	
Predictor	Parameter estimate	p value	Parameter estimate	p value
Visits with reported URI‡	–0.25	0.14	1.13§	0.01
Visits with current fever	–1.41	<0.001	–1.61	0.11
Visits with reported diarrhea	0.23	0.27	–1.05	0.06
Visits during which child was observed crawling in unsanitary conditions	–0.05	0.65	–0.72	0.02

*BMI, body mass index; URI, upper respiratory infection.
†Models were adjusted for maternal schooling/training (<7 or >7 y), sex and birth weight. R^2: BMI age 24 mo, 0.08; BMI age 22 y, 0.03.
‡A model that used number of visits instead of proportion of visits identified very similar findings, as did a model that limited analysis to those with >10 visits only.
§The parameter estimate corresponds to the change in BMI when participants with no visits (0%) with infection are compared to those with all visits (100%) with infection. Alternatively, a 10% increase in reported visits with infection corresponds to an increase of 10% of the parameter in BMI in adulthood. For example, a 10% increase in visits with reported URI corresponds to a 0.113 kg/m^2 increase in adult BMI.

In summary, our study demonstrated an association between frequency of symptomatic infectious diseases during infancy and BMI in adults. Prospective studies in well-characterized subjects are needed to confirm these findings, delineate mechanisms, and assess the magnitude of the effects of early childhood infectious diseases on weight.

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Inadequate Antibody Response to Rabies Vaccine in Immunocompromised Patient

Eran Kopel, Gal Oren, Yechezkel Sidi, and Dan David

We describe an inadequate antibody response to rabies vaccine in an immunocompromised patient. A literature search revealed 15 additional immunocompromised patients, of whom 7 did not exhibit the minimum acceptable level of antibodies after a complete postexposure prophylaxis regimen. An international rabies registry is needed to provide a basis for determining appropriate vaccination protocols.

Rabies is a rapidly progressive viral encephalitis caused by RNA viruses of the family *Rhabdoviridae*, genus *Lyssavirus*. Dogs are the major reservoir for these viruses worldwide and usually transmit the virus by conveying their infected saliva through the penetrated skin of bitten humans or animals. The usual incubation period in humans ranges from 10 days to 1 year (average 20–60 days). Rabies causes 30,000–70,000 human deaths throughout the world each year. The rabies-related death rate is ≈100% in unvaccinated patients. Thus, preexposure prophylaxis and postexposure prophylaxis (PEP) are the main effective approaches for treating the disease (*1*–*3*).

We describe a case in which an acceptable antibody response to rabies vaccine did not develop in an immunocompromised patient. We also searched the literature for similar cases and summarize the demographic, clinical, and epidemiologic characteristics of such case-patients to date.

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The Patient

A 74-year-old woman was hospitalized at the Chaim Sheba Medical Center in August 2011; she reported progressive general weakness that had begun several months before her admission. Her blood count on admission showed severe lymphopenia (250 lymphocytes/μL). In addition, her recent medical history suggested that she had experienced a category II or III exposure (*4*) to a monkey's bite, as classified by the World Health Organization (WHO), 10 days before admission, while she was traveling in a country where rabies was endemic.

The patient was treated with the standard PEP regimen for immunocompromised patients in accordance with Israel Ministry of Health guidelines at the time she was admitted (*5*). These guidelines also corresponded to the latest guidelines of WHO and of the American Advisory Committee on Immunization Practices (ACIP) regarding PEP for immunocompromised patients (*4*,*6*). In brief, 5 doses of cell culture rabies vaccine, of which both purified Vero cell vaccine (PVRV) and purified chick embryo cell vaccine are available in Israel, are administered intramuscularly on days 0 (together with 20 IU/kg of human rabies immune globulin), 3, 7, 14, and 28.

The PEP regimen for the patient began 12 days after her potential exposure to rabies virus through the monkey bite with the administration of the PVRV vaccine (Verorab, batch E1036; Sanofi Pasteur SA, Lyon, France). On day 15 of the PEP regimen, 2 vials of serum and 1 vial of cerebrospinal fluid (CSF), each of which contained >2 mL of fluid from routine samples, were tested for rabies virus neutralizing antibodies (VNA) by the National Rabies Laboratory at Kimron Veterinary Institute. These samples were adequately cooled until the time of analysis. VNA titers were measured by using the rapid fluorescent focus inhibition test (*7*).

No detectable levels of VNA were measured either in CSF (<0.04 IU/mL) or in the serum samples (<0.07 IU/mL in both vials). The acceptable WHO cut-off level, indicating an adequate adaptive immune response, is 0.5 IU/mL (*4*); the ACIP cut-off level is 0.1 IU/mL (complete virus neutralization at serum dilution of 1:5) (*6*).

Before the fifth PVRV could be administered, the patient died of sepsis, most likely of nosocomial origin, induced by her rapidly progressive immunodeficient condition. The pathologic and histologic findings from a lymph node biopsy specimen were concordant with the diagnosis of advanced B-cell lymphoma.

Because of the challenging clinical conditions that we encountered (an immunocompromised patient in need of rabies PEP without an apparent adequate adaptive immune response to the standard regimen), we searched the medical literature for similar reported cases to describe more completely, and in proper context, the epidemiologic and

public health issues that were evoked by our case-patient. We conducted a search in the MEDLINE database using the PUBMED website (http://www.ncbi.nlm.nih.gov/pubmed) on September 15, 2011. We used various combinations of the following search terms or Medical Subject Heading terms: "rabies," "vaccine," "failure," "immune response," "human," and "immunocompromised host."

By this strategy, we found 5 publications (*8*–*12*), which reported 15 immunocompromised patients who were possibly exposed to rabies and were given a PEP regimen (Table). Various underlying illnesses were responsible for the immunodeficiency states of these patients. Eight patients had AIDS (*8*,*10*), defined as laboratory confirmation of HIV infection and CD4+ T-lymphocyte count of <200 cells/μL for patients >13 years of age or if the criteria for HIV infection were met and at least 1 of the AIDS-defining conditions had been documented for patients 18 months to <13 years of age (*13*). Five patients were infected with HIV, of whom ≥1 patients had AIDS, but this information was not further specified in the original publication (*9*). One patient had high-grade B-cell lymphoma (*11*), and 1 patient had received a kidney transplant (*12*).

Of these 15 patients, 7 did not show the acceptable WHO cut-off VNA titer level at any of the reported measurement points during and after administration of the initial PEP regimen. Whether an adequate immune response had eventually developed in a patient due to any additional vaccine doses beyond the original PEP protocol was not accounted for in our literature summary.

Conclusions

We report a patient who had an inadequate rabies antibody response to the standard PEP regime probably due to an underlying immunodeficiency condition. Besides this case-patient, from a survey of the literature, we found reports of an additional 7 immunocompromised patients who also demonstrated a lack of acceptable response level. These patients had followed various rabies PEP regimens with different vaccine types, administration methods, number of injection sites, and doses. However, despite those measures, an adequate immune response did not develop in these 7 patients during the entire follow-up period of each study.

The WHO cut-off titer level of 0.5 IU/mL (*4*), an equivalent to complete virus neutralization at a serum dilution of ≈1:50 by the rapid fluorescent focus inhibition test, as well as the lower titer recommend by ACIP (serum dilution of 1:5) (*6*) are arbitrary laboratory values that do not correlate directly with seroprotection. Moreover, the WHO cut-off titer was originally based on the adequate immune response levels required when repeatedly monitoring healthy patients who needed a prophylactic preexposure regime on a regular basis, for example, veterinarians (*14*).

The VNA titer response ideally should be determined 2–4 weeks (WHO) or 1–2 weeks (ACIP) after the last dose of vaccine to assess whether an additional dose is needed (*4*,*6*). This points to a limitation of the current study, which had 1 measurement point on day 15; thus, the measurement time in our study might merely reflect an immune response to the first 3 doses (days 0, 3, and 7). The same limitation also applies to the previously published studies (*8*–*12*), all

Table. Summary of published reports on inadequate antibody response to rabies vaccine in immunocompromised patients*

Characteristic	Case-patient 1 (*8*)	Case-patients 2–6 (*9*)	Case-patients 7–13 (*10*)	Case-patient 14 (*11*)	Case-patient 15 (*12*)	Case-patient in this study
Age, y	6	NA	7–38	55	55	74
Sex	F	NA	4 F, 3 M	M	M	F
Country	Thailand	Thailand	Thailand	Israel	Mexico	Israel
Vaccination year	1998	NA	1998–1999	1999	2009	2011
Underlying illness	AIDS	HIV infection	AIDS	Advanced B-cell lymphoma	Kidney transplant recipient	Advanced B-cell lymphoma
Leukocyte count at baseline	44 $CD4^+$/μL	111–250 $CD4^+$/μL	25–199 $CD4^+$/μL	NA	NA	250 lymphocytes/μL
Vaccine type (dose)	PVRV (0.1 mL)	PVRV (0.1 mL)	PVRV (0.1 mL)	PCECV (1.0 mL)	PVRV (0.5 mL)	PVRV (0.5 mL)
Standard PEP regimen (d)	4 sites, ID (0, 3, 7); 2 sites, ID (60, 90)	2 sites, ID (0, 3, 7); 1 site, ID (28, 90)	4 sites, ID (0, 3, 7); 2 sites, ID (60, 90)	1 site, IM (0, 3, 7, 14, 28)	1 site, IM (0, 3, 7, 14, 28)	1 site, IM (0, 3, 7, 14)†
Ig at day 0 (dose)	NA	Equine rabies Ig (40 IU/kg)	Human rabies Ig (20 IU/kg)	Human rabies Ig (20 IU/kg)	Human rabies Ig (20 IU/kg)	Human rabies Ig (20 IU/kg)
VNA titer in case-patients without adequate vaccine response (d of last measurement)	<0.07 IU/mL in serum (90)	Undetectable and <0.5 IU/mL in serum samples for 2 patients (90)	<0.04 IU/mL and 0.23 IU/mL in samples for 2 patients (90)	0.2 IU/mL in serum (30)	0.31 IU/mL in serum (28)	<0.07 IU/mL in serum and <0.04 IU/mL in CSF (15)

*NA, not available; PVRV, purified Vero cell vaccine; PCECV, purified chick embryo cell vaccine; PEP, postexposure prophylaxis; ID, intradermal; IM, intramuscular; VNA, rabies virus neutralizing antibodies; CSF, cerebrospinal fluid.
†The patient died before the scheduled fifth dose on day 28.

of which had the last VNA titer measurement on the same day or only a few days after the end of the vaccination regimen. Nevertheless, not even a slight increase in the VNA titers was observed on day 15 in either the CSF or serum samples, which could possibly imply a further lack of immune response. Low or undetectable VNA levels on day 90, the last day of the PEP regimen, were similarly observed in some of the previous studies (*8*–*10*). Thus, we could not expect the antibody titer to rise much further, even if additional measurements would have been taken 1–4 weeks after the last vaccine dose as indicated by the guidelines.

In conclusion, current epidemiologic knowledge and existing PEP regimens might not provide enough reassurance for public health experts and attending clinicians when advising and treating immunocompromised patients. Establishing a collaborative international rabies registry with a particular emphasis on immunocompromised patients could therefore provide evidence that would contribute to decisions regarding the appropriate vaccination protocol.

Dr. Kopel is a public health physician and an epidemiologist. Among his research interests are field epidemiology, infectious diseases of public health significance, and population-based cohorts.

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Yersinia enterocolitica Outbreak Associated with Ready-to-Eat Salad Mix, Norway, 2011

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In 2011, an outbreak of illness caused by *Yersinia enterocolitica* O:9 in Norway was linked to ready-to-eat salad mix, an unusual vehicle for this pathogen. The outbreak illustrates the need to characterize isolates of this organism, and reinforces the need for international traceback mechanisms for fresh produce.

Yersiniosis, a notifiable disease in Norway, is the fourth most common cause of acute bacterial enteritis registered by the Norwegian Surveillance System for Communicable Diseases. Approximately 30 domestic cases are reported annually (2010 incidence rate 0.5 cases/100,000 population). In Norway, >98% of cases of *Yersinia enterocolitica* infection are caused by serotype O:3, which is also the dominant serotype in Europe, Japan, and parts of North America (*1*). Infection by *Y. enterocolitica* is often associated with ingestion of pork because pigs commonly harbor the pathogenic serotypes O:3 and O:9 (*1*). Recent foodborne outbreaks have been associated with pork products (*2*,*3*) and pasteurized milk (*4*).

In Norway, fecal specimens from patients who have acute gastroenteritis are routinely tested for the presence of *Y. enterocolitica*. Presumptive *Y. enterocolitica* O:3 and O:9 isolates are sent by primary laboratories to the National Reference Laboratory (NRL) at the Norwegian Institute of Public Health, where they are routinely verified, serotyped against a range of O antisera, biotyped if relevant, and tested for *Yersinia* virulence plasmid (pYV). If the strains are pathogenic *Y. enterocolitica*, they are typed by use of multiple-locus variable-number tandem repeat analysis (MLVA), using multicolor capillary electrophoresis (*5*). In March 2011, a multidisciplinary investigation was initiated after the NRL received 5 isolates of *Y. enterocolitica* O:9 from humans in disparate areas of the country. All had an identical MLVA profile, which had not been previously seen in Norway. An international request for information produced no reports of similar outbreaks.

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The Study

A confirmed case-patient was defined as a person in Norway after January 1, 2011, who had laboratory-confirmed *Y. enterocolitica* O:9 infection that matched the MLVA profile of the outbreak strain. By May 5, the NRL had registered 21 outbreak case-patients (median age 37 years [range 10–63 years]), of whom 15 were female. Case-patients resided in 10 geographically dispersed municipalities throughout the country (Figure 1). Most case-patients became ill during February 7–March 20 (Figure 2).

We interviewed the first 7 case-patients by using a standard trawling questionnaire, which was subsequently shortened and used to test hypotheses in a matched case–control study. Two of the case-patients initially interviewed stated that they had eaten a specific blend and brand of salad mix. Nine case-patients were included in the case–control

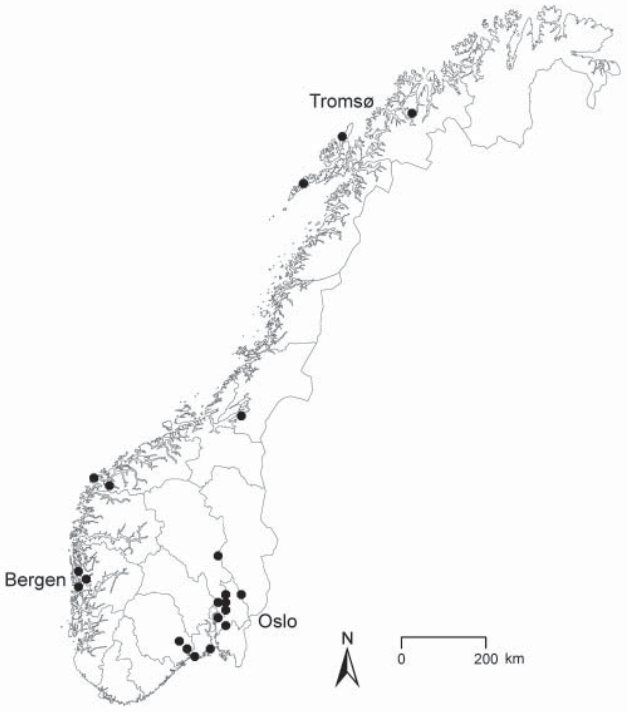

Figure 1. Geographic distribution of 21 outbreak cases (dots) of *Yersinia enterocolitica* O:9 infection, Norway, February–April 2011. Scale bar represents 100 kilometers.

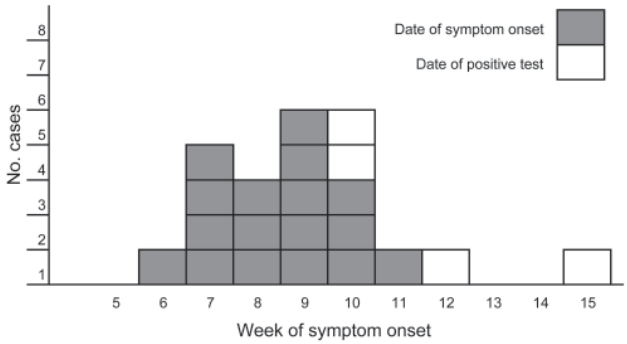

Figure 2. Week of symptom onset or positive test result for 21 persons with *Yersinia enterocolitica* O:9 infection, Norway, 2011. Dark gray, date of symptom onset for 17 case-patients; light gray, date of positive test result for 4 case-patients for whom the date of symptom onset was not available.

study. Three controls for each case-patient were selected from the National Population Register. Controls and case-patients were matched by age, sex, and municipality of residence and were interviewed in person or by telephone.

In the univariate analysis, ready-to-eat salad mix, diced ham, chicken breast, and arugula were statistically associated with illness (Table). Diced ham and chicken breast were rejected as likely sources of infection because the case-patients reported eating different brands purchased at different stores. Because arugula was an ingredient in the salad mix in question, it was not possible to otherwise distinguish it in the univariate analysis from the salad mix itself. The only food item that remained significant in the final multivariable model was salad mix. Sixteen of the identified case-patients reported having eaten salad mix.

We traced the suspected salad mix to a single Norwegian company. Under the auspices of the Norwegian Food Safety Authority, we conducted an environmental investigation, including a traceback investigation and a review of production and cleaning procedures at the company. The suspected salad mix contained 4 salad green types: arugula, radicchio rosso, iceberg lettuce, and endive. These ingredients came, unprocessed, from 12 suppliers in 2 European countries. After delivery to the company in Norway, the greens were washed in 2 cold water baths, cut, and packaged. We found no indications of inadequate routines for ingredient control, hygiene, or sampling within Norway. We identified radicchio rosso, a leaf chicory, as the likely source of infection because it can be stored for several months and was the only ingredient included in the suspected salad mix that had delivery, production, and storage dates consistent with the outbreak period. The company in Norway traced the radicchio rosso to 1 of 3 possible growers in 1 European country but was not able to identify the source of contamination. The Norwegian company voluntarily withdrew all salad mixes containing radicchio rosso from the market. After withdrawal of the implicated ingredients, no new outbreak cases were reported.

We collected 37 food samples from the homes of case-patients, retail locations, and the company that processed the salad mix products and analyzed them at the Norwegian Veterinary Institute. We cold-enriched all samples for 21 days and analyzed them according to conventional culture methods. Using PCR, we examined all enriched broths and isolated colonies for the *ail* gene, an indicator of pathogenic *Y. enterocolitica* that was found in the outbreak strain (*6*,*7*) (www.nmkl.org/kronologisk.htm). We cultured *Y. enterocolitica*–positive samples to isolate *Yersinia* colonies. We isolated presumptive *Yersinia* spp. from 11 of the salad ingredients, including 2 strains that were consistently positive for the *ail* gene and that were isolated from mixed salad and radicchio rosso samples obtained directly from the company. All of the specimens were sent to the NRL for characterization to the species level, biotyping, serotyping, and testing for pYV. The isolate found in the mixed salad sample was *Y. enterocolitica* biotype 1A. It did not agglutinate in antiserum 0 through 34 and was pYV negative, whereas the radicchio rosso isolate was identified as *Y. kristensenii* and agglutinated in O:11,24 antiserum.

Conclusions

This outbreak of *Y. enterocolitica* was associated with ingestion of ready-to-eat salad mix. Despite a thorough traceback investigation, the likely source of contamination for the outbreak, radicchio rosso, remains unproved. As

Table. Results from univariate conditional logistic regression analyses of a *Yersinia enterocolitica* outbreak, Norway, February–April 2011

Exposure	No. cases, n = 9	No. controls, n = 26*	Matched odds ratio† (95% CI)	p value
Ready-to-eat salad mix	6	3	13.7 (1.6–116.3)	0.02
Diced ham, ham pieces	5	3	6.3 (1.2–32.9)	0.03
Chicken breast	8	9	10.0 (1.2–83.6)	0.03
Arugula	7	8	9.8 (1.2–83.6)	0.04
Pork chops	4	3	8.4 (0.9–78.6)	0.06
Bean sprouts	3	1	8.2 (0.8–79.3)	0.07
Sugar peas	6	8	3.7 (0.9–16.1)	0.08
Iceberg lettuce	8	13	6.6 (0.8–57.5)	0.09

*n = 25 for ready-to-eat salad mix.

found in many outbreaks linked to salad products (*8*–*10*), more women were affected than men or children. The outbreak was detected rapidly through systematic characterization of *Y. enterocolitica* isolated from humans to a level that enabled identification of an outbreak of non-O:3 *Y. enterocolitica* infections, which were linked by MLVA. Few European countries regularly type *Y. enterocolitica*, which might explain why international requests for information produced no similar reports from other countries.

To our knowledge, isolates of pathogenic *Y. enterocolitica* are seldom recovered from food samples. Previous survey studies of *Yersinia* spp. in prepared salads have recovered primarily nonpathogenic and environmental strains, such as *Y. enterocolitica* biotype 1A and *Y. kristensenii* (*11*,*12*). In addition, evidence is increasing that the *ail* gene is not always associated with pathogenic *Y. enterocolitica*, suggesting that *ail*-based detection methods for potentially pathogenic *Y. enterocolitica* in food should be supplemented by isolating the strain itself for further characterization or by investigating for other virulence markers. Strains of *Y. enterocolitica* biotype 1A, which are considered nonpathogenic, harbor the gene (*13*,*14*), whereas it might be absent from some pathogenic strains (*15*). Although we could not conclusively link any of the isolates from the salad ingredients to the human *Y. enterocolitica* isolates, finding nonpathogenic *Yersinia* in packaged salads reinforces that the environment of this food product is conducive to the persistence of the bacterium. No further outbreak cases were reported after the salad withdrawal, supporting our conclusion.

The necessity of epidemiologic, environmental, and traceback investigations in addition to microbiological investigation in outbreak situations cannot be underestimated. Considering the increasing number of outbreaks associated with fresh produce, improving traceback among these products should be emphasized. Despite the ultimately inconclusive traceback investigation, this outbreak shows the value of targeted surveillance and microbiological typing as a means for quickly identifying and investigating small foodborne disease outbreaks with international implications.

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Acanthamoeba polyphaga mimivirus Virophage Seroconversion in Travelers Returning from Laos

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During January 2010, a husband and wife returned from Laos to France with probable parasitic disease. Increased antibodies against an *Acanthamoeba polyphaga mimivirus* virophage indicated seroconversion. While in Laos, they had eaten raw fish, a potential source of the virophage. This virophage, associated with giant viruses suspected to cause pneumonia, could be an emerging pathogen.

An ameba-associated giant virus, *Acanthamoeba polyphaga mimivirus* (APM), was first described in 2003 after its discovery in water collected from a cooling tower in the United Kingdom (*1*). In 2008, a new APM strain (mamavirus), along with an APM virophage, was discovered in water from a cooling tower in France (*2*). The APM virophage is a virus that infects giant viruses. The pathogenicity of giant viruses is still a matter of debate (*3*). We describe seroconversion to antibodies against the APM virophage in 2 humans.

The Study

The patients were a 29-year-old woman and her 36-year-old husband, each born in Laos, who had immigrated to France in 2008 and 2000, respectively. During December, 20, 2009, through January 22, 2010, they traveled to Laos with their 4-month-old baby to visit friends and relatives. This was their first return to Laos since immigration. While in Laos, they ate local food, including raw fish. Five days after their return to France, they experienced asthenia, low-grade fever, myalgia, and nausea. They had no other history of travel, and their baby showed no clinical signs.

Blood test results indicated hypereosinophilia, hepatic cytolysis, and cholestasis (Table, Appendix, wwwnc.cdc.gov/EID/article/18/9/12-0099-T1.htm). At 7 days after the onset of symptoms, the patients were hospitalized; physical examination of the man detected no abnormalities, and examination of the woman detected tachycardia. Echocardiography of the woman showed a thin pericardial effusion, but chest radiographs showed no abnormalities. Serum from the woman contained antibodies against toxocara, trichinellae, and *Fasciolia* trematodes; and serum from the man contained antibodies against *Fasciolia* trematodes. No parasites were recovered from stool samples from either patient. The patients received antiparasitic treatment (praziquantel for 3 days and albendazole for 15 days), after which they recovered completely and their eosinophil counts returned to reference levels.

We routinely test all serum samples for antibodies against intracellular microorganisms discovered at the World Health Organization Collaborative Center for Rickettsioses and Arthropod Borne Bacterial Diseases. For the patients reported here, we conducted microimmunofluorescence assays by using APM virophage and mamavirus APM strain antigens, obtained after amebal coculture with *Acanthamoeba castellanii*. From the first positive serum sample from each patient (acute-phase samples), we detected elevated IgG and IgM against the APM virophage (Table, Appendix). For the woman, serum obtained when she had been pregnant, 5 months before disease onset, was negative. The APM antibody titer in the 3 samples was either negative or lower than the significant cutoff point (Table, Appendix).

To determine specificity of the APM virophage antibodies, we tested 2 positive serum samples (1 from each patient) and the negative serum sample from the woman by Western blotting and 2-dimensional gel electrophoresis with purified APM virophage, mamavirus antigens, and *A. castellanii* antigens. Protein spots were excised from the silver-stained gels (Figure). All spots were excised for the APM virophage, and only immunoreactive spots were excised for mamavirus and *A. castellanii*. Peptide digestion and mass spectrum analyses were performed by using a matrix-assisted laser desorption ionization spectrometer (MALDI-TOF/TOF Bruker Ultraflex II; Bruker Daltonics, Wissembourg, France) (*2*). The negative serum showed no immune reaction against virophage proteins, whereas the positive serum showed high-intensity immune reactions for 2 groups of spots, identified as open reading frames (ORFs) 21 and 14 (Figure).

After protein spots were excised, we obtained a reference proteome map for APM virophages with only 2 identified ORFs (21 and 14), although several isoforms of these proteins were recovered from different parts of the gel (Figure). For the 2 patients, the immunoreactive spots corresponded to specific antibodies against virophage

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proteins. For mamavirus and *A. castellanii*, we observed immunoreactive spots of only slight intensity that were strictly identical in the 3 samples. Mass spectrometry was performed for these spots, but their identity was not verified. Whether these spots were artifacts or represented low antibody titers remains unknown. Various body fluids recovered during patient follow-up were screened with molecular testing (PCR and reverse transcription PCR with in-house primers and probes) and amebal coculture to seek APM virophages and/or APM. Results of molecular testing (Table) and amebal coculture (*2*,*5*) were negative.

Human exposure to virophages is unknown. We searched for environmental occurrences of APM virophage–like sequences in environmental metagenomic datasets (*6*). A BLASTP (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Protens) search of APM virophage-translated ORFs was performed against all metagenomic ORF peptides from Sanger reads (5,634,288,892 nt). These included translated peptide sequences of ORFs identified on all metagenomic sequence reads and excluded reads generated from Roche 454 GS-20 FLX and Titanium pyrosequencer (Roche Applied Science, Mannheim, Germany).

With no minimal e-value and an alignment of 25 reads per query, we obtained 347 hits from the different metagenomic datasets. When an increased stringency (e-value<10^{-4}) was used, 112 reads were still recovered. Most hits (29 reads and 7 ORFs) were returned from the environment of Lake Gatun, a large artificial freshwater lake in the Republic of Panama. Numerous mimivirus-related sequences were also found in the Lake Gatun metagenome (561 reads with an e-value of <10^{-4} among 228 different ORFs), suggesting that the virophage and its host are common in this environment.

Conclusions

Each patient was probably infected with a yet-unidentified parasite, although they each had positive test results for ≥1 foodborne helminthiases endemic to Southeast Asia. Nonetheless, the broad-spectrum antiparasitic treatment was effective (*7*).

For each patient, antibodies against the APM virophage were elevated. We cannot rule out serologic cross-reaction between APM virophage proteins and proteins of other origin, as described for the major capsid protein of *A. polyphaga mimivirus* that was recognized in serum of patients infected by *Francisella tularensis* (*8*). However, our method indicates that only human antibodies specific to APM virophage proteins were produced. The negative results of our molecular testing might have been caused by polymorphisms in the sequences chosen for amplification (*9*). Cross-reactivity with proteins of other virophages, such as Mavirus-infecting Cafeteria roenbergensis virus or Organic Lake virophage–infecting phycodnaviridae, is, however, the most plausible explanation because these virophages have some protein homology similar to that for capsid (*10*,*11*). Our environmental analysis indicated recovery of the APM virophage and viral host sequences from the environment, particularly a freshwater lake. Along with raw fish-borne parasites, aquatic environments could be a source of human exposure to the APM virophage, as they are for other ameba-associated microorganisms emerging as causal agents of pneumonia (*12*,*13*).

It is noteworthy that specific antibodies against the APM virophage but not APM were detected. Because the APM virophage is associated with a host giant virus, human exposure to APM virophages and to giant viruses should be concomitant. However, for the patients reported here, APM virophages might have been associated with an undescribed giant virus that cannot be detected with current

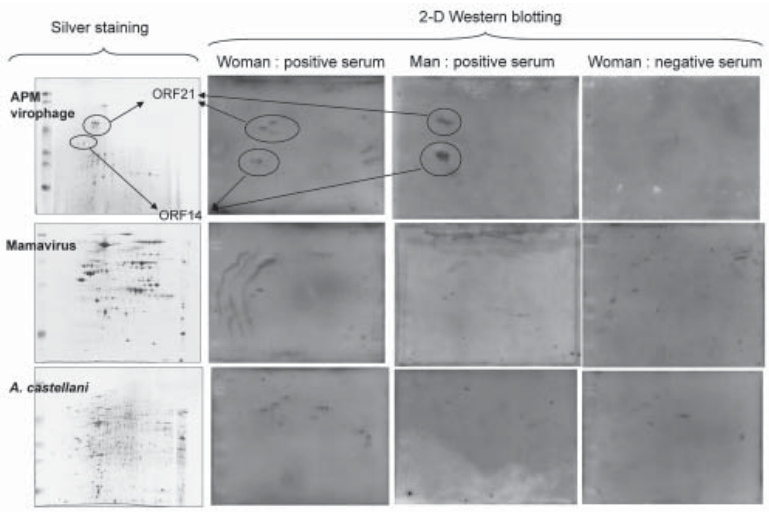

Figure. Two-dimensional (2-D) gel electrophoresis with silver stain results (on left) and Western blot results (on right) for 3 serum samples from patients who had visited Laos. The proteins were resolved by using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Protean II xi chamber; Bio-Rad, Hercules, CA, USA). After migration, the gels were processed either by a silver-staining method compatible with mass spectrometry (*4*) or by transfer onto nitrocellulose membranes in a semidry blotting apparatus (Semi-Phor unit; Hoefer Scientific Instruments, San Francisco, CA, USA). The membranes were probed with horseradish peroxidase–conjugated goat anti-human secondary antibodies (Southern Biotech, Birmingham, AL, USA), and detection was achieved by enhanced chemiluminescence (ECL; GE Healthcare, Vélizy, France). APM, *Acanthamoeba polyphaga mimivirus*; ORF, open reading frame; *A. castellani*, *Acanthamoeba castellani*.

laboratory techniques (*9*,*14*). Indeed, we have found that APM virophages infect distinct but related giant viruses of the family *Mimiviridae* (B. La Scola, unpub. data). Patients might have been directly exposed to free virophages that are apparently present in high numbers in fresh as well as saline water. For example, during the natural cycle involving virophages, phycodnaviruses, and algae, populations of each evolve over time (*10*). This potential direct exposure could explain why antibodies against giant viruses were not detected in the patients reported here. Virophages could also be associated with viruses that infect various protozoa or parasites (*15*).

We cannot exclude the possibility that each patient seroconverted while still in France, during the 5 months before their trip. It seems, however, more probable that they seroconverted while in Laos. Each patient ate raw fish, a potential source of the APM virophage. Human seroconversion against the APM virophage suggests that virophages could potentially be listed as emerging human pathogens.

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Rapid Detection of Carbapenemase-producing *Enterobacteriaceae*

Patrice Nordmann, Laurent Poirel, and Laurent Dortet

To rapidly identify carbapenemase producers in *Enterobacteriaceae*, we developed the Carba NP test. The test uses isolated bacterial colonies and is based on in vitro hydrolysis of a carbapenem, imipenem. It was 100% sensitive and specific compared with molecular-based techniques. This rapid (<2 hours), inexpensive technique may be implemented in any laboratory.

Multidrug resistance is emerging worldwide at an alarming rate among a variety of bacterial species, causing both community-acquired and nosocomial infections (*1*). Carbapenems, the last line of therapy, are now frequently needed to treat nosocomial infections, and increasing resistance to this class of β-lactams leaves the health care system with almost no effective drugs (*1*). However, reports of carbapenem-resistant *Enterobacteriaceae* have increased (*2*,*3*). Resistance may be related to association of a decrease in bacterial outer-membrane permeability, with overexpression of β-lactamases with no carbapenemase activity or to expression of carbapenemases (*2*,*4*,*5*). Spread of carbapenemase producers is a relevant clinical issue because carbapenemases confer resistance to most β-lactams (*2*). Various carbapenemases have been reported in *Enterobacteriaceae*, such as the following types: *Klebsiella pneumoniae* carbapenemase (KPC; Ambler class A); Verona integron–encoded metallo-β-lactamase (VIM), imipenemase (IMP), New Delhi metallo-β-lactamase (NDM) (all Ambler class B); and oxacillinase-48 (OXA-48; Ambler class D) (*2*,*4*–*6*). In addition, carbapenemase producers are usually associated with many other non–β-lactam resistance determinants, which give rise to multidrug- and pandrug-resistant isolates (*2*,*3*,*7*).

Potential carbapenemase producers are currently screened first by susceptibility testing, using breakpoint values for carbapenems (*2*,*8*). However, this technique is time-consuming, and many carbapenemase producers do not confer obvious resistance levels to carbapenems. There is a need for laboratories to search for carbapenemase producers (*9*). Phenotype-based techniques for identifying in vitro production of carbapenemase, such as the modified Hodge test, are not highly sensitive and specific (*2*,*8*,*10*). Detection of metallo-β-lactamase producers (IMP, VIM, NDM) and of KPC producers may be based on the inhibitory properties of several molecules but requires additional expertise and time (usually an extra 24–48 hours) (*2*,*8*,*11*,*12*). Furthermore, no inhibitors are available for detecting OXA-48–type producers that are spreading rapidly, at least in northern Africa, the Middle East, and Europe (*2*). Molecular detection of carbapenemase genes remains costly and requires substantial expertise. Both the phenotype-based techniques and molecular tests are time-consuming (at least 12–24 hours) and are poorly adapted to the clinical need for isolating patients rapidly to prevent nosocomial outbreaks.

We developed a novel test, described here, based on a technique designed to identify the hydrolysis of the β-lactam ring of a carbapenem. This test is rapid, sensitive and specific, and adaptable to any laboratory in the world.

The Study

We included in the study 162 carbapenemase-producing strains of various enterobacterial species isolated from clinical samples (e.g., blood cultures, urine, sputum) and of global origin (Table 1). This collection of strains also included 46 strains that were fully susceptible to carbapenems or showed a decreased susceptibility to carbapenems as a consequence of non–carbapenemase-based mechanisms (Table 2). Antibiograms were carried out for all strains on Mueller-Hinton agar (Becton Dickinson, Le Point de Chaix, France) according to guidelines of the Clinical and Laboratory Standards Institute (*13*). The Carba NP (Carbapenemase Nordmann-Poirel) test was performed as follows. One calibrated loop (10 μL) of the tested strain directly recovered from the antibiogram was resuspended in a Tris-HCl 20 mmol/L lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific Pierce, Rockford, IL, USA), vortexed for 1 minute and further incubated at room temperature for 30 minutes. This bacterial suspension was centrifuged at 10,000 × *g* at room temperature for 5 minutes. Thirty μL of the supernatant, corresponding to the enzymatic bacterial suspension, was mixed in a 96-well tray with 100 μL of a 1-mL solution made of 3 mg of imipenem monohydrate (Sigma, Saint-Quentin Fallavier, France), pH 7.8, phenol red solution, and 0.1 mmol/L $ZnSO_4$ (Merck Millipore, Guyancourt, France). The phenol red solution was prepared by mixing 2 mL of a phenol red (Merck Millipore) solution 0.5% (wt/vol) with 16.6 mL of distilled water. The pH value was then adjusted to 7.8 by adding drops of 1 N NaOH. A mixture of the phe-

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Table 1. Carbapenemase-producing clinical enterobacterial isolates subjected to the Carba NP test*

Ambler class, carbapenemase type	Species	β-Lactamase	No.	MIC range, mg/L			Carba NP test result
				IMP	ERT	MER	
Class A							
KPC-type	*Klebsiella pneumoniae*	KPC-2	27	0.5–>32	4–>32	1–>32	+
		KPC-3	3	0.5–8	4–>32	1–8	+
	Klebsiella ozaenae	KPC-3	1	>32	>32	2	+
	Escherichia coli	KPC-2	5	0.5–4	0.5>32	0.5–2	+
	Enterobacter cloacae	KPC-2	7	1–24	1.5–32	0.75–16	+
	Enterobacter aerogenes	KPC-2	1	8	>32	8	+
	Citrobacter freundii	KPC-2	2	8–>32	1.5–>32	1.5–3	+
	Serratia marcescens	KPC-2	2	>32	>32	>32	+
	Salmonella spp.	KPC-2	1	4	1	0.25	+
NMC-A	*E. cloacae*	NMC-A	1	16	>32	16	+
SME-type	*S. marcescens*	SME-1	1	32	4	12	+
		SME-2	1	32	4	12	+
GES-type	*E. cloacae*	GES-5	1	>32	>32	>32	+
IMI-type	*Enterobacter asburiae*	IMI-2	1	>32	>32	>32	+
Class B							
NDM-type	*K. pneumoniae*	NDM-1	16	0.5–>32	2–>32	1–>32	+
		NDM-4	1	>32	>32	>32	+
	E. coli	NDM-1	7	1–16	3–>32	1–16	+
	E. cloacae	NDM-1	1	2	16	2	+
	C. freundii	NDM-1	1	>32	>32	>32	+
	Providencia stuartii	NDM-1	1	12	0.38	1.5	+
	Proteus rettgeri	NDM-1	1	3	0.5	1.5	+
VIM-type	*K. pneumoniae*	VIM-1	15	0.5–>32	0.5–>32	0.38–>32	+
		VIM-19	1	8	16	4	+
	E. coli	VIM-1	2	1.5–3	0.38–1.5	0.5–1	+
		VIM-2	2	2–4	0.5–1.5	0.38–0.5	+
		VIM-19	1	8	16	4	+
	E. cloacae	VIM-1	4	1–>32	0.38 to >32	0.5–>32	+
	S. marcescens	VIM-2	1	>32	>32	>32	+
IMP-type	*K. pneumoniae*	IMP-1	5	0.5–8	2–4	1–8	+
		IMP-8	2	0.5–1	0.5–1	0.5	+
	E. coli	IMP-1	2	0.5	3–4	0.5–1	+
		IMP-8	1	6	8	3	+
	E. cloacae	IMP-1	12	8–>32	>32	2–>32	+
		IMP-8	2	0.75–1.5	0.5–1	0.5–1	+
	S. marcescens	IMP-1	2	8–>32	>32	2–>32	+
		IMP-11	1	8	>32	2	+
Class D							
OXA-48 type	*K. pneumoniae*	OXA-48	15	0.38–>32	0.38–>32	0.38–>32	+
		OXA-181	2	0.5-1	2–4	0.5–1	+
	E. coli	OXA-48	6	0.38–3	0.5–16	0.12–1	+
	E. cloacae	OXA-48	3	0.5–1	0.5–16	0.5–1.5	+
	P. rettgeri	OXA-181	1	8	1	2	+

*IMP, imipenem; ERT, ertapenem; MER, meropenem; KPC, *K. pneumoniae* carbapenemase; NMC-A, non–metallo-enzyme carbapenemase; SME, *S. marcescens* enzyme; GES, Guiana extended-spectrum β-lactamase; IMI, imipenem-hydrolysing β-lactamase; NDM, New Delhi metallo-β-lactamase; VIM, Verona integron–encoded metallo- β-lactamase; IMP, imipenemase; OXA-48, oxacillinase-48.

nol red solution and the enzymatic suspension being tested was incubated at 37°C for a maximum of 2 hours. Test results were interpreted by technicians who were blinded to the identity of the patients who gave the samples.

All strains had previously been characterized for their β-lactamase content at the molecular level. MICs of carbapenems were determined by using the Etest (AB bioMérieux, Solna, Sweden), and results were recorded according to US guidelines (Clinical and Laboratory Standards Institute), as updated in 2012 (*13*). The breakpoints used were those for imipenem and meropenem: susceptibility <1 μg/mL, resistance >4 μg/mL, and for ertapenem, susceptibility <0.25 μg/mL, resistance > μg/mL.

When the Carba NP test was used, the color of the wells turned from red to orange or yellow (Figure 1) for all tested strains that were producing carbapenemases (Table 1), whereas wells corresponding to bacterial extracts of isolates that did not produce carbapenemase remained red, whatever their level of carbapenem susceptibility (Table 2). The color changed from red to yellow as early as 5–10

Table 2. Non–carbapenemase-producing clinical enterobacterial isolates subjected to the Carba NP test*

β-Lactamase type, species	β-Lactamase	No.	MIC, mg/L			Carba NP test result
			IMP	ERT	MER	
ESBLs						
Klebsiella pneumoniae	CTX-M-3	1	0.12	0.12	0.12	–
	CTX-M-14	1	0.12	0.12	0.12	–
	CTX-M-15	3	0.12	0.12	0.12	–
Escherichia coli	CTX-M-1	1	0.12	0.12	0.12	–
	CTX-M-3	1	0.12	0.12	0.12	–
	CTX-M-14	2	0.12	0.12	0.12	–
	CTX-M-15	2	0.12	0.12	0.12	–
	VEB-1	1	0.12–0.25	0.12	0.12	–
Enterobacter cloacae	CTX-M-15	3	0.12	0.12	0.12	–
	VEB-1	1	0.12	0.12	0.12	–
Plasmid-mediated AmpC or chromosomal AmpC + decreased membrane permeability						
K. pneumoniae	DHA-1	1	>32	>32	>32	–
	DHA-2	1	0.12	0.5	0.12	–
E. coli	Extended-spectrum cephalosporinase	1	0.12	0.12	0.12	–
	CMY-2	1	0.12	0.12	0.12	–
	CMY-10	1	0.12	0.38	0.12	–
	DHA-1	1	0.12	0.12	0.12	–
	ACC-1	1	0.12	0.12	0.12	–
	Overexpressed cephalosporinase	1	16	>32	2	–
Proteus mirabilis	ACC-1	1	0.25	0.12	0.12	–
E. cloacae	Overexpressed cephalosporinase	7	0.12–16	1–>32	0.12–>32	–
Enterobacter aerogenes	Overexpressed cephalosporinase	1	1	4	0.75	–
Morganella morganii	Overexpressed cephalosporinase	2	1.5–2	0.12	0.5	–
ESBL + decreased membrane permeability						
K. pneumoniae	CTX-M-15	8	0.25–8	1–>32	1–>32	–
	SHV-28	1	1	4	1	–
	SHV-2a	1	0.25	2	0.38	–
Enterobacter sakazakii	CTX-M-15	1	0.25	1.5	0.25	–
Citrobacter freundii	TEM-3	1	1	8	1	–

*IMP, imipenem; ERT, ertapenem; MER, meropenem; ESBLs, extended-spectrum β-lactamases.

minutes after incubation for KPC producers began. In most cases, incubation for 30 minutes was sufficient for obtaining a frank color change for carbapenemase producers. The test's specificity and sensitivity were 100% when results were compared with those from molecular-based methods, the reference standard for identifying carbapenemase genes. All tests were performed in triplicate, giving identical and reproducible results.

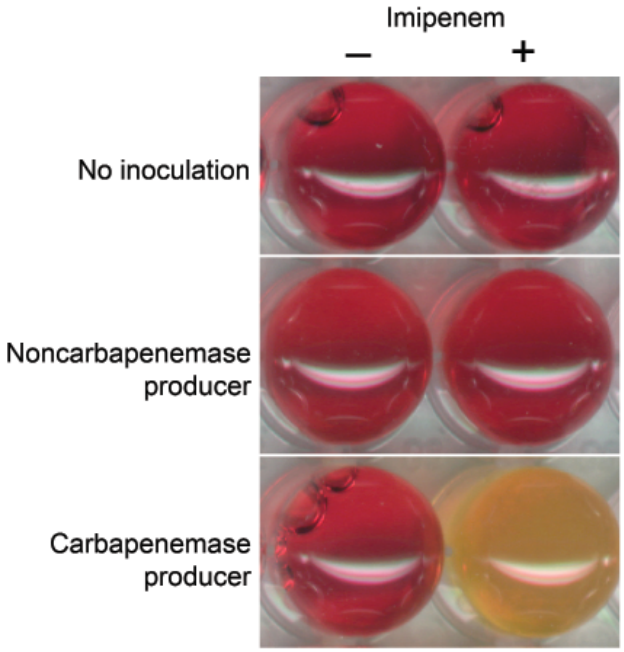

Figure 1. Representative results of the Carba NP test. The Carba NP test was performed with a noncarbapenemase producer (*Escherichia coli* producing the extended-spectrum β-lactamase CTX-M-15, upper panel) and with a carbapenemase producer (*Klebsiella pneumoniae*–producing New Delhi metallo-β-lactamase-1, lower panel) in a reaction medium without (left panel) and with (right panel) imipenem. Uninoculated wells are shown as controls. Photographs were taken after a 1.5-hour incubation. A color version of this figure is available online (wwwnc.cdc.gov/EID/article/18/9/12-0355-F1.htm).

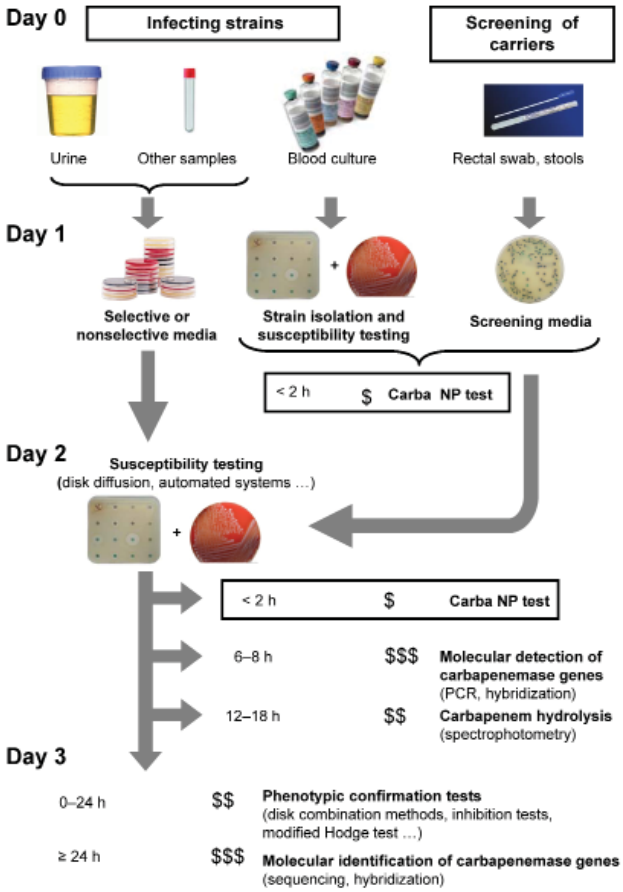

Figure 2. Strategy for identification of carbapenemase-producing *Enterobacteriaceae*. The time needed to perform the test is indicated before each test. The number of flasks indicates the degree of specialization needed to perform the test; the number of $ indicates the relative cost of each test. A color version of this figure is available online (wwwnc.cdc.gov/EID/article/18/9/12-0355-F2.htm).

The Carba NP test perfectly differentiates carbapenemase producers (Table 1) from strains that are carbapenem resistant due to non–carbapenemase-mediated mechanisms, such as combined mechanisms of resistance (outer-membrane permeability defect associated with overproduction of cephalosporinase and/or extended-spectrum β-lactamases) or from strains that are carbapenem susceptible but express a broad-spectrum β-lactamase without carbapenemase activity (extended-spectrum β-lactamase, plasmid and chromosome-encoded cephalosporinases) (Table 2). Interpretable positive results were obtained in <2 hours, making it possible to implement rapid containment measures to limit the spread of carbapenemase producers.

Conclusions

The Carba NP test has multiple benefits. It is inexpensive, rapid, reproducible, and highly sensitive and specific. It eliminates the need for using other techniques to identify carbapenemase producers that are time-consuming and less sensitive or specific. Using this accurate test would improve detection of patients infected or colonized with carbapenemase producers. The test has been routinely implemented in our microbiology department at Hôpital de Bicêtre and is giving excellent results (data not shown). In addition, use of the Carba NP test has greatly decreased the laboratory technicians' workload and simplified the clinical management of potential carbapenemase producers.

This test could be used, for example, for directly testing 1) bacteria obtained from antibiograms of blood culture or 2) bacterial colonies grown on culture media before antimicrobial drug susceptibility testing (Figure 2). Further studies will evaluate its clinical value for antimicrobial drug stewardship on bacteria isolated directly from clinical samples (Figure 2). When the Carba NP test is used for that purpose, we expect that the time to detect carbapenemase producers will decrease by at least 24 hours (Figure 2).

The test could also be used to quickly identify carbapenem-resistant isolates from fecal specimens screened for multidrug-resistant bacteria (Figure 2). This capability would be valuable in preventing outbreaks. To determine positive and negative predictive values of the test, additional evaluations will be required with strains isolated from clinical samples screened on different types of selective media. The use of the Carba NP test may also support novel antimicrobial drug development by facilitating patient enrollment in pivotal clinical trials. Its use as a home-made test may contribute to the global surveillance network.

The Carba NP test can efficiently indicate the strains to be further tested by PCR or submitted to sequencing for a detailed identification of the carbapenemase genes. Last, the test could be used in low-income countries that are large reservoirs for carbapenemase producers (*2*). It offers a practical solution for detecting a main component of multidrug resistance in *Enterobacteriaceae*. Use of the Carba NP test will contribute to a better stewardship of carbapenems by changing the paradigm of controlling carbapenemase producers worldwide

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Dr Nordmann is professor of medical microbiology, South-Paris University, Paris, and director of the INSERM U914 Emerging Resistance to Antibiotics program. His main field of research interest includes the genetics, biochemistry, and molecular epidemiology of resistance in gram-negative bacteria.

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Multiple-Insecticide Resistance in *Anopheles gambiae* Mosquitoes, Southern Côte d'Ivoire

Constant V.A. Edi, Benjamin G. Koudou, Christopher M. Jones, David Weetman, and Hilary Ranson

Malaria control depends on mosquito susceptibility to insecticides. We tested *Anopheles gambiae* mosquitoes from Côte d'Ivoire for resistance and screened a subset for target site mutations. Mosquitoes were resistant to insecticides of all approved classes. Such complete resistance, which includes exceptionally strong phenotypes, presents a major threat to malaria control.

Targeting the mosquito vector is the most effective way to prevent malaria transmission; worldwide, this method accounts for more than half of malaria control expenditures (*1*,*2*). During the past decade, increased use of insecticide-treated bed nets and indoor residual spraying have made a pivotal contribution toward decreasing the number of malaria cases (*1*). However, these gains are threatened by the rapid development and spread of insecticide resistance among major malaria vectors in Africa (*3*). To keep vector resistance from undermining control programs, insecticide-resistance management strategies must reduce the current overreliance on pyrethroids. These compounds are used widely for indoor residual spraying and uniquely for insecticide-treated bed nets. However, having a limited number of insecticides available for malaria vector control restricts options for effective insecticide resistance management. Only 4 classes of insecticide, which share 2 modes of action, are approved by the World Health Organization (WHO).

A mutation at a single target site can result in mosquito resistance to DDT and pyrethroids or to organophosphates and carbamates. Furthermore, mosquitoes can express multiple insecticide-resistance mechanisms (*4*). For example, in several populations of the major malaria vector in Africa, *Anopheles gambiae* s.l. mosquitoes, mutations in the DDT/pyrethroid target site, known as knockdown resistance (*kdr*) alleles, have been found in conjunction with resistance alleles of the acetylcholinesterase gene (*Ace-1*^{R}), the target site of organophosphates and carbamates (*5*). To date, however, these cases of multiple-insecticide resistance have been restricted by the relatively low prevalence of organophosphate/carbamate resistance and the limited effect that *kdr* mutations alone have on pyrethroid-based interventions (*6*). We report a population of *An. gambiae* mosquitoes from a rice-growing area of southern Côte d'Ivoire that have high frequencies of *kdr* and *Ace-1*^{R} alleles and unprecedentedly high levels of phenotypic resistance to all insecticide classes available for malaria control.

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The Study

During May–September 2011, mosquito larvae were collected in irrigated rice fields surrounding Tiassalé, southern Côte d'Ivoire (5°52′47″N; 4°49′48″W) and reared to adults in insectaries on a diet of MikroMin (Tetra, Melle, Germany) fish food. A total of 1,571 adult female *An. gambiae* s.l. mosquitoes, 3–5 days of age, were exposed to 1 of 5 insecticides (0.1% bendiocarb, 1.0% fenitrothion, 0.75% permethrin, 0.05% deltamethrin, 4% DDT) or a control paper for 1 hour, according to standard WHO procedures (*7*). Mosquito deaths were recorded 24 hours later. DNA was extracted from individual mosquitoes according to the LIVAK method (*8*), and a subsample of 500 mosquitoes were all found to be the M molecular form of *An. gambiae* s.s. by using the SINE-PCR method (*9*). The target site mutation G119S in the *Ace-1* gene (*Ace-1*^{R}) and L1014F and L1014S *kdr* mutations were screened by using restriction fragment length polymorphism (*10*) or TaqMan assays (*11*), respectively.

According to WHO criteria, *An. gambiae* mosquitoes from Tiassalé are resistant to all insecticide classes, and resistance is extremely prevalent; more than two thirds of mosquitoes survived the diagnostic dose for 4 of the 5 insecticides tested (Table 1). To assess the level of resistance, we exposed the Tiassalé population and a susceptible laboratory population of *An. gambiae* (Kisumu) mosquitoes to the pyrethroid deltamethrin or

Table 1. Prevalence of insecticide resistance in *Anopheles gambiae* mosquitoes, M form, from Tiassalé, Côte d'Ivoire, 2011

Insecticide	No. tested*	No. dead	% Dead (95% CI)
Permethrin	288	69	24.0 (19.1–29.3)
Deltamethrin	282	90	31.9 (26.5–37.7)
DDT	306	25	8.2 (5.4–11.8)
Fenitrothion	296	219	74.0 (68.6–78.9)
Bendiocarb	299	37	12.4 (8.9–16.6)

*Measured by death within 24 h, after 1h exposure to each insecticide. All mosquitoes were resistant according to World Health Organization classification (<80% dead) (7).

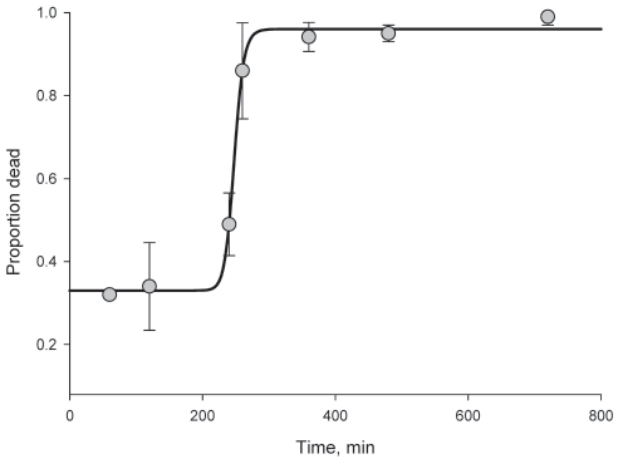

Figure 1. Time-mortality curve for wild-caught *Anopheles gambiae* mosquitoes from Tiassalé, southern Côte d'Ivoire, exposed to deltamethrin (median time to death = 248 minutes). Logistic regression line was fitted to time-response data by using SigmaPlot version 11.0 (www.sigmaplot.com). R^2 = 0.96. Error bars indicate SEM.

the carbamate bendiocarb for a range of exposure times and assessed deaths 24 hours later (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/12-0262-Techapp.pdf). We found an unexpectedly strong resistance phenotype to the 2 insecticides (Figures 1, 2). For deltamethrin, 4 hours of exposure were required to kill 50% (median lethal time, [LT_{50}]); in comparison, the LT_{50} for the susceptible Kisumu strain was <2 minutes (resistance ratio = 138) (online Technical Appendix). Similarly, the LT_{50} for bendiocarb was nearly 5 hours for the Tiassalé strain yet <12 minutes for the susceptible strain (resistance ratio = 24) (online Technical Appendix).

To investigate the causes of this resistance, we screened a subset of mosquitoes for the target site mutations, *kdr* 1014F and 1014S. Only the 1014F *kdr* mutation was detected, and this resistance allele was found at high frequency (83%). There was a significant association between presence of the 1014F *kdr* allele and ability to survive exposure to DDT but not to either pyrethroid (Table 2). In contrast, the *Ace-1*^{R} allele was strongly associated with survival after exposure to bendiocarb and fenitrothion (Table 2).

Conclusions

Pyrethroid resistance in *An. gambiae* mosquitoes was first reported from Côte d'Ivoire in 1993 (*12*); carbamate resistance was detected in the 1990s (*13*). Nevertheless, ≈2 decades later, it is surprising and worrying to find complete resistance to all insecticides tested, particularly—for deltamethrin and bendiocarb—at such high levels. Resistance mechanisms seem to be varied. *Ace-1*^{R} is strongly associated with organophosphate and carbamate resistance, and the absence of 119S homozygotes might be attributable to the high fitness cost of the *Ace-1*^{R} allele in the absence of insecticide (*14*). Presence of the 1014F *kdr* allele alone does not confer the ability to survive diagnostic doses of pyrethroids; thus, alternative mechanisms must be responsible for the high-level pyrethroid resistance in this population.

The selective pressures responsible for this intense multiple-insecticide resistance in Tiassalé mosquitoes are unclear. There is a high coverage of insecticide-treated bed nets, but this coverage does not differ from that in other parts of the continent, and indoor residual spraying has not been conducted in this region. Use of insecticides in agriculture has been linked to resistance in malaria vectors. This use is perhaps the most likely explanation in this district of intense commercial production of rice, cocoa, and coffee.

Whatever the cause, the implications of this resistance scenario for malaria control are severe. With no new classes of insecticides for malaria control anticipated until 2020 at the earliest (*15*), program managers have few options available when confronted with multiple-insecticide resistance. Assessing the effect of pyrethroid resistance on the efficacy of insecticide-treated bed nets is complex because of the poorly understood associations between net integrity, insecticide content, net usage, and net efficacy. Nevertheless, resistance levels, such as those reported here, combined with continual selection pressure will inevitably lead to suboptimal mosquito control by use of insecticide-treated bed nets and indoor residual spraying. If unchecked, this resistance could spread rapidly and threaten the fragile gains that have been made in reducing malaria across Africa.

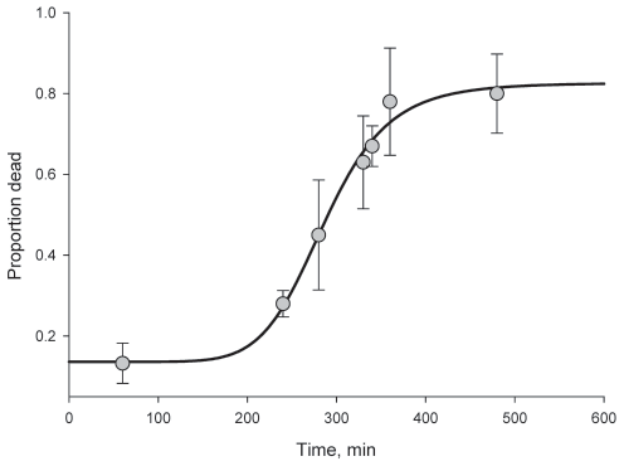

Figure 2. Time-mortality curve for wild-caught *Anopheles gambiae* mosquitoes from Tiassalé, southern Côte d'Ivoire, exposed to bendiocarb (median time to death = 286 minutes). Logistic regression line was fitted to time-response data by using SigmaPlot version 11.0 (www.sigmaplot.com). R^2 = 0.88. Error bars indicate SEM.

Table 2. Association between genotype and mosquito survival after insecticide exposure*

Insecticide	No. tested	Status	No.	No. per genotype			Frequency†	Odds ratio§	p value
				LL	LF	FF	1014F‡		
DDT	73	Alive	48	2	7	39	88.5	4	0.02
		Dead	25	2	10	13	72		
Permethrin	88	Alive	44	1	12	31	84.1	1.23	0.82
		Dead	44	3	12	29	79.5		
Deltamethrin	89	Alive	45	1	12	32	84.4	0.82	0.86
		Dead	44	2	9	33	85.2		
				GG	GS	SS	119S¶		
Bendiocarb	86	Alive	49	0	49	0	50	100	0.40×10^{-12}
		Dead	37	25	12	0	16.2		
Fenitrothion	100	Alive	50	0	50	0	50	1,176	0
		Dead	50	48	2	0	2		

*F and L represent mutant resistant alleles (phenylalanine) and wild-type alleles (leucine), respectively; S and G represent mutant resistant alleles (serine) and wild-type alleles (glycine), respectively. No resistant homozygotes GG were found among the 186 mosquitoes genotyped for *Ace-1*^{R} by restriction fragment length polymorphism (a subset of 48 was further screened by using the TaqMan assay; congruence between the 2 methods was 100%).
†The frequencies were calculated for each insecticide and mosquito status (alive/dead) after exposure.
‡1014F represent the *kdr* frequencies.
§Genotypic odds ratios (ORs) are shown because these exceed allelic ORs for DDT (recessive model), bendiocarb, and fenitrothion (both overdominant models), and are similar for permethrin and deltamethrin. For bendiocarb and fenitrothion absence of GG genotypes in the "Alive" group means that ORs are infinity, therefore ORs are shown if one GG was present. F and L represent mutant resistant alleles (phenylalanine) and wild-type alleles (leucine), respectively; S and G represent mutant resistant alleles (serine) and wild-type alleles.
¶119S represents the *Ace-1*^{R} frequencies.

Mr Edi is a PhD student at the Liverpool School of Tropical Medicine. His research interests are the causes and consequences of insecticide resistance in malaria vectors.

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etymologia

Anopheles

[ə-nof′ə-lēz]

From the Greek *an* (“not”) + *ophelos* (“benefit”), a genus of mosquitoes, many species of which are vectors of malaria. *Anopheles* was first described by German entomologist Johann Wilhelm Meigen in 1818. Although some sources translate *Anopheles* as “harmful,” it would be decades before Ronald Ross showed in 1897 that these mosquitoes transmit malaria parasites, and Meigen was most likely using *Anopheles* in a more literal interpretation as “useless.” That said, the connotation of “harmful” was prophetic in describing a mosquito that, even today, is indirectly responsible for ≈1 million deaths per year.

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Schmallenberg Virus in Domestic Cattle, Belgium, 2012

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To determine prevalence of antibodies against Schmallenberg virus in adult cows and proportion of infection transmitted to fetuses, we tested serum samples from 519 cow/calf pairs in Belgium in spring 2012. Of cattle within 250 km of location where the virus emerged, ≈91% tested positive for IgG targeting nucleoprotein. Risk for fetal infection was ≈28%.

In the summer and fall of 2011, a nonspecific febrile syndrome, characterized by hyperthermia, drop in milk production, and watery diarrhea, was reported among adult dairy cows on farms in northwestern Europe (*1*,*2*). In addition, in November 2011, an enzootic outbreak emerged in several European countries; sequelae included abortion, stillbirth, and birth at term of lambs, kids, and calves with neurologic signs or malformations of the head, spine, or limbs (*3*,*4*). Both syndromes were associated with the presence in the blood (adult animals) or in the central nervous system (newborn animals) of the RNA of a new Shamonda-like orthobunyavirus, provisionally named Schmallenberg virus after the town in Germany where the first positive samples were identified (*3*,*4*). Because this new viral disease in cattle emerged recently, information on its epidemiology is limited. The objectives of this study were to determine the prevalence of antibodies against Schmallenberg virus in adult cows living within ≈250 km of the location where the virus emerged, 9 months after the emergence, and to determine the proportion of fetal transmission of the virus.

The Study

During February 13–April 22, 2012, serum samples were obtained at random from blood drawn by field veterinarians from 519 cow/calf pairs at 209 farms located in southeastern (195 farms; Figure 1, rectangle A) or southwestern (14 farms; Figure 1, square B) Belgium. Samples were obtained from 1–7 cow/calf pairs at each farm. None of the 519 calves exhibited neurologic signs of disease at birth through 10 months of age. Serum specimens were also obtained from a cohort of adult cattle in spring 2010 (n = 71) and the first quarter of 2011 (n = 40). We used the ID Screen Schmallenberg Virus Indirect ELISA kit (ID.vet Innovative Diagnostics, Montpellier, France) to determine if the serum samples contained IgG antibodies against the recombinant nucleoprotein of the emerging Schmallenberg virus. Results are expressed as percentages of the reference signal yielded by the positive control serum, with serologic status defined as negative (<60%), doubtful (>60% and <70%), or positive (>70%) by the manufacturer. Contingency tables were analyzed by using χ^2 analysis to determine 1) if there was an association between sampling date and occurrence of seroconversion and 2) if there was an association between farm location and occurrence of seroconversion. Significance level was set at $p<0.05$.

All serum samples collected during spring 2010 and spring 2011 were negative for antibodies against Schmallenberg virus, which is consistent with the emergence of the new virus during the summer and fall of 2011 (*1*). Of the 209 farms sampled, only 13 were categorized as having seronegative cattle, each on the basis of the single paired sample that was available. These farms were not clustered by location. In each of the 196 remaining farms, >75% cows had seroconverted. Overall, apparent seroprevalence among adult cows was 90.8% (95%, CI 88.3–93.2, Figure 2). Association between farm location and seroconversion was not significant ($p = 0.607$), with results of 92.3%, 88.3%, 90.0%, and 92.0% in eastern, southern, western, and central areas, respectively (Figure 1). Acquired herd immunity against the new virus was thus quite high in the adult cattle population sampled, which suggests that this virus has spread quickly throughout the region since its emergence ≈250 km northeast of these areas in the late summer of 2011. Furthermore, a significant association between week of sampling and occurrence of seroconversion was found ($p = 0.039$), with a progressive increase of apparent seroprevalence: 87.8% (weeks 7–9, 95% CI 82.6–93.1), 90.4% (weeks 10–11, 95% CI 85.8–95.0), and 93.0% (weeks 12–16, 95% CI 89.6–96.4). This finding suggests that the virus was still circulating in the stables during the period examined. This results is not surprising because biting midges of the genus *Culicoides*, which are believed to transmit Schmallenberg virus (*5*), were recently shown to be able to complete their life cycle in animal enclosures (*6*).

Of the calves born to seropositive cows, 116 (24.6%) of 471 (95% CI 20.7–28.5; Figure 2) also tested positive, and no association was found between anti–Schmallenberg virus IgG in the newborn calves' serum samples before

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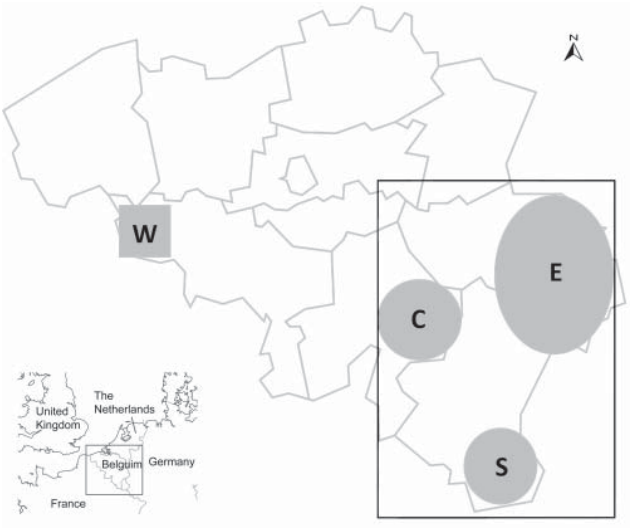

Figure 1. Location of 209 farms in Belgium from which 519 pairs of cow/calf serum samples were obtained from blood drawn by field veterinarians in southeastern (rectangle, 195 farms) or southwestern (square, 14 farms) areas in 2012. The area represented by the rectangle is centered on the village of Béthomont (50°08′N, 5°65′E) and measures ≈150 km from north to south and 100 km from east to west. Areas C (center), E (east), and S (south) refer to 3 distinct spatial clusters within the area represented by the rectangle. The area represented by the small shaded square (W) is centered on the village of Taintignies (50°54′N, 3°34′E); each side measures ≈20 km.

they received colostrum and farm location (p = 0.639), with 23.8%, 23.0%, 31.6%, and 25.3% seroprevalence in eastern, southern, western, and central areas, respectively (Figure 1). Infection of pregnant cows by Schmallenberg virus thus often results in transmission to the fetus across the placenta, and this transmission does not lead automatically to abortion, stillbirth, or congenital deformities. Because none of the calves examined here showed the neurologic or musculoskeletal signs typically associated with unrestricted replication of Schmallenberg virus (*3*,*4*), maternal infection probably occurred after the fetuses became immunocompetent, that is, after the 150th day of gestation (*7*). Therefore, on the basis of the corresponding dates of artificial inseminations, we conclude that the 38 seropositive calves born during March 19–April 22, 2012, were infected after November 1, 2011.

Because the corresponding farms were not clustered spatially, we deduced that the new virus and its vectors actively circulated throughout southern Belgium in November 2011. Again, a significant association was detected between week of sampling and occurrence of seroconversion during gestation (p = 0.023), with a progressive decrease of apparent seroprevalence among calves born to seropositive cows: 28.4% (weeks 7–9, 95% CI 21.1–35.6), 21.0% (weeks 10–11, 95% CI 14.7–27.4), and 19.2% (weeks 12–16, 95% CI 13.9–24.4). Thus, the apparent rate of the virus crossing the placenta seems to decrease over time. Because the biology of the new virus would not have changed in a few weeks, this decreased rate of transmission could be attributed to the gradual increase in the relative proportion of cows that were infected before the development of the placenta enables placental transfer of the virus, i.e., before the 30th day of gestation (*7*). Therefore, to estimate the risk for fetal infection among immunologically naive cows, we focused on the group of seropositive cows whose gestation began long before the supposed emergence of the new virus. Of the 519 pregnancies, 148 resulted from artificial inseminations performed during May 2–22, 2011. The probability that the cows had been infected before the 30th day of gestation by a virus that emerged in late summer is close to zero. On the basis of data from this pertinent subset, we found that ≈28% of the calves born to cows primo-infected after the first placentome developed (which permitted the virus to cross the placenta) were indeed infected. This finding closely fits with the ≈30% reported for Akabane virus, a close phylogenetic relative of Schmallenberg virus (*7*).

In conclusion, this study confirms that the emerging virus was absent from the area examined in spring 2011 and provides evidence that 1 year later almost all adult cattle had seroconverted. Furthermore, the results suggest that the risk for infection of the fetus in an immunologically naive herd is ≈28% and that in utero infections can occur without sequelae visible at birth if the infection occurs when the fetus's immune system is mature enough to control virus spread. In the case of Akabane virus, the cow's natural

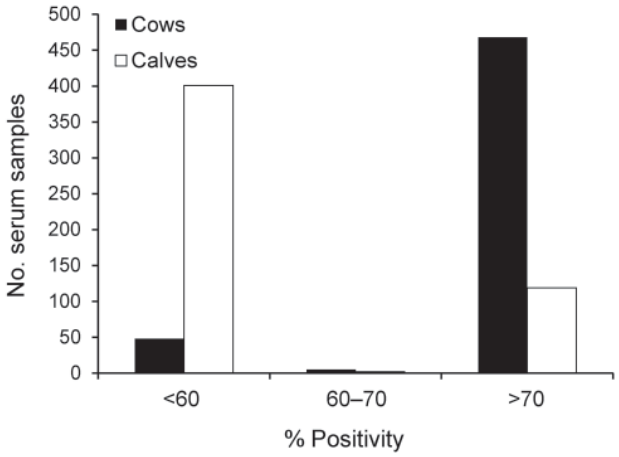

Figure 2. Frequency distribution of the results yielded by indirect ELISA for detecting IgG targeting recombinant nucleoprotein of the emerging Schmallenberg virus in serum samples from 519 cows, Belgium, 2012. Results are expressed as percentages of the reference signal yielded by the kit positive control serum, with serologic status defined as negative (<60%), doubtful (>60% and <70%), or positive (>70%) by the manufacturer.

immunity prevents subsequent infections of the fetus (*8*). It seems likely, therefore, that the Schmallenberg virus infection itself, and its resulting economic effects on farms in the regions concerned, might disappear in 2012.

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Antimicrobial Drug Use and Macrolide-Resistant *Streptococcus pyogenes*, Belgium

Liesbet Van Heirstraeten, Samuel Coenen, Christine Lammens, Niel Hens, Herman Goossens, and Surbhi Malhotra-Kumar

In Belgium, decreasing macrolide, lincosamide, streptogramins B, and tetracycline use during 1997–2007 correlated significantly with decreasing macrolide-resistant *Streptococcus pyogenes* during 1999–2009. Maintaining drug use below a critical threshold corresponded with low-level macrolide-resistant *S. pyogenes* and an increased number of *erm*(A)-harboring *emm*77 *S. pyogenes* with low fitness costs.

Macrolide resistance in *Streptococcus pyogenes* results primarily from modification of the drug target site by methyltransferases encoded by *erm* genes, *erm*(A) and *erm*(B) or by active efflux mediated by a *mef*-encoded efflux pump. Of these, *erm*(A) is inducibly expressed (*1*) and generally confers low-level resistance to macrolides, whereas lincosamides and streptogramins B (MLS_B), which share overlapping binding sites, remain active against *erm*(A)-harboring *S. pyogenes* (*2*). Conversely, *erm*(B) can be constitutively or inducibly expressed and confers high-level resistance to MLS_B (*2*). *mef*(A) also is constitutively expressed but confers low to moderate resistance to 14- and 15-membered macrolides and susceptibility to 16-membered MLS_B (*2*).

That macrolide use is the main driver of macrolide resistance in streptococci has been well demonstrated at the population and individual levels (*3*,*4*). Because *erm* and *mef* are cocarried with *tet* genes on mobile elements, tetracycline use also affects macrolide resistance (*4*). In addition, acquisition of resistance often confers a cost to bacteria, the magnitude of which is the main parameter influencing the rate of development and stability of the resistance mechanisms and, conversely, the rate at which resistance would decrease under decreasing use of antimicrobial drugs (*5*). We investigated temporal changes in the molecular epidemiology of macrolide-resistant *S. pyogenes* during 1999–2009 in relation to strain fitness (i.e., ability of bacteria to survive and reproduce) and to outpatient use of MLS_B and tetracycline in Belgium.

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The Study

We screened 11,819 *S. pyogenes* isolates from patients with tonsillopharyngitis or invasive disease in Belgium during 1999–2009 for macrolide resistance. We used double-disk diffusion, MIC testing, and multiplex PCR to detect *erm* and *mef* genes and investigated their clonality by *emm* typing and by pulsed-field gel electrophoresis (*6*). The prevalence of macrolide-resistant *S. pyogenes* decreased from 13.5% to 3.3% during 1999–2006 and remained low from 2006 onward (Figure 1); most isolates harbored *erm*(B) (395 [46.5%]) or *mef*(A) (383 [45.1%]). We detected *erm*(A) in only 85 (10.0%) resistant strains; however, their proportions among macrolide-resistant strains increased from 1 (1.2%) of 81 in 1999 to 36 (76.6%) of 47 in 2009. *erm*(A)-harboring *S. pyogenes* isolates primarily belonged to *emm*77 (50/85[5.8%]). *mef*(A) was mostly associated with *emm*1, *emm*4, and *emm*12 and *erm*(B) with *emm*11, *emm*22, and *emm*28 (Figure 2, Appendix, wwwnc.cdc.gov/EID/article/18/9/12-0049-F2.htm). During 1999–2009, proportions of *mef*(A)- and *erm*(B)-associated *emm* types decreased gradually, whereas those of *erm*(A)-harboring *emm*77 (*erm*(A)-*emm*77) increased steadily from 2006 onward (Figure 2). *erm*(A)-*emm*77 became predominant in 2008-2009, representing 10–28 (32.2%-59.6%) of total macrolide-resistant *S. pyogenes* isolates during those 2 years (Figures 1, 2). Most (97.8%) *erm(A)-emm*77 belonged to the same pulsed-field gel electrophoresis cluster and harbored *tet*(O), indicating gene linkage.

Next, we used data on outpatient use of MLS_B and tetracycline collected by the Belgian National Institute for Health and Disability Insurance during 1997–2008 and aggregated at the active substance level (World Health Organization Collaborating Center for Drug Statistics Methodology, www.whocc.no/atc/structure_and_principles/) to model the data obtained for macrolide-resistant *S. pyogenes*. MLS_B and tetracycline use was expressed in packages/1,000 inhabitants/day, a better proxy for prescriptions than defined daily doses in Belgium, where the number of defined daily doses per package or prescription had increased during the previous decade (*7*). MLS_B and tetracycline use decreased from 1997 to 2004 (1.16–0.53 packages/1,000 inhabitants/day) and remained stable at this level (0.50–0.53 packages/1,000 inhabitants/day) from 2004 onward (Figure 1). Total outpatient use of antimicrobial drugs also decreased (3.75–2.4 packages/1,000 inhabitants/day) during 1997–2007, as did use of penicillins, whereas proportional use of amoxicillin–

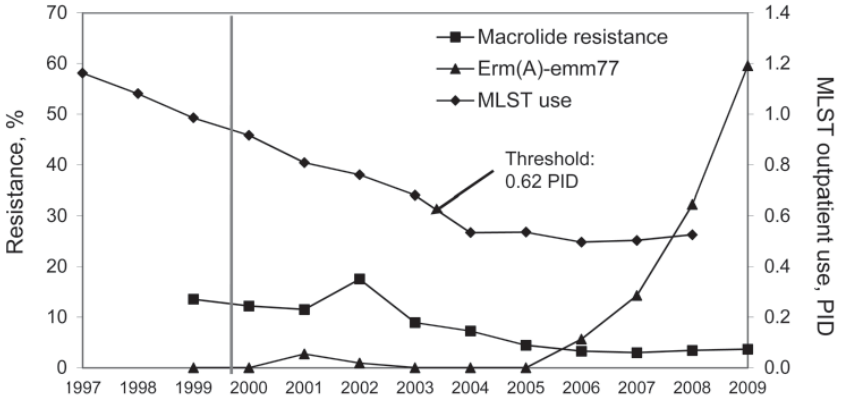

Figure 1. Prevalence of macrolide-resistant *Streptococcus pyogenes* and proportions of the *erm*(A)-*emm*77 geno-*emm*-type among macrolide-resistant strains during 1999–2009, and macrolides, lincosamides, streptogramins B, and tetracycline (MLST) use data expressed in packages/1,000 inhabitants/day (PID) during 1997–2007 in Belgium. Threshold indicates the critical level of macrolide, lincosamide, streptogramins B, and tetracycline use below which low-level macrolide-resistant *S. pyogenes* and selection of an inducible resistance mechanism with a lower fitness cost might be facilitated. Dotted line indicates start of the public health campaigns to reduce antimicrobial drug prescribing. The sharp increase in macrolide resistance in 2002 was linked to a local clonal outbreak of *mef(A)-emm1* harboring *S. pyogenes*.

clavulanate acid increased transiently soon after public campaigns began in Belgium (*8*). Yearly proportions of macrolide-resistant strains among total isolates correlated with MLS_B and tetracycline use in generalized linear models with a negative binomial distribution and a log-link (GLM, PROC GENMOD, SAS Institute, Cary, NC, USA). Using an interval of 2 years, we observed a highly significant positive correlation between decreasing use of MLS_B and tetracycline during 1997–2007 and decreasing levels of macrolide-resistant *S. pyogenes* during 1999–2009 ($p<0.0001$). The consistent decrease in MLS_B and tetracycline use since 1997 was further accentuated by the start of public health campaigns in December 2000 that also were directed toward prescribers and successfully reduced antimicrobial drug prescribing in Belgium (Figure 1) (*8*). A similar trend was observed in Finland, where a nationwide increase in erythromycin use and resistant *S. pyogenes* led to issuance of national recommendations to reduce outpatient use of MLS_B; erythromycin-resistant *S. pyogenes* declined after 2 years of reduced MLS_B use (*9*). Nonetheless, for *S. pyogenes*, these correlations are not always clear, primarily because of frequent clonal fluctuations for this organism. For instance, despite a 21% decrease in macrolide use in Slovenia, resistance doubled among noninvasive *S. pyogenes* isolates (*10*).

Notwithstanding clonal changes, the fitness costs (i.e., an organism's decreased ability to survive and reproduce because of a genetic change, expressed as a decreased bacterial growth rate) associated with particular resistance mechanisms is another major factor governing the relation between use and resistance. Mathematical models have shown threshold levels of antimicrobial drug use below which the frequency of resistance would not increase if resistance imposes a fitness cost for the bacteria (*11*). We further hypothesized that the frequency of certain macrolide-resistant geno-*emm*-types might differ if antimicrobial drug use remains below a certain threshold. In concordance with the models, we found a negative correlation between use of MLS_B and tetracycline and proportions of *erm*(A)-*emm*77 among macrolide-resistant *S. pyogenes* ($p = 0.0002$), and we identified 0.62 packages/1,000 inhabitants/day as the critical threshold volume of MLS_B and tetracycline use below which proportions of *erm*(A)-*emm*77 among macrolide-resistant *S. pyogenes* would increase significantly ($p<0.0001$). Next, we compared the fitness of *erm*(A)-*emm*77 with that of 6 other major macrolide-resistant geno-*emm*-types in Belgium during 1999–2009 (Figure 2). After growth-competition experiments (*12*), initial and final proportions of competing strains were determined by multiplex PCR to detect *erm*(B), *erm*(A), or *mef*(A) in 50 randomly selected colonies per plated mixture. Number of generations and relative fitness of competed pairwise strains were calculated as described (*13*). The inducible *erm*(A) in an *emm*77 background was more fit (67%) than most of the geno-*emm*-types that predominated during the previous years of higher MLS_B and tetracycline use (Table). Only the *mef*(A)-*emm*1 and *erm*(B)-*emm*28 geno-*emm*-types were equally as fit as *erm*(A)-*emm*77. Foucault et al. (*14*) showed that in the noninduced state, the inducible *vanB* gene had no effect on fitness of enterococci and might explain the low fitness cost of *erm*(A) carriage in *emm*77 strains. A predominance of *erm*(A)-harboring strains during 1993–2002, with 30% in an *emm77* background, was also reported in Norway, a country with a low prevalence of resistance (2.7%) and antimicrobial drug use (*15*). Of note here is the combination of *erm*(A) and *emm*77 as geno-*emm*-type because the fitness benefit (i.e., lack of fitness cost) was not as remarkable for other *erm*(A)-harboring *emm* types

Table. Characteristics of macrolide-resistant *Streptococcus pyogenes* used in competition experiments and relative fitness* of the *erm*(A)-*emm*77 geno-*emm*-type against competitor strains, Belgium, 1999–2009

Geno-*emm*-type	Macrolide MIC, mg/L		Relative fitness (SD)†		p value, *t* test
	Erythromycin	Clindamycin	*erm(A)-emm77*	Competitor	
erm(A)-*emm77*	2	0.125	ND	ND	ND
erm(B)-*emm28*	>512	>512	1.03 (0.09)	0.98 (0.09)	0.662
erm(B)-*emm22*	>512	>512	1.27 (0.15)	0.79 (0.09)	0.080
erm(B)-*emm11*	>512	256	2.12 (0.28)	0.48 (0.06)	0.013
mef(A)-*emm12*	8	0.125	1.29 (0.18)	0.78 (0.11)	0.105
mef(A)-*emm4*	8	<0.03	1.55 (0.15)	0.65 (0.06)	0.047
mef(A)-*emm1*	16	0.5	1.01 (0.05)	1.00 (0.06)	0.934

*The ability of the bacteria to survive and reproduce. ND, no data.
†Average of duplicate experiments.

(data not shown). The mechanisms underlying the higher fitness benefit conferred by an *emm*77 versus another *emm* background for the *erm*(A) genetic element remain to be investigated and might be related to differences in basal gene expression or compensatory changes in the *emm*77 genome or might result from differences in the genetic element harboring *erm*(A) in *emm*77.

Conclusions

Using macrolide-resistant *S. pyogenes* as a marker for use of MLS_B and tetracycline, we showed a decrease in use of these antimicrobial drugs, accentuated by successful public health campaigns, reflected a steady decline of macrolide-resistant *S. pyogenes* in Belgium. Furthermore, successfully maintaining use below a critical threshold resulted in maintenance of low-level macrolide-resistant *S. pyogenes* and emergence of the inducibly expressed and low-level resistant *erm*(A)-*emm*77 geno-*emm*-type. Maintaining antimicrobial drug use below a critical threshold might facilitate stabilization of low-level antimicrobial drug resistance and of milder resistance mechanisms with lower fitness costs.

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Influenza A(H1N1) pdm09 Virus among Healthy Show Pigs, United States

Gregory C. Gray, Jeffrey B. Bender, Carolyn B. Bridges, Russell F. Daly, Whitney S. Krueger, Michael J. Male, Gary L. Heil, John A. Friary, Robin B. Derby, and Nancy J. Cox

Within 5 months after the earliest detection of human influenza A(H1N1)pdm09 virus, we found molecular and culture evidence of the virus in healthy US show pigs. The mixing of humans and pigs at swine shows possibly could further the geographic and cross-species spread of influenza A viruses.

Cross-species infections with influenza A viruses readily occur between humans and pigs. Pigs often have been infected by human epidemic viruses (*1*), and swine workers and their family members are at increased risk for swine influenza virus (SIV) infection (*2–4*). We studied swine shows as a setting for influenza A virus transmission (*5*).

The Study

After acquiring informed consent, we recruited persons >7 years of age showing pigs at 3 state fairs in Minnesota (2008, 2009) and South Dakota (2009). Exhibitors were eligible for the study if they reported working with pigs at least 1 cumulative hour per week and had no current immunocompromising condition. Enrolled participants completed a questionnaire and permitted collection of nasal swab specimens from their show pigs. Before data were collected, multiple institutional review boards, the Institutional Animal Care and Use Committee of the University of Minnesota and the University of Iowa, and state fair officials approved the study.

We used the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) real-time reverse transcription PCR (rRT-PCR) (*6*) to screen for influenza A virus. Swab specimens (run in duplicate) with cycle threshold (C_t) values <35 were considered positive for influenza Avirus; specimens with C_t values of 35 to <40 were suspected to be positive; and specimens with C_t values >40 were considered negative. In a blinded fashion, aliquots of swab specimens from pigs were shared with the Minnesota Veterinary Diagnostic Laboratory (St. Paul, MN, USA), where rRT-PCRs for matrix, hemagglutinin (HA), and neuraminidase (NA) genes were performed. Specimens were then shared with the National Veterinary Services Laboratory (Ames, IA, USA) and later with CDC for further molecular and sequencing studies.

Positive and suspected-positive rRT-PCR specimens were cultured in shell vials on MDCK cells by using standard techniques. Sequence-based analyses of the influenza A virus isolates were performed by the CDC influenza division, using full or partial genome sequencing approaches for all 8 gene segments. Sequences were compared by using BLASTn alignment search techniques (http://blast.ncbi.nlm.nih.gov).

Questionnaires were completed by 121 (98%) participants. Participants were predominantly male (71%), and their median age was 34.9 years (range 9–75 years); 24% of participants were <18 years of age. Some pig exhibitors were children with <1 year of pig exposure (Table); others were pig farmers with numerous years of pig exposure. Participants reported an average of 18.7 years of pig exposure.

Nasal swab specimens were collected from a total of 149 pigs (from Minnesota, 47 in 2008 and 57 in 2009; from South Dakota, 45 in 2009). Almost all (97%) swabbed pigs were <1 year of age, and 40% were female. All pigs were observed to be healthy by a veterinarian before they were permitted to enter the show.

In 2008, nasal swab specimens from show pigs showed no molecular or viral culture evidence of influenza A virus. However, in 2009, a number of pigs were positive for influenza A virus. Comparing the molecular results of the 3 laboratories and using conservative rRT-PCR result interpretations on which all laboratories agreed, we determined that influenza A virus was detected in 12 (12%) of 102 swine respiratory samples by rRT-PCR; 11 (19%) of these were from among the 57 pigs swabbed at the Minnesota state fair (Figure). Viral culture yielded 7 influenza isolates, 5 of which were recultured and sequenced by CDC. Sequence for 4 of the isolates (A/Swine/Minnesota/074A/2009, A/Swine/Minnesota/115A/2009, A/Swine/Minnesota/130A/2009, and A/Swine/Minnesota/136B/2009) were similar to influenza A(H1N1)pdm09 viral gene sequences: identity scores ranged from 99.8% to 100% for polymerase basic 1, polymerase basic 2, polymerase acidic protein, HA, nucleocapsid protein, NA,

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Table. Characteristics of 123 persons enrolled in study of influenza A virus among show pigs, Minnesota and South Dakota state fairs, 2008 and 2009

Characteristic	No. (%)
State fair where enrolled	
Minnesota	90 (73.2)
South Dakota	33 (26.8)
Year enrolled	
2008	48 (39.0)
2009	75 (61.0)
Age group, y*	
<8	29 (23.6)
18–39	37 (30.1)
40–59	46 (37.4)
>60	8 (6.5)
Sex*	
M	86 (69.9)
F	35 (28.5)
Work performed in the last 30 d†	
Unemployed	8 (6.5)
Farmer	108 (87.8)
Swine farmer	100 (81.3)
Government employee	8 (6.5)
Veterinarian/veterinary assistant	2 (1.6)
Receipt of vaccination for human influenza in past 2 y*	
Yes	35 (28.5)
No	76 (61.8)
Unknown	7 (5.7)
Respiratory illness in past 12 mo*	
Yes	64 (52.0)
No	55 (44.7)
Unknown	2 (1.6)
Worked with swine, y*	
<1	3 (2.4)
≥1–4	10 (8.1)
5–10	39 (31.7)
>10	50 (40.7)

*These covariates have missing data.
†Responses are not mutually exclusive.

matrix, and nonstructural protein genes (Global Initiative on Sharing Avian Influenza Data [GISAID] accession nos. EPI295284–EPI295327). The fifth isolate (A/Swine/South Dakota/152B/2009) was cultured from an asymptomatic pig at the South Dakota state fair. After sequence studies of the HA and NA genes, this isolate was classified as a triple reassortant H1N2 virus (GISAID accession nos. EPI295328–EPI295335) similar to recent US swine isolates.

A follow-up telephone survey of study participants identified 2 with influenza-like illness (ILI) within 7 days after the fair: an adult, with ILI with onset 1 day after his pigs arrived at the fair (and 4 days before pig swabbing), and his daughter who developed an ILI on the last day of the fair (3 days after pig swabbing). Three pigs exhibited by the child tested positive for influenza A virus.

Conclusions

We found a 19% prevalence of influenza A virus among the 57 show pigs swabbed at the 2009 Minnesota state fair, which occurred during the second wave of the 2009 pandemic. Temporal analysis of the results indicated that most pigs with rRT-PCR–positive results were sampled within 24 hours after arriving at the fair, suggesting that they probably were infected before their arrival (Figure). None of the pigs with molecular or viral culture evidence of influenza A virus infection had clinical signs of influenza illness at the time of sampling. This finding validates previous pig show–linked human cases (*5*,*7*) and suggests that exposure to apparently healthy pigs at shows is a possible source of influenza A virus transmission.

These detections of A(H1N1)pdm09 virus in the United States (reflecting the ease of transmission from humans to pigs) were soon followed by multiple other detections in US pigs (*8*,*9*) and in numerous pigs in other countries. Such observations are now common (*10*), leading to speculation that just as the human-origin subtype H3N2 virus variant became enzootic in pigs (*11*), the A(H1N1)pdm09 virus and related viruses are now enzootic. Our findings of asymptomatic A(H1N1)pdm09 virus infections in pigs is supported by other data suggesting that as few as 10% of infected pigs might show clinical signs of A(H1N1)pdm09 virus infection (*12*).

Of concern is that new reassortants between A(H1N1)pdm09, enzootic SIVs, and possibly other human- or avian-origin viruses might emerge and possibly spread to humans who have contact with asymptomatic pigs (*10*,*13*,*14*). Clinicians who care for persons in whom influenza A illness develops, particularly when human influenza is not widely circulating, should ask about pig exposures and consider further molecular testing to rule out human infection with a nonhuman-origin influenza A virus. In addition, to minimize potential interspecies transmission of influenza viruses, it might be prudent to develop guidelines for the exhibition of pigs.

Because of the possibility of novel virus generation in pigs and of human-to-pig and pig-to-human transmission of influenza virus, routine influenza A virus surveillance

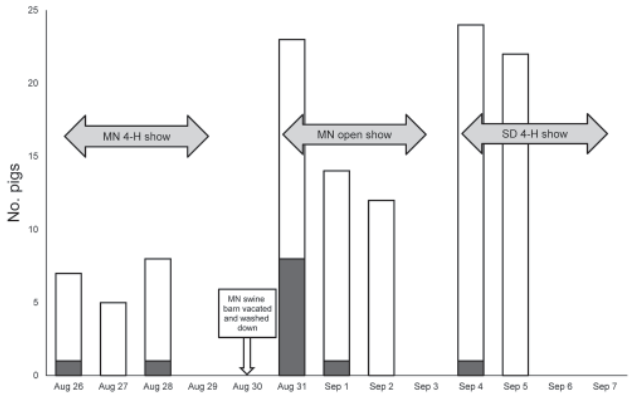

Figure. Show pigs with nasal swab specimens positive for influenza A virus by real-time reverse transcription PCR, Minnesota and South Dakota state fairs, 2009. MN, Minnesota; SD, South Dakota. White, total number of pigs swabbed; gray, pigs testing positive for influenza A by real-time reverse transcription PCR.

among pigs and influenza A virus testing of ill persons exposed to pigs is needed to ensure timely detection of novel influenza viruses in humans and pigs (*4*). Early detection is essential for development of effective vaccines and initiation of other means to prevent the spread of novel influenza A viruses. However, considerable barriers exist to conducting surveillance in pigs and pig-exposed persons, not the least of which is the threat that such surveillance could economically harm the pork industry (*10*). To improve influenza surveillance, additional ways are needed for pig farmers, the pork industry, the US Department of Agriculture, and public health professionals to collaborate. Swine workers, food animal veterinarians, and persons involved in raising show pigs are at high risk for zoonotic influenza infection (*4*,*15*). They should be strongly encouraged to receive seasonal influenza vaccines and to take measures to reduce zoonotic disease transmission, including using personal protective equipment. They also should limit their contact with pigs when they or the pigs have symptoms of respiratory illness (*4*,*10*).

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Aeromonas spp. Bacteremia in Pregnant Women, Thailand–Myanmar Border, 2011

To the Editor: *Aeromonas* spp. bacteria cause a broad spectrum of human infections (*1*). Invasive infections can be associated with exposure to contaminated water and occur frequently in patients with underlying immunosuppression or trauma but only infrequently in pregnant women (*2*). During January–May 2011, *Aeromonas* spp. bacteremia was identified in 3 pregnant Myanmar women of Karen ethnicity, who sought care at migrant/refugee clinics on the Thailand–Myanmar border.

In January 2011, a 31-year-old woman in week 12 of pregnancy sought care at the Wang Pha migrant clinic. She reported a brief history of fever, headache, abdominal pain, and vaginal bleeding. She was tachycardic and tachypneic. A complete blood count indicated mild thrombocytopenia (104 × 10^3/μL). Ampicillin, gentamicin, and metronidazole were empirically prescribed. Ultrasonography confirmed a nonviable fetus, and products of conception were surgically evacuated. Four days later, treatment was changed to oral amoxicillin and ciprofloxacin because of clinical improvement and isolation of *A. hydrophila* from blood collected at admission. The patient recovered and was discharged after 6 days.

In March 2011, a 25-year-old primigravid woman in week 12 of pregnancy sought care at the Maela refugee camp; she had a 24-hour history of fever, chills, vomiting, diarrhea, and vaginal bleeding. She had recently sought treatment from a traditional birth attendant, who reportedly had inserted 3 sticks into the cervix and performed uterine massage. At admission, the patient was febrile and in shock. Bimanual examination revealed an open cervix with malodorous discharge. Ultrasonography revealed only products of conception. The patient received intravenous fluids and was empirically treated with ampicillin, gentamicin, and metronidazole. After surgical evacuation of the products of conception, the patient's condition continued to deteriorate. Despite referral to the local hospital, the woman died the next day with clinical features suggestive of septic shock and disseminated intravascular coagulopathy. Complete blood count results were unavailable. *A. veronii* biovar sobria was isolated from blood collected at admission.

In May 2011, a 50-year-old woman in week 12 of pregnancy was admitted to the Wang Pha Clinic with a history of vaginal bleeding. She was febrile and in shock. Vaginal examination detected an open cervix with malodorous discharge. Complete blood count indicated reference level leukocytes and mild thrombocytopenia (116 × 10^3 cells/μL). Blood was collected for culture. The patient received intravenous fluids and was empirically treated with ampicillin, gentamicin, and metronidazole. Products of conception were removed under ultrasonographic guidance. Three days later, treatment was changed to ceftriaxone and metronidazole because of continuing fever and positive blood culture findings (gram-positive cocci and gram-negative bacilli). These organisms were subsequently identified as *Streptococcus pyogenes* and *A. veronii* biovar sobria. The patient's condition improved rapidly, and after 5 days she was discharged with prescriptions for oral amoxicillin and ciprofloxacin.

Blood culture isolates were provisionally identified as *Aeromonas* spp. by Gram stain, oxidase test, and API 20NE and 20E kits (bioMérieux, Marcy L'Étoile, France). The Aerokey II algorithm was used to identify the isolates to the species level (Table) (*3*). Susceptibilities to antimicrobial drugs were obtained by the agar disk–diffusion method, according to Clinical and Laboratory Standards Institute guidelines (*4*).

One other case of invasive *Aeromonas* spp. infection during pregnancy (*A. hydrophila* sepsis at week 24 of gestation) has been reported; the focus of infection in that case was hepatobiliary, and normal pregnancy outcome was documented (*2*). For the 3 case-patients reported here, miscarriage occurred for all. Although products of conception were not submitted for culture, septic abortion was suspected clinically. Of note, *A. veronii* biovar sobria has been associated with abortion in water buffaloes (*5*). Nonmedical abortion, including the use of sticks to terminate pregnancy, is reportedly conducted on the Thailand–Myanmar border (*6*), and its use in 1 of the case-patients reported here was confirmed. Such practices predispose women to infectious complications as a result of the use of nonsterile instruments. *Aeromonas* spp. can colonize the human genital tract (*7*). Therefore, the organisms could have gained entry on contaminated abortion sticks or, if the vagina was colonized, as a result of the trauma of the procedure. An alternative hypothesis to explain our clinical findings is that fetal loss occurred as a result of *Aeromonas* spp. sepsis originating from another exposure.

The isolates were susceptible to a variety of antimicrobial drugs (Table). Empirically prescribed antimicrobial drug protocols for treatment of sepsis during pregnancy (e.g., ampicillin, gentamicin, and metronidazole or an extended-spectrum cephalosporin and metronidazole) should be effective against *Aeromonas* spp. However, if *Aeromonas* spp. are isolated, targeted treatment with a fluoroquinolone, extended-spectrum cephalosporin, or carbapenem is more appropriate (*1*).

Table. Characteristics of *Aeromonas* spp. isolates from blood of 3 pregnant women, Thailand–Myanmar border, January–May 2011*

Characteristic	Patient no., organism		
	1, *A. hydrophila*	2, *A. veronii* biovar sobria	3, *A. veronii* biovar sobria
Species identification results			
Growth in 1% NaCl	+	+	+
Growth in 6% NaCl	–	–	–
Growth on TCBS agar (colonies)	+/– (yellow)	+/– (yellow)	+/– (green)
Glucose fermentation	+	+	+
Oxidase	+	+	+
O129 susceptibility (10 µg/150 µg)	–/–	–/–	–/–
Esculin hydrolysis	+	–	–
Indole	+	+	+
Voges-Proskauer	+	+	+
Gas from glucose (TSI)	+	+	+
Acid from arabinose	–	–	–
Cephalothin susceptibility (30 µg)	–	+	–
Antimicrobial drug susceptibilities (zone diameter, mm)			
Ampicillin (10 µg)	NA (6)	NA (6)	NA (6)
Ceftazidime (30 µg)	S (30)	S (31)	S (32)
Ceftriaxone (30 µg)	S (26)	S (40)	S (31)
Meropenem (10 µg)	S (30)	S (28)	S (23)
Chloramphenicol (30 µg)	S (30)	S (31)	S (28)
Ciprofloxacin (5 µg)	S (33)	S (35)	S (26)
Co-trimoxazole (25 µg)	S (24)	S (22)	S (23)
Gentamicin (10 µg)	S (20)	S (19)	S (18)

*TCBS, thiosulfate-citrate-bile salts-sucrose; O129, disks impregnated with 2,4-diamino-6,7di-iso-propylpteridine phosphate for the presumptive identification of *Vibrio* spp. from other gram-negative rods; TSI, triple sugar iron; NA, not applicable; S, susceptible (derived according to criteria from [*4*]).

We speculate that contamination of sticks used to induce abortion might play a role in these infections in this setting, although obtaining a definitive history to substantiate this speculation is difficult. Also, the scarcity of diagnostic microbiology laboratories in resource-poor settings results in underidentification of the causative pathogens.

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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.

bla$_{OXA-181}$–positive *Klebsiella pneumoniae*, Singapore

To the Editor: Nordmann et al. (*1*) raised concern over the global spread of carbapenemase-producing *Enterobacteriaceae*. In their article, they called attention to the oxacillinase-48 (OXA-48) type carbapenemases because bacteria that produce these enzymes do not have a distinctive antimicrobial drug susceptibility profile, and there is less awareness of this mechanism of carbapenem resistance. We report the recent isolation of *bla*$_{OXA-181}$–positive *Klebsiella pneumoniae* from 2 patients from Bangladesh who were admitted to separate hospitals in Singapore within a short period of each other.

The first patient was a 64-year-old man who had a recent heart attack and was transferred from a hospital in Dhaka, Bangladesh, to a hospital in Singapore for treatment for pancytopenia. He had no other history of recent travel. While in Bangladesh, the patient had *Pseudomonas* spp. bacteremia and had received meropenem and vancomycin. In Singapore, his antimicrobial drug treatment regimen was changed to ciprofloxacin, linezolid, and amikacin. Blood samples obtained on the day of admission were cultured and grew a vancomycin-resistant *Enterococcus* spp. and a carbapenem-resistant *K. pneumoniae* (isolate DB53879_11).

Two days after admission, when the results of his blood culture were known, the patient's antimicrobial drug treatment regimen was changed to oral linezolid (600 mg every 12 hours), intravenous tigecycline (initially 50 mg every hour but later increased to 100 mg every 12 hours), and intravenous polymyxin E (initially 3 MU/d but later increased to 3 MU every 12 hours). Blood cultured for *K. pneumoniae* showed positive results for 5 days after the patient was hospitalized before clearing.

Isolate DB53879_11 was resistant to many antimicrobial drugs as determined by Etest (bioMérieux, Marcy l'Etoile, France) (Table). It was strongly positive for carbapenemase production as determined by use of a modified Hodge test (*2*) and showed a negative result with the KPC + MBL Confirm ID Kit (Rosco Diagnostica A/S, Taastrup, Denmark).

Using PCR, we amplified and sequenced a product identical to the complete sequence of *bla*$_{OXA-181}$. Primers designed for known flanking regions of *bla*$_{OXA-181}$ (GenBank accession no. JN205800) failed to amplify any product. Like described isolates (*3*–*5*), DB53879_11 was also positive for *bla*$_{OXA-1}$ and *bla*$_{CTX-M-15}$, but it also was positive for *bla*$_{CMY-4}$. An attempt to transfer *bla*$_{OXA-181}$ to azide-resistant *Escherichia coli* J53 by plate mating was unsuccessful.

Two weeks after we received the specimen from the first patient, we were referred 2 carbapenem-resistant *K. pneumoniae* strains isolated from sputum (isolate DX1083_11) and blood (isolate BL21479_11) from a 30 year-old man admitted to another hospital in Singapore. He had been treated in the same hospital in Dhaka as the first patient for multiorgan failure secondary to dengue shock syndrome. Antimicrobial drug susceptibility phenotypes and resistance gene complements for DX1083_11 and BL21479_11were similar to those for the isolate from the first patient. The second patient received intravenous tigecycline, polymyxin B, and meropenem.

All 3 isolates were identical when tested by random amplification of polymorphic DNA (*6*) and by pulsed-field gel electrophoresis after restriction endonuclease digestion of chromosomal DNA with *Spe*I. Multilocus sequence typing showed that DX1083_11 belonged to sequence type 14 (www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). This sequence type is the same as that for *bla*$_{OXA-181}$–positive *K. pneumoniae* reported from New Zealand (*3*). Both patients died of their illnesses.

OXA-181 is a close relative of OXA-48 from which it differs by 4 aa (*4*). *bla*$_{OXA-181}$–positive *K. pneumonia* infections were first described in India but imported cases have since been described in Oman, the Netherlands,

Table. Antimicrobial drug susceptibilities of 3 *bla*$_{OXA-181}$–positive *Klebsiella pneumoniae* isolates, Singapore*

	MIC (mg/L)		
Drug	DB53879_11	DX1083_11	BL21479_11
Ampicillin	≥256	≥256	≥256
Amoxicillin/clavulanate	≥256	≥256	≥256
Piperacillin/tazobactam	≥256	≥256	≥256
Cefuroxime	≥256	≥256	≥256
Ceftriaxone	≥32	≥32	≥32
Ceftazidime	≥256	≥256	≥256
Cefepime	≥256	≥256	≥256
Ertapenem	≥32	≥32	≥32
Imipenem	16	≥32	16
Meropenem	≥32	≥32	≥32
Doripenem	≥32	≥32	≥32
Ciprofloxacin	≥32	≥32	≥32
Levofloxacin	≥32	≥32	≥32
Gentamicin	≥256	≥256	≥256
Amikacin	≥256	≥256	≥256
Sulfamethoxazole/trimethoprim	≥32	≥32	≥32
Tetracycine	≥256	≥256	8
Tigecycline	4	4	4
Polymyxin B	0.5	1	4

*OXA-181, oxacillinase-181.

and New Zealand (*3–5*,*7*). We were unable to confirm the original source of these isolates, and continuous surveillance for carbapenemase producers in our hospital has not uncovered any $bla_{OXA-181}$–positive isolates since 1996. To our knowledge, there are no reports of $bla_{OXA-181}$–positive isolates in Bangladesh. However, this country borders India, which is a source of $bla_{OXA-181}$–positive *Enterobacteriaceae*. These cases highlight potential problems that may arise from medical tourism (the rapidly increasing practice of traveling across international borders to obtain health care) and document the expanding range of a newly emerging mechanism of carbapenem resistance.

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Dengue Fever in South Korea, 2006–2010

To the Editor: Dengue fever is an acute, febrile disease caused by a flavivirus and is transmitted by *Aedes* spp. mosquitoes (*1*). South Korea is not considered as a region to which dengue virus is endemic because it is located above 35°N latitude and has an isotherm of 10°C in winter, which potentially limits year-round survival of *Aedes aegypti* mosquitoes (*1*,*2*). Thus, dengue fever was seldom recognized as a public health concern in South Korea. However, the first case of dengue fever in South Korea was reported in 1995 in a woman who had traveled to Sri Lanka (*3*). A second case was found in a sailor who had worked in countries in Africa in 2000 (*4*).

Since 2001, dengue fever has been a notifiable infectious disease in South Korea because of concerns about increasing international travel as a source of infection and because the less efficient potential dengue vector, *Ae. albopictus* mosquitoes, were found in this country. All cases reported through the surveillance system should be complemented by thorough epidemiologic investigations to determine whether a case was imported or originated in South Korea. Thus, we analyzed dengue fever–associated data from the Korea Centers for Disease Control and Prevention.

During 2006–2010, a total of 367 suspected cases were reported by physicians through the National Infectious Disease Surveillance System. IgM ELISA and reverse transcription PCR results identified 324 cases as dengue fever. Investigation of 34 cases could not be completed because some cases were in foreigners and in Korean persons who resided in foreign countries, left South Korea after diagnosis, or could not be reached by the contact information that was provided. Investigation of 290 cases was completed by reviewing medical records and by interviews. Interviews were conducted by provincial and Korea Centers for Disease Control and Prevention Epidemic Intelligence Service officers, who used a standardized investigation form.

All 290 case-patients had a history of international travel before onset of dengue fever symptoms.

Destination information was available for all 290 case-patients; 17 countries were identified. Visitors to the Philippines (34.1%) contributed the largest number of cases, followed by visitors to Indonesia, India, Thailand, Vietnam, Cambodia, Laos, Malaysia, Myanmar, Bangladesh, China, East Timor, Maldives, Palau, Sri Lanka, Brazil, and Nigeria (Table). These countries are in areas to which dengue fever is endemic or have reported cases (*1*).

The time interval between the last day of travel and symptom onset was known for 272 (93.8%) of the 290 case-patients. A total of 271 case-patients had traveled within 14 days before symptom onset, and 89 (32.7%) had symptom onset or were given a diagnosis of dengue fever during travel. Symptoms developed within 7 days after travel in 171 (62.9%) persons and 8–14 days after travel in 11 (4.0%) persons. Mean ± SD duration from the last day of travel to symptom onset among 182 case-patients who had symptom onset after travel was 3.20 ± 2.61 days.

Our results indicate that all investigated case-patients had a history of international travel and times of symptom onset during or after travel but within the incubation period for dengue infection. One case-patient had a time to symptom onset of <34 days. This person was eventually given a diagnosis of infection with Epstein-Barr virus but was tested for dengue virus. Because the incubation period exceeded that for dengue virus incubation, this case was classified as an asymptomatic dengue virus infection and an Epstein-Barr virus infection.

Most dengue cases in South Korea are likely imported, and most presumptive countries from which dengue fever originated are in Southeast and southern Asia. These countries are popular holiday destinations for persons from South Korea. Because of distances, few tourists from South Korea travel to Africa and South America (*5*). China is the most popular destination for travelers from South Korea. However, the proportion of persons who acquired dengue infection in China was low (0.7%) because most persons who traveled to China went to Beijing or Shanghai, not to areas in southern China where dengue epidemics have occurred (*5*,*6*).

We report that all dengue fever cases in South Korea during 2006–2010 were imported by persons who had traveled abroad. Global expansion of dengue virus and an increase in international travelers have increased the likelihood of additional cases of dengue fever. In addition, *Ae. albopictus* mosquitoes have been detected in South Korea and can potentially transmit autochthonous dengue infection, as reported in Croatia, France, and Hawaii, USA (*7*–*9*). Thus, more intensified surveillance and investigations should be focused on dengue transmission by *Ae. albopictus* mosquitoes in South Korea.

Table. Presumptive country of origin of imported dengue fever cases, South Korea, 2006–2010

Region, country	No. (%)
Southeast Asia	242 (83.4)
The Philippines	99 (34.1)
Indonesia	37 (12.8)
Thailand	30 (10.3)
Vietnam	24 (8.3)
Cambodia	17 (5.9)
Laos	6 (2.1)
Malaysia	5 (1.7)
Myanmar	5 (1.7)
East Timor	2 (0.7)
Palau	1 (0.3)
Other	16 (5.5)
Southern Asia	40 (13.8)
India	32 (11.0)
Bangladesh	4 (1.4)
Maldives	2 (0.7)
Sri Lanka	1 (0.3)
Other	1 (0.3)
Eastern Asia	
China	2 (0.7)
Africa	
Nigeria	1 (0.3)
South America	
Brazil	1 (0.3)
Other	4 (1.4)
Total	290 (100)

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Brucellosis in Takins, China

To the Editor: Brucellosis is a highly contagious bacterial disease and one of the world's major zoonoses. It is responsible for enormous economic losses in livestock, and it threatens human health and wildlife populations (*1*). In most host species, brucellosis primarily affects the reproductive system, leading to concomitant loss in productivity of affected animals (*1*). Brucellae have been found in wildlife, such as bison, elk, and wild boar, potentially posing a threat for zoonosis (*2*). Currently, the genus *Brucella* comprises 10 species, which are divided according to host specificity and ability to cause chronic infections in human and animals (*3*,*4*). Most *Brucella* species are associated primarily with certain hosts, presumably the result of evolutionary adaptation to a successful host. *Brucella melitensis* is the species most pathogenic in humans and the species most commonly involved in ovine and caprine brucellosis.

In January 2009, in the nature reserve in Qinling Mountains, China, hygromas were found on the knees, stifles, hocks, haunches, and bursae between the nuchal ligament and the primary thoracic spines of 10 free-ranging takins (*Budorcas taxicolor*). The hygroma contents and tissue samples were collected by using aseptic technique, packed separately, cooled immediately, and stored frozen at -20°C until cultured. The samples were streaked onto blood agar and MacConkey agar and incubated aerobically or anaerobically with 5% CO_2 at 37°C for 4 days.

Tiny gram-negative coccobacilli were isolated. The organism was nonmotile at 20°C and 37°C, and it stained red with the Stamp modification of the Ziehl-Neelsen method. The organism was identified as *B. melitensis* by the Vitek 2 GN identification system (bioMérieux, Marcy l'Étoile, France). The isolate was urease positive, catalase positive, and oxidase positive. It did not require carbon dioxide for growth and did not produce hydrogen sulfide. The isolate could be agglutinated by A-monospecific antiserum but not by M-monospecific antiserum or rough *Brucella*-specific antiserum. It was sensitive to Berkeley and Iz phages at routine test dilution but not sensitive to Tbilisi, Weybridge, Firenze, and R/C phages. According to classical biotyping methods, the isolate was identified as *B. melitensis* biotype 2 (*5*).

Molecular identification by 16S rRNA gene sequencing was used in this study (*6*). According to nucleotide–nucleotide GenBank search by using BLAST (http://blast.ncbi.nlm.nih.gov/), the sequence was 100% identical to the sequences of 16S rDNA of brucellae, especially reference strains including *B. melitensis* 16M (GenBank accession no. NC_003317), *B. abortus* biovar 1 str. 9–941 (NC_006932), *B. suis* 1330 (NC_004310), *B. canis* American Type Culture Collection 23365 (NC_010103), and *B. ovis* American Type Culture Collection 25840 (NC_009505). The isolate was further confirmed as *B. melitensis* according to the 731-bp product by using AMOS-PCR, which discriminates among species by the unique locations of the IS*711* element (*7*,*8*). The restriction pattern of the *omp2b* gene by *Hin*f I was accordant with pattern 3 reported by Cloeckaert et al. (*9*); this finding further indicated that the isolate was *B. melitensis* (*9*).

The takin (*Budorcas taxicolor*) is a ruminant belonging to the family Bovidae, subfamily Caprinae, genus *Budorcas* (Figure). Takins are found in eastern Asia and Southeast Asia and are listed as "vulnerable A2cd" by the International Union for Conservation of Nature (*10*). Brucellosis might pose a major direct or indirect threat to the conservation of endangered species,

Figure. Takin (*Budorcas taxicolor*).

such as takins, and can be a source of conflicts among stakeholders in conservation efforts.

Several antelopes, such as takins, serows (*Capricornis sumatraensis*), and gorals (*Naemorhedus goral*), occur sympatrically in the Qinling Mountains of China. Because brucellae are often transmitted by direct contact or exposure to a contaminated environment, it is possible that rather than being a natural reservoir for the bacteria, takins are infected horizontally by contact with birth exudates from other infected animals (*2*). However, information on brucellosis prevalence in those sympatric ruminants in China is insufficient. Therefore, further investigation and research are needed to test this hypothesis. Also, brucellosis is endemic among livestock and human populations in western China. Because domestic sheep and goats are grazed in the mountains, infections in livestock can spill over into wildlife, such as takins. Brucellosis in humans might also be caused by exposure to infected animals during activities like the handling, skinning, and eviscerating of the carcasses of infected animals.

Whether takins are the reservoir host or an accidental host for *B. melitensis* is still unclear. To further understand the interaction of brucellae among wildlife, domestic animals, and humans, and for purposes of brucellosis management and control, systematic investigations of brucellosis prevalence among wildlife should be conducted.

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Measles and Secondary Hemophagocytic Lymphohistiocytosis

To the Editor: We found interesting the article by Lupo et al. about a case of fatal measles in an immunocompetent 29-year-old woman (Fatal measles without rash in immunocompetent adult, France; http://dx.doi.org/10.3201/eid1803.111300). Perhaps, however, the possible diagnosis of secondary hemophagocytic lymphohistiocytosis (HLH) should also have been considered in that setting.

HLH is a potentially fatal hyperinflammatory syndrome characterized by histiocyte proliferation and hemophagocytosis. HLH may be inherited (i.e., primary, familial, generally occurring in infants) or may occur at any age secondary to infection, malignancy, or rheumatologic disease. Secondary HLH is determined according to clinical criteria from the HLH Study Group of the Histiocyte Society, which require >5 of the following for a diagnosis: fever; splenomegaly; cytopenia (affecting >2 cell lineages); hypertriglyceridemia or hypofibrinogenemia; hemophagocytosis in the bone marrow, spleen, or lymph nodes; low or absent natural killer cell cytotoxicity; hyperferritinemia; and elevated levels of soluble CD25.

We conducted a PubMed search and found 5 articles that described 6 cases of HLH in patients with measles (*1*–*5*). Pneumonia was described in all of them (*1*–*5*), and central nervous system involvement was described in 3 (*1*,*4*). Four cases occurred in children, 3 of them immunocompetent (*1*,*3*–*5*). The 2 adults were an immunocompetent 18-year-old man who had acute respiratory distress (*2*) and a 19-year-old man with acute lymphocytic leukemia who had measles pneumonia and acute hemorrhagic leukoencephalitis (*1*). The only fatal case occurred in an immunocompromised 8-year-old boy with giant-cell pneumonia (*3*).

The identification of hemophagocytosis in bone marrow aspirate represents only 1 of the 5–8 criteria needed for a diagnosis of HLH; conversely, a bone marrow aspirate lacking hemophagocytosis does not rule out the diagnosis of HLH. Still, we believe HLH should be considered for any patient with fever and pancytopenia, especially in the presence of respiratory distress or multiorgan dysfunction. An appropriate therapy could save the patient (Secondary hemophagocytic syndrome in adults: a case series of 18 patients in a single institution and a review of literature; http://dx.doi.org/10.1002/hon.960).

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In Response: We thank Iaria et al. (*1*) for their comments on our letter reporting an unusual case of fatal measles without rash in an immunocompetent woman who manifested cytopenias and an intractable acute respiratory distress syndrome (*2*). The authors suggest that secondary hemophagocytic lymphohistiocytosis (HLH) could have been considered in this patient.

Our reply supplies supplementary clinical and laboratory findings that could be useful for discussion.

During our patient's hospitalization, we were able to investigate 6/8 diagnostic criteria for HLH proposed by the Histiocyte Society (*3*). Of these, only 2 or 3 were found: persistent fever at 38.5°C; hypertriglyceridemia at 267 mg/dL (analysis performed at day 7); and cytopenias, which preferentially affected erythrocytes and lymphocytes. (Thrombocytopenia was moderate at >100 × 10^9 platelets/L, and no neutropenia was found [>2 × 10^9 neutrophils/L].) Liver function was not affected; no hepatomegaly was found, and alanine aminotransferase, aspartate aminotransferase, and bilirubin levels remained within reference ranges. Physical examinations did not detect splenomegaly, and laboratory findings did not show hypofibrinogenemia or ferritin level exceeding 500 ng/mL. A bone marrow biopsy performed on day 2 of hospitalization did not show hemophagocytosis. Studies of natural killer cell function and soluble CD25, which are also proposed diagnostic criteria for HLH, were not performed.

Overall, we found that the arguments in favor of HLH were too limited to consider this diagnosis and initiate an aggressive therapeutic approach based on immunosuppressive drugs. Even if, in the event of HLH, an early and appropriate treatment can be life-saving, the destruction of the remaining immune functions might also be lethal for the patient.

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Contaminated Soil and Transmission of Influenza Virus (H5N1)

To the Editor: Highly pathogenic avian influenza (HPAI) virus (H5N1) has been responsible for 603 confirmed human cases worldwide, including 356 that resulted in death, and for >7,000 epizootic outbreaks (*1*,*2*). Direct contact between hosts is the main mechanism of transmission for avian influenza viruses, but the possible role of the environment as a source of HPAI virus (H5N1) infection has been rarely studied, particularly in the context of countries where the virus is enzootic or epizootic (*3*–*7*). To determine if contaminated soil contributes to the transmission cycle of HPAI virus (H5N1), we used experimental and simulated field conditions to assess possible transmission in chickens.

All experiments were conducted by using HPAI virus (H5N1) strain A/chicken/Cambodia/LC1AL/2007 (GenBank accession nos. HQ200574–HQ200581). All animal experiments were conducted in the biosafety level 3 laboratory of Institut Pasteur in Cambodia (IPC), in compliance with the European Community 86/609/CEE directive and approved by the Animal Ethics Committee of IPC (permit: AEC/IPC/003/2010). Specific pathogen–free (SPF) chickens were provided by the National Veterinary Research Institute of Cambodia.

We used 3 types of soil: 1) sandy topsoil collected from around rice fields in Phnom Penh Province, Cambodia; 2) building sand purchased from a local building company; and 3) soil-based compost purchased from a local tree nursery. Physicochemical and microbiologic parameters were measured for water extracts obtained for each type of soil (online Technical Appendix Table, www.cdcnc.gov/EID/pdfs/12-0402-Techapp.pdf), and low- and high-dose contamination protocols (online Technical Appendix Figure) were used to experimentally contaminate each soil type. In brief, we seeded the soil samples with 1–56 infectious units of contaminated feces; 1 infectious unit was defined as 1 g feces from an SPF duck mixed with 1 × $10^{7.8}$ 50% egg infective dose of HPAI virus (H5N1) particles. The contaminated soil was then sprinkled on the bottom of an isolator (surface area 0.2 m^2) in which the chickens were housed. Oropharyngeal and cloacal swab samples and feathers were collected daily from the chickens and underwent quantitative reverse transcription PCR (qRT-PCR) testing targeting the H5 hemagglutinin gene (*8*). Surviving birds were killed humanely at the end of the experiments, and postmortem examination and collection of serum and organ samples were conducted on all animals. Organ samples were tested by using qRT-PCR, and serum samples were tested

by using hemagglutination inhibition assay (*9*).

No clinical symptoms, deaths, or seroconversion for HPAI virus (H5N1) were observed in chickens exposed to contaminated sandy topsoil, regardless of the dose protocol used (Table). However, for building sand and soil-based compost, the high-dose contamination protocol, starting with 8 infectious units, resulted in a 100% mortality rate by day 4. Low-dose protocols, starting at 1 infectious unit, resulted in survival of all birds at day 24, with no clinical symptoms and no virus detected in the samples collected postmortem. However, seroconversion for HPAI virus (H5N1) was observed in 33% and 50% of the chickens exposed to building sand and compost, respectively (Table).

Soil-based compost and building sand, although existing in natural settings, are not the most common substrates found in places where free-ranging poultry are raised in Cambodia. Therefore, despite the high mortality rate observed in our study after exposure to highly contaminated soils, the role of these soil types in transmission of HPAI virus (H5N1) infection to poultry or other species, including humans, appears limited when replaced in actual epizootic or enzootic field conditions. Our results also show that exposure of chickens to moderately contaminated soil may result in a protective immune response.

Sandy topsoil, on the other hand, did not allow any transmission of HPAI virus (H5N1) from the environment to chickens. This type of soil, which covers ≈40% of the rice-growing areas of Cambodia (*10*) and is abundant in neighboring countries of the Mekong region, is the most common ground on which local poultry are found wandering and the typical soil found at the sites of HPAI virus (H5N1) outbreaks. This sandy topsoil is acidic and poorly buffered, which explains the differences observed between our indirect pH measures and the direct measures reported in specialized literature (*10*). The soil's low pH may inactivate enveloped viral particles, as well as bacteria (online Technical Appendix Table).

In Cambodia, as in several other countries affected by HPAI virus (H5N1), decontamination of the environment after an outbreak is recommended by authorities; for example, disinfectants such as cresols are sprayed over environmental surfaces. However, because of resource limitations, only limited areas can be treated. Our results provide evidence that, even when abundantly contaminated, some soil types are unlikely to allow transmission of the virus to poultry and, consequently, probably not to other animals or to humans. These results suggest that limited resources could be better concentrated in high-risk areas, where the nature of the soils would be more likely to lead to poultry infection after natural contamination. These data may aid in the design of more cost-effective and solid-based decontamination measures for preventing transmission of HPAI virus (H5N1) to humans and animals.

Table. Clinical, virologic, and serologic results obtained from chickens exposed to soils experimentally contaminated with influenza virus (H5N1)*

Protocol	Sandy topsoil (rice fields)	Building sand	Soil-based compost
Low-dose contamination			
Clinical signs	None	None	None
Mortality rate	None	None	None
Virus in postmortem samples	NA	NA	NA
Seroconversion rate	None	33% at day 24	50% at day 24
High-dose contamination			
Clinical signs	None	None	None
Mortality rate	None	100% by day 4	100% by day 4
Virus in postmortem samples	NA	Yes	Yes
Seroconversion rate	None	NA	NA

*Low-dose contamination protocol, exposure of the chickens to soil inoculated with 1 infectious unit on day 0, 2 on day 6, 4 on day 12, and 8 on day 18. High-dose contamination protocol, exposure of the chickens to soil inoculated with 8 infectious units on day 0, 12 on day 6, 16 on day 12, and 20 on day 18. In each experiment, 10–20 chickens were exposed to contaminated soils. Each experiment was repeated twice. For each experiment, a control group was established (same number of chickens exposed to noncontaminated soils; no deaths were observed in control groups). NA, not applicable.

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Rickettsia raoultii–like Bacteria in *Dermacentor* spp. Ticks, Tibet, China

To the Editor: *Rickettsia raoultii* is an obligate intracellular gram-negative bacterium belonging to the spotted fever group (SFG) of the genus *Rickettsia*. Genotypes RpA4, DnS14, and DnS28, originally isolated from ticks from Russia in 1999 (*1*), were designated as *Rickettsia raoultii* sp. nov. on the basis of phylogenetic analysis (*2*). *R. raoultii* has been found mainly in *Dermacentor* spp. ticks in several countries in Europe (*3*). It was detected in a *Dermacentor marginatus* tick from the scalp of a patient with tick-borne lymphadenitis in France (*2*), which suggests that it might be a zoonotic pathogen. We determined the prevalence of *R. raoultii*–like bacteria in *Dermacentor* spp. in highland regions in Tibet.

Ticks from sheep (*Ovis aries*) near Namuco Lake (a popular tourist destination 4,718 m above sea level) were collected and identified morphologically as *D. everestianus* and *D. niveus* ticks (*4*). Genomic DNA was extracted from individual specimens by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). All DNA samples were amplified by using PCRs specific for the citrate synthase (*gltA*, 770 bp) gene (*5*) and the outer membrane protein A (*ompA*, 629 bp) gene (*6*). Some samples were amplified by using a PCR specific for the *ompB* (2,479 bp) gene (*7*).

Randomly selected amplicons for *gltA* (n = 27), *ompA* (n = 31), and *ompB* (n = 7) were cloned into the pGEM-T Easy vector (Promega, Shanghai, China) and subjected to bidirectional sequencing (Sangon Biotech, Shanghai, China). Sequences obtained were deposited in GenBank under accession nos. JQ792101–JQ792105, JQ792107, and JQ792108–JQ792166. Phylogenetic analysis was conducted for sequences we identified and sequences of recognized SFG rickettsial species available in Genbank by using the MegAlign program (DNASTAR, Inc., Madison, WI, USA) and MEGA 4.0 (*8*).

Of 874 tick specimens, 86 were *D. everestianus* ticks (13 male and 73 female), and 788 were *D. niveus* ticks (133 male and 655 female). Samples positive for *gltA* and *ompA* were considered SFG rickettsial species. Using this criterion, we found that 739 tick specimens (84.6%) were positive for *Rickettsia* spp. Of 86 *D. everestianus* ticks, 85 (98.8%) were positive for *Rickettsia* spp. and of 788 *D. niveus* ticks, 654 (83.0%) were positive. Infection rates for male and female *D. niveus* ticks were 87.9% and 82.1%, respectively. We found an overall prevalence of 84.6% for *R. raoultii*–like bacteria in *Dermacentor* spp. in the highland regions in Tibet.

Nucleotide sequence identities ranged from 99.2% to 100% (except for isolate WYG55, which had an identity of 98.6%) for the *ompA* gene and from 99.2% to 99.9% (except for isolate XG86, which had an identity of 98.5%) for the *ompB* gene. These results indicated that homology levels of most isolates were within species thresholds (*ompA* ≈98.8% and *ompB* ≈99.2%) (*9*). Isolate WYG55 showed the lowest identity (98.2%) among *gltA* gene sequences and the lowest identity (98.6%) among *ompA* gene sequences. Isolate XG86 showed lowest identity (98.5%) among *ompB* gene sequences. These results suggest that other *Rickettsia* spp. were among the investigated samples.

A BLASTn search (www.ncbi.nlm.nih.gov/) for the obtained sequences was conducted. The best matches (highest identities) detected were with sequences of *R. raoultii*. However, comparison of our sequences with corresponding sequences of *R. raoultii* in GenBank showed identity ranging from 98.0% to 99.0% for

ompA and from 98.1% to 99.0% for *ompB*, which did not meet the threshold (*9*) for *R. raoultii*. We compared the new sequences with corresponding reference sequences of universally recognized SFG group *Rickettsia* spp. in Genbank and constructed 2 phylogenetic trees (Figure). The new sequences were placed into separate branches, which were closely related to *R. raoultii* branches.

Prevalence of *R. slovaca* and *R. raoultii* was 6.5% and 4.5% in *D. silvarum* ticks in Xinjiang Uygur Autonomous Region of China (*10*). In contrast, we found that the overall prevalence of *R. raoultii*–like bacteria might be ≤84.6% in *D. everestianus* and *D. niveus* ticks in Dangxiong County in Tibet.

Our findings suggest that *D. everestianus* and *D. niveus* ticks are potential vectors of *R. raoultii*–like bacteria and indicate that spread of *R. raoultii*-like bacteria encompasses a large area in China. In the study sites, yak and Tibetan sheep are the major domestic animals, and rodents are the major wild animals. Rodents are also the major hosts of *Dermacentor* spp. ticks, which can transmit *R. raoultii* transstadially and transovarially (*2*). Animals bitten by infected ticks can acquire the pathogen and serve as natural reservoirs.

On the basis of phylogenetic analysis, we found that the *Rickettsia* spp. in ticks investigated represents a novel species, which can be designated *Candidatus* Rickettsia tibetani. However, additional phylogenetic studies are needed to obtain more information on the molecular biology of these bacteria.

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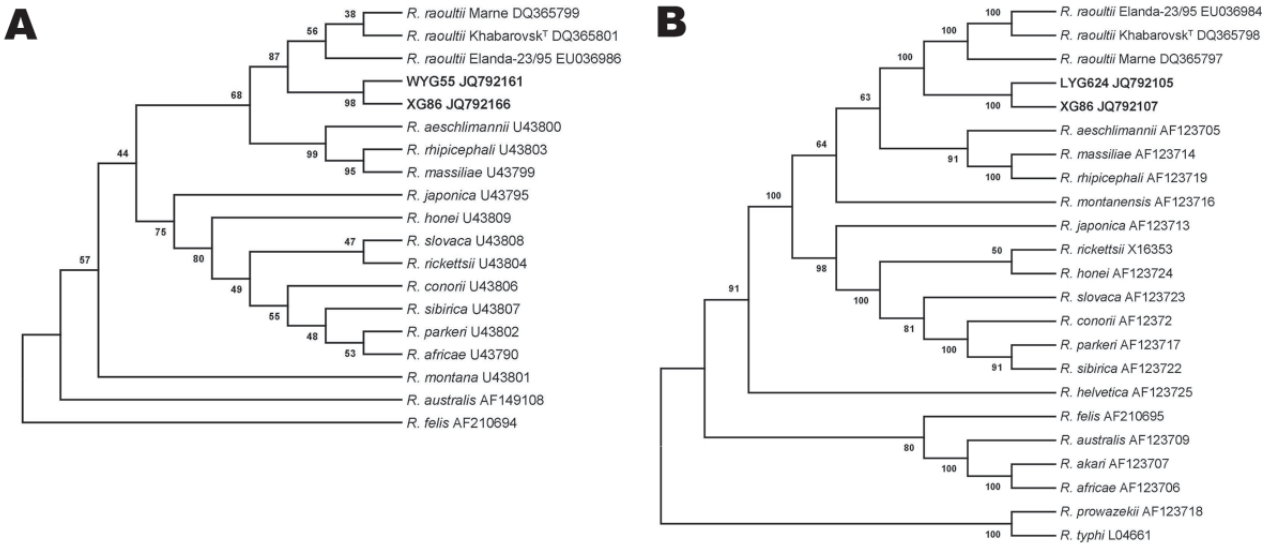

Figure. Unrooted phylogenetic trees inferred from comparison of A) outer membrane protein A (*ompA*) and B) *ompB* gene sequences of rickettsial species by using the neighbor-joining method. Sequences in **boldface** were obtained during this study. Numbers at nodes are the proportion of 100 bootstrap resamplings that support the topology shown.

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Leishmania (*Viannia*) *guyanensis* Infection, Austria

To the Editor: Infection with *Leishmania* spp. was diagnosed in an asymptomatic soldier during an explorative national cross-sectional serologic screening of soldiers volunteering for United Nations missions at the Military Hospital Vienna in 2009. Diagnosis was made by using a commercial ELISA kit (Ridascreen *Leishmania*; R-Biopharm, Darmstadt, Germany). One year later, the soldier was reassessed for persisting antibodies by using the same ELISA and for *Leishmania* DNA in a blood sample stored in EDTA by using the *Leishmania* OligoC-Test (Coris BioConcept, Gembloux, Belgium). (The study was approved by the Research Ethics Committee of the Austrian Armed Forces and written informed consent was obtained from the person investigated.) Because the results of both tests were positive, an additional PCR was performed for identification below genus level with the LITSR/L5.8S primer pair (*1*). To confirm the PCR results, we sequenced the amplicon in both directions in 2 independent setups and compared the obtained 299-bp sequence with published sequences from GenBank by performing a multiple sequence alignment. Our sequence (strain EN10) showed 100% (299/299 bp) identity with several strains from the *Leishmania (Viannia) guyanensis* complex, including the *L. guyanensis* strain MHOM/SR/87/TRUUS4 and the *L. panamensis* strains FJ948438, FJ948439, and FJ948446. Sequence homology to representatives of the *L. (V.) braziliensis* complex was ≈93%; to representatives of the *L. (Leishmania) mexicana* complex, 61%–68%; and to the *L. (L.) donovani* complex, 70%–71%. The *L. (V.) guyanensis* complex traditionally includes the species *L. guyanensis*, *L. panamensis,* and *L. shawi*, but *L. panamensis* seems to be a subspecies or even a synonym of *L. guyanensis* (*2*). We thus classified our strain as *L. guyanensis*. Sequence data were deposited at GenBank (accession no. JN671917).

L. guyanesis/panamensis is found in 9 countries in Central and South America (*3*). It is a common cause of zoonotic cutaneous leishmaniasis in humans. The sloths *Choloepus didactylus* (*L. guyanensis*) and *C. hoffmanni* (*L. panamensis*) are believed to be the principal reservoir hosts and the sandfly species *Lutzomyia umbratilis* (*L. guyanensis*) and *Lu. trapidoi* (*L. panamensis*) to be the principal vectors (*3*). Also, dogs can act as reservoirs for the *L. (V.) guyanensis* complex (*4*).

The infected soldier had never been to Central or South America and had no history of blood transfusions. His lifetime travel history included Italy, Spain, Greece, Germany, Croatia, New York City, and military assignments in Kosovo. Thus, how and where the infection had been acquired remain open for discussion.

Although sandflies are not as robust as *Anopheles* spp., for example, the most plausible scenario is that either an *L. guyanensis*–infected sandfly or a noninfected but transmissive sandfly from a disease-endemic area was transported in a ship or airplane (comparable to the well-known "airport malaria" situation) to an area where the patient had traveled. In recent years, *Lu. vexator* has become widespread and abundant in upstate New York (*5*). Although, this is not a known vector for *L. guyanensis*, its spread in New York State shows that *Lutzomyia* spp. can rapidly adapt to new and distant areas. Of the areas where the infected person had traveled, at least in New York City and Spain, regular introduction of *L. guyanensis* by immigrants, travelers, or dogs from Central and South America is very likely. Moreover, *Leishmania*

parasites are known to remain viable for a lengthy period in infected humans and animals and even in those that have received treatment.

However, alternative scenarios with other sandfly species, possibly even those found in Europe, acting as vectors cannot be totally excluded. Approximately 25 sandfly species are found in Europe, of which at least 6 are vectors for *Leishmania* spp. (*6*). Whether *L. guyanensis* can be transmitted by *Phlebotomus* sandfly species is unknown. When *L. infantum*, originally transmitted by *Phlebotomus* spp., was introduced from Europe to Central and South America in the post-Columbian era, it readily adapted to several vectors of the genus *Lutzomyia* (*3*). Adoption of new reservoir hosts and new vector species has also been observed in other species (*7*). Members of the *Leishmania* subgenus develop in the midgut, and representatives of the *Viannia* subgenus develop in the hindgut and the midgut. Nevertheless, several *Lutzomyia* species can transmit both, representatives of the *Viannia* and *Leishmania* subgenera. In general, most sandflies appear to be vector competent for >1 *Leishmania* spp. The New World species *Lu. longipalpis* and the Old World species *Ph. argentipes*, *Ph. arabicus*, *Ph. halepensis,* and *Ph. perniciosus* enabled the maturation of almost all *Leishmania* species tested under experimental conditions (*8*). The presence of sandflies in Austria was reported very recently (*9*), but the vector competence of the species found (*P. mascittii*) has still not been elucidated. Moreover, this finding likely reflects an increased population density rather than an introduction of a previously nonendemic species.

Nonvector transmission is also a possibility. The infected person did not remember ever having received blood products; however, transmission is generally possible by all forms of blood contact, including through needle sharing among persons who use injection drugs and through sexual intercourse (*10*).

The authors declare that there is no conflict of interest and that all experiments performed comply with the currents laws of Austria.

The study was supported by the Medical University of Vienna and by the Military Hospital Vienna, Austria. Results of the study were presented at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17–20, 2011, Chicago, Illinois, USA (abstract P-929).

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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.

Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo

To the Editor: Bats are hosts for various emerging viruses, including the zoonotic paramyxoviruses Hendra virus and Nipah virus, which occur in Australia and Southeast Asia, respectively, and cause severe disease outbreaks among humans and livestock (*1*). Antibodies and henipavirus-related RNA have also been found in the straw-colored fruit bat, *Eidolon helvum*, in Ghana, West Africa (*2*,*3*). These bats are a chief protein source for humans in sub-Saharan Africa and are therefore targeted by hunters (*4*,*5*). This practice raises special concern about the risk for virus transmission from bats to humans.

To investigate the risk of zoonotic disease emergence through hunting and preparation and consumption of bats, in October 2009, we obtained animals from local hunters. This meat was destined to be sold at markets in downtown Brazzaville, Republic of Congo. All bats were *E. helvum*, one of the most frequently hunted and traded fruit bat species in Africa (*4*,*5*). According to hunters, bats were captured with nets in an area near the capital (4°22′40″S, 15°06′27″E) during the night and collected in the morning. Animals were maintained in cages until they were sold alive in the market. For this study, living bats were brought to the National Laboratory in Brazzaville.

All animals appeared clinically healthy on arrival at the laboratory. Animals were euthanized, and samples were stored immediately in RNA or later in liquid nitrogen; additional organ samples were transferred into a 10% buffered formalin solution. Neither macroscopic pathologic changes nor histopathologic evidence for viral infection was found. A total of 339 samples collected from 42 bats were tested for paramyxovirus RNA by PCR targeting L-gene sequences of respirovirus, morbillivirus, and henipavirus (*6*). Fifteen samples from 11 individual bats yielded a product of the expected size of 494 bp. These amplicons were cloned and underwent Sanger sequencing. Virus load in tissue samples, as determined by use of specific real-time PCR, ranged from 1.1×10^2 to 3.4×10^4 copies per piece (≈0.3 cm^3). Four samples could not be quantified, probably because copy numbers were too low. Virus load in urine was 1.8×10^6 per mL. For 4 of the 14 positive samples, we gathered additional sequence information by using pan-*Paramyxovirinae* primers targeting the most conserved genomic region (*6*). Sequencing of the cloned urine sample resulted in 2 distinct sequences for each fragment. Details regarding positive samples and dataset composition are found in the Technical Appendix (wwwnc.cdc.gov/EID/pdfs/11-1607-Techapp.pdf).

In a phylogenetic tree, *Eidolon* paramyxovirus (EPMV) sequences are shown to form at least 3 distinct groups in the *Paramyxoviridae* family (Figure, Appendix, panel A, wwwnc.cdc.gov/EID/article/18/9/11-1607-F1.htm) and seem to be highly diverse compared with other paramyxovirus genera. At least 1 bat appeared to be infected with 2 different strains. Despite a geographic distance of >2,000 km among bats sampled, no spatial distinction was found between sequences from bats from Ghana and bats from the Republic of Congo. The same result can be seen when phylogenetic trees are built on the basis of the *Paromyxovirinae* fragment (Figure, Appendix, panel B). In both trees, henipaviruses cluster in between EPMV sequences. Because EPMV and henipaviruses originate from fruit bats, this finding is not surprising. All animals in this study originate from a single locality just outside Brazzaville, the capital of the Republic of Congo. *E. helvum* bats are one of the most abundant species of fruit bats in sub-Saharan Africa; they roost in large colonies comprising up to 1 million animals. Bats in this species migrate up to 2,500 km per year, probably following seasonal changes in food availability (*7*). The diversity of distinct EPMV lineages recovered by this study at a single site, and the variable clustering with sequences retrieved from animals in Ghana, demonstrate that different strains are exchanged over large distances by migratory *E. helvum* bats.

Humans are exposed to these paramyxoviruses primarily by 2 mechanisms: 1) through bushmeat hunting (using nets or shotguns), handling, and consumption; and 2) through environmental contamination with bat excretions and saliva. *E. helvum* bats frequently roost in the middle of cities, and viral load in bat urine has been shown to be high. In Bangladesh, humans became infected with Nipah virus after consuming palm sap contaminated by bat urine and saliva (*8*). Infection of domestic pigs in Ghana (*9*) might also be a result of contact with bat excreta, which is especially troubling because pigs have acted as amplifying hosts in previous Nipah virus outbreaks in humans (*10*).

Despite the substantial exposure suggested by this study, to our knowledge, no human infection associated with bat paramyxoviruses has been reported in Africa, and elevated numbers of deaths have not been observed in bat hunters. Nevertheless, the existence of isolated cases cannot be excluded because underreporting is widespread, and many cases are undiagnosed. Additional studies on virus-host ecology, along with clinical surveys of exposed persons (hunters, vendors, cooks, etc.), are required to assess the zoonotic risk of these viruses and, ultimately, diminish the threat of a novel paramyxovirus entering and spreading in human populations.

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Severe Measles, Vitamin A Deficiency, and the Roma Community in Europe

To the Editor: The Roma community in Europe is a subgroup of the Romani people, whose origins are in northern India and who have been known in English-speaking countries as "gypsies." Measles outbreaks, including severe cases, were reported in the European Roma community during 2008-2010 (*1*,*2*). We describe the potential roles of malnutrition and vitamin A deficiency as risk factors for severe measles in adults from this community.

In Europe, >25,000 cases of measles, more than half of which occurred in France, were reported during a 2011 outbreak (*3*). The exact proportion of measles cases occurring among the Roma community in France during the outbreak are not available (*2*). Measles epidemiology has changed; the disease now mainly affects children <1 year old and young adults, the latter of whom are mostly unvaccinated or have unknown vaccination status (*2*,*4*). Roma people in Europe experience some of the worst health conditions in the industrialized world: they live in overcrowded conditions and have limited access to prevention programs and to healthcare services (*2*,*5*). In such populations, deficiencies of vitamins, such as A, C, and E, have been reported (*6*). Vitamin A deficiency has been associated with severe cases of measles in children in developing countries (*7*,*8*). To date, we did not find published data associating vitamin A deficiency with severe measles among adults. We describe 6 adults from the Roma community in Marseille, France, who had measles and low levels of vitamin A; 2 of these persons had severe measles.

Case-patients 1 and 2 were men who were 21 and 25 years of age, respectively. They were admitted to North Hospital, Marseille, France, with typical signs of measles (fever, cough, and maculopapular rash). They had no medical history of serious illness, including no immunocompromising conditions, and no history of measles vaccination. For both patients, the diagnosis of measles was confirmed by the results of PCR performed on pharyngeal and urinary samples. In case-patient 1, acute meningoencephalitis developed, and he was transferred to the intensive care unit for 3 days. During his stay, the patient was found to have active viral hepatitis B. Case-patient 2 had the following signs and symptoms: abdominal pain and vomiting, severe hepatitis (serum transaminases level 10× higher than the upper reference limit; total bilirubin within reference range), and keratitis. Other causes of viral or bacterial hepatitis were ruled out by serologic testing, and the patient did not frequently drink alcohol.

Case-patients 1 and 2 had vitamin A deficiency with values of 0.31 mg/L and 0.2 mg/L, respectively, (reference range 0.5–0.8 mg/L). We measured vitamin A levels in blood samples from the next 4 consecutive hospitalized patients with measles, all of whom were from the Roma community. They did not have complications of measles, but were hospitalized for infection-control reasons. All 4 patients had low levels of vitamin A (0.16–0.34 mg/L) (Table). In case-patient 4, the blood level of retinol-binding protein was 0.026 g/L (normal range 0.02–0.05 g/L), confirming vitamin A deficiency. Vitamin A supplementation was administered intramuscularly as recommended by the World Health Organization (200,000 IU followed by a second dose the next day) to each patient (*8*). The 6 patients progressed to recovery. Low levels of vitamin A (0.36–0.46 mg/L) were also found for 2 other patients from the Roma community who did not have measles (Table).

Serum vitamin A concentrations do not always reflect total vitamin A stores (*9*). In severe protein–calorie malnutrition, and during intercurrent infection, serum retinol levels could be artificially low in relation to a decrease in retinol-binding protein level (*9*). However, the diagnosis of vitamin A deficiency is usually supported by low levels of serum vitamin A and levels of retinol-binding protein within the reference range as described for at least 1 of the case-patients reported here.

Vitamin A deficiency affects the severity of illness and the rate of deaths associated with measles, and it is known to induce severe measles-related complications in children, delaying recovery and promoting xerophthalmia, corneal ulcer, and blindness (*7*,*8*,*10*). Acute measles precipitates vitamin A deficiency by depleting vitamin A stores and increasing its utilization, leading to more severe ocular injury (*7*,*8*). Vitamin A supplementation given to children with measles has been associated with better outcomes (*7*,*8*). Although it is too early to associate vitamin A deficiency with severe measles in adult patients, such an association is possible. Adults with low levels of vitamin A but not infected with measles could be at higher risk for more severe disease if they become infected with the virus.

We conclude that all adults who have measles should be assessed for vitamin A and retinol-binding protein levels and should be considered for vitamin A supplementation, as are children (*8*). A prospective case-control study assessing vitamin A deficiency in the Roma adult community is necessary to assess its consequences on measles outcome. Aside from preventing complications among the Roma people, improving vaccine coverage in this nomadic population is crucial for reducing measles virus circulation among the general population.

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Table. Characteristics of 6 measles case-patients and 2 control patients with vitamin A deficiency, Roma community, France*

Participant	Age, y/ sex	Signs and symptoms	Defining characteristics	Vitamin A level, mg/L†	Outcome	No. days in hospital
Case-patient 1	21/M	Rash, meningo-encephalitis	Positive PCR results for pharyngeal and urinary samples	0.31	Recovered	12
Case-patient 2	25/M	Rash, hepatitis, keratitis	Positive PCR result for pharyngeal and urinary samples	0.20	Recovered	4
Case-patient 3	26/M	Rash	Positive PCR result for pharyngeal sample	0.27	Recovered	2
Case-patient 4	22/M	Rash	Positive PCR for result urinary, nasal, and pharyngeal samples; Positive serologic test result	0.27	Recovered	3
Case-patient 5	15/M	Rash	Positive PCR result for nasal sample	0.16	Recovered	1
Case-patient 6	17/M	Rash, hepatitis, Koplik spots	Clinical signs	0.34	Recovered	2
Control 1	34/F	None	NA	0.46	NA	NA
Control 2	12/M	None	NA	0.36	NA	NA

*Control participants did not have measles. NA, not applicable.
†Reference range 0.5–0.8 mg/L.

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Picobirnaviruses in the Human Respiratory Tract

To the Editor: Picobirnaviruses (family *Picobirnaviridae*) are nonenveloped, double-stranded RNA viruses of vertebrates with a bisegmented genome. Segment 1 (2.2–2.7 kb) encodes the capsid protein, and segment 2 (1.2–1.9 kb) encodes the RNA-dependent RNA polymerase. On the basis of sequence diversity in segment 2, picobirnaviruses are classified into 2 genogroups (*1*–*4*). Picobirnaviruses have been detected in fecal samples from humans with and without gastroenteritis; in patients co-infected with known enteric pathogens, including rotaviruses, caliciviruses, and astroviruses (*1*,*4*); and in a wide range of animals, such as pigs, calves, dogs, monkeys, and snakes. The pathogenicity of picobirnaviruses largely remains to be determined, but studies in immunocompromised persons suggest that picobirnaviruses may be opportunistic enteric pathogens (*5*,*6*).

Recently, we identified picobirnaviruses in the respiratory tract of pigs in Asia, and this identification expanded the knowledge on the tropism and host range of picobirnaviruses (*7*). No respiratory or other clinical signs were observed in these pigs at the time of sampling, making it unclear whether picobirnaviruses are indeed respiratory pathogens (*7*). To determine whether picobirnaviruses could also be present in the human respiratory tract, we performed a diagnostic genogroup I picobirnavirus PCR, with degenerated primers, that targeted the RNA-dependent RNA polymerase coding region (*1*,*4*,*8*) on 309 bronchoalveolar lavage specimens collected from 309 patients with respiratory disease of unknown origin in the Netherlands during 2003–2006. (All study procedures were performed in compliance with relevant laws and institutional guidelines and in accordance with the Declaration of Helsinki.)

Samples from 3 patients were confirmed by sequencing to be positive for genogroup I picobirnaviruses. To determine genetic relationships between human genogroup I picobirnaviruses from the respiratory tract and genogroup I picobirnaviruses detected in wastewater and in human and porcine fecal samples, we constructed a phylogenetic tree on the basis of a ≈165-nt fragment of the RNA-dependent RNA polymerase gene as described (*8*) (Figure, Appendix, wwwnc.cdc.gov/EID/article/18/9/12-0507-F1.htm). Before tree construction, 75 groups were created from the ≈300 available picobirnavirus sequences by using FastGroup II (*10*). Because the average pair-wise Jukes-Cantor distance was 0.46, a neighbor-joining tree was created by using the Jukes-Cantor model, with a bootstrap replication of 1,000 (Figure). One of the 3 genogroup I picobirnavirus sequences found in this study, PBVI/Homo sapiens/VS2000057/2003, showed <95% sequence identity with previously described picobirnavirus sequences and is shown as a separate branch in the phylogenetic tree. The genogroup I picobirnavirus nucleotide sequences from the respiratory tracts of persons in the Netherlands showed 58% to 97% similarity with each other. They belonged to different phylogenetic clades and did not group with other picobirnaviruses according to year of isolation or host species.

In conclusion, the identification of new picobirnaviruses in respiratory tract samples from pigs (*7*) prompted us to look for the presence of picobirnaviruses in the respiratory tracts of humans. Genogroup I picobirnaviruses could be identified in some of the bronchoalveolar lavage specimens obtained from patients with unexplained respiratory disease in the Netherlands. This observation expands our knowledge of picobirnaviruses in humans

and provides a clear example of how epidemiologic baseline information on virus host range and tropism in animals may provide indications for the presence of similar viruses in the same organ system of humans. To clarify the epidemiology and pathogenicity of picobirnaviruses in humans, additional surveillance should be carried out in persons with and without respiratory and enteric disease.

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New Delhi Metallo-β-Lactamase 4–producing *Escherichia coli* in Cameroon

To the Editor: The metallo-β-lactamase (MBL) group of enzymes inactivates many β-lactam antimicrobial drugs. First identified from a *Klebsiella pneumoniae* strain recovered from a patient hospitalized in India, the New Delhi metallo-β-lactamase-1 (NDM-1), particularly in *Enterobacteriaceae*, is now the focus of worldwide attention (*1*). Whereas India and Pakistan were considered as the main reservoirs of the $bla_{\text{NDM-1}}$ gene (*2*) that produces this MBL, several NDM-1–producing *Enterobacteriaceae* isolates have been reported from the Balkan states and the Middle East, suggesting that those areas might be secondary reservoirs (*2*).

Since 2010, 3 NDM-1 point-mutation variants have been described (*3*–*5*). The first variant, NDM-2, was identified from an *Acinetobacter baumannii* isolate collected from a patient transferred from a hospital in Egypt to Germany (*4*). Subsequently, a clonal dissemination of NDM-2–producing *A. baumanni* was described in Israel (*6*). The second variant, NDM-4, which was identified in *Escherichia coli* from a patient hospitalized in India, possessed a higher carbapenemase activity compared with NDM-1 (*5*). The most recent variant, NDM-5, was identified in *E. coli* from a patient who had a history of hospitalization in India (*3*).

As recommended for the detection of carbapenemase producers (*7*), a rectal swab specimen was collected from a patient transferred from Cameroon to France. The *E. coli* strain FEK was isolated from the specimen.

He had been hospitalized for 1 month in Douala for an inflammatory syndrome associated with a kidney failure before his transfer to Paris. No history of travel in India was reported for this patient. Susceptibility testing was performed by disk diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France), and results were interpreted according to the updated guidelines of the Clinical and Laboratory Standards Institute (Wayne, PA, USA; www.clsi.org). The MICs were determined by using Etest (bioMérieux, La Balmes-Les-Grottes, France) on Mueller-Hinton agar at 37°C.

E. coli FEK was fully resistant to all β-lactam antimicrobial drugs, including imipenem, meropenem, ertapenem, and doripenem (MICs >32 mg/L for all carbapenems). This isolate was also resistant to aminoglycosides, except amikacin, and to fluoroquinolones. We performed PCR amplification followed by sequencing on whole-cell DNA, as described (*8*). We identified the bla_{NDM-4}, $bla_{CTX-M-15}$, and bla_{OXA-1} genes. *E. coli* FEK also harbored the *aacA4* gene encoding the AAC(6′)-Ib acetyltransferase that confers high-level resistance to aminoglycosides, except amikacin. Results of multilocus sequence typing analysis performed as described (*5*) showed that the isolate belonged to sequence type (ST) ST405. Identification of this ST type among NDM-producing *E. coli*, compared with NDM-4– and NDM-5–producing *E. coli* in ST648, demonstrated that the spread of NDM-4 occurred among unrelated *E. coli* clonal backgrounds (*3*,*5*).

Plasmid DNA of *E. coli* FEK was extracted and analyzed as described (*5*). A single, ≈120-kb plasmid was identified. Direct transfer of the β-lactam resistance marker into *E. coli* J53 was attempted by liquid mating-out assays at 37°C. With the exception of the aminoglycoside amikin, transconjugants from *E. coli* were resistant to β-lactam antimicrobial drugs. MICs of imipenem, meropenem, ertapenem, and doripenem were 6, 3, 6, and 4 mg/L, respectively. The transconjugants harbored an 120-kb plasmid carrying bla_{NDM-4} and the $bla_{CTX-M-15}$, bla_{OXA-1}, and *aacA4* genes. We performed PCR-based replicon typing as described (*5*) and showed that this bla_{NDM-4}–positive plasmid belonged to the IncFIA incompatibility group. The IncF incompatibility group was previously reported to be associated with bla_{NDM-4} and bla_{NDM-5} (*3*,*5*).

By analyzing genetic structures surrounding the bla_{NDM-4} gene, performed by PCR mapping as described (*8*), we identified insertion sequence IS*Aba125* upstream and the bleomycin resistance gene ble_{MBL} downstream of the bla_{NDM-4} gene. The same genetic environment has been observed for most NDM-1–positive enterobacterial isolates (*8*). We showed in previous research that expression of ble_{MBL} conferred high-level resistance to bleomycin and bleomycin-like molecules (*9*); accordingly, the *E. coli* clinical isolate and its transconjugant were highly resistant to bleomycin (MIC >512 μg/mL) (*9*).

The patient had a history of Hodgkin lymphoma treated by 8 sessions of bleomycin chemotherapy 1 year before his hospitalization. This anticancer drug is widely distributed throughout the body following intravenous administration, and plasmatic concentrations increase in proportion with the increase of the dose (*10*). Because the patient was successively treated with 30 mg of bleomycin, the serum levels achieved (≈2–5 mg/mL) might have contributed to selection of the ble_{MBL} gene. Similarly, the multiple courses of antibacterial drug therapy administered in Cameroon (including carbapenems) could have contributed to selection of the bla_{NDM-4} gene.

By culturing rectal swab samples from the patient, we identified fecal carriage of *E. coli* carrying a plasmid-encoded bla_{NDM-1} gene. That strain had a distinct ST type (ST5) compared with the index strain. The plasmid carrying the bla_{NDM-1} gene with the bla_{OXA-1} and *aacA4* genes belonged to the IncFIA incompatibility group.

β-Lactamase NDM-4 displaying increased carbapenemase activity compared with NDM-1 was described in a patient hospitalized in India (*5*). This study shows that NDM-4 producers are also present in Africa; specifically, in the highly populated city of Douala, providing an environment that may promote the dissemination of those strains. We showed that the same patient was carrying strains expressing 2 NDM variants**,** possibly indicating ongoing evolution of NDM variants.

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Salmonella enterica Serovar Agbeni, British Columbia, Canada, 2011

To the Editor: Infection with *Salmonella enterica* serovar Agbeni is rare. In Canada, it was reported 8 times during 2000–2010 and never in the province of British Columbia (2011 population 4.5 million) (Public Health Agency of Canada, unpub. data). In June 2011, an outbreak of *S. enterica* ser. Agbeni affecting 8 persons was identified in British Columbia; pulsed-field gel electrophoresis patterns for all isolates were identical. Although no specific source was identified, 2 features were noted: 1) diagnosis through urine specimens for 3 of 8 persons and 2) a longer than typical incubation period for *Salmonella* spp. infection.

In British Columbia, public health authorities interview all reported *Salmonella* spp.–infected persons by using a standard questionnaire (www.bccdc.ca/discond/CDSurveillanceForms) to collect information about potential exposures during the 3 days before date of illness onset. Seven of the ill persons in British Columbia had attended the same wedding on May 14, 2011, which was outside the 3-day period about which they were asked. The person with the earliest reported case (May 16) was not associated with the wedding or with the other ill persons.

We reviewed wedding food sources and preparation. The 7 persons with wedding-associated illness were reinterviewed by using a menu-specific questionnaire; no obvious food source was implicated. The first wedding guest to be reported with enteric symptoms was visiting from outside British Columbia and had assisted with food preparation. In April and May 2011, five persons from the same jurisdiction outside British Columbia in which this wedding guest resided were identified with *S. enterica* ser. Agbeni infection; isolates from these persons had the same pulsed-field gel electrophoresis pattern as those in British Columbia. Also, the ill person who was not associated with the wedding had traveled to that same jurisdiction before onset of symptoms. The original source of infection was probably outside of British Columbia.

Average age of the 8 ill persons was 52.8 years (range 21–82 years). Six were men. One person reported hospital admission. No underlying conditions were documented in any of the 8 ill persons.

Culture results of urine samples were positive for 3 (38%) of the 8 ill persons; feces were not tested. All 3 persons had symptoms of urinary tract infection (UTI), and 2 had fever. All were men and were the oldest persons reported. Two had gastrointestinal (GI) symptoms before UTI symptoms. For 1 person, the interval between onset of GI and UTI symptoms was 15 days.

Approximately 1% of nontyphoidal *Salmonella* spp. infections are detected in urine (*1*,*2*). In British Columbia, ≈3% of *Salmonella* isolates submitted to the reference laboratory are isolated from urine (British Columbia Centre for Disease Control's Public Health Microbiology and Reference Laboratory, unpub. data). *Salmonella* spp. are more often recovered from urine in adults >60 years of age, children (*2*,*3*), and female patients (*2*,*4*). Immunocompromising conditions and urinary tract structural abnormalities also are risk factors for isolating the organism in urine (*2*,*3*). Also, certain *Salmonella* serogroups or serotypes are more likely than others to be isolated from urine (*2*,*3*). GI symptoms concurrent with or preceding UTI are rare (*4*,*5*). We found no literature to suggest whether *S. enterica* ser. Agbeni is more likely to cause systemic illness or UTI. The

only risk factor identified among the persons reported here was older age. Unlike persons in other reports, persons in our report were all men, and 2 reported GI symptoms. The mechanism for UTI in these cases is unclear but could have included ascending and hematogeneous spread.

We calculated incubation periods for GI symptoms for 6 persons as the time between onset of GI symptoms and the May 14 wedding (5 persons) or last travel date (1 person). The incubation period was 5–7 days (average 5.5 days). The incubation period for UTI, which could be calculated for 2 persons, was an average of 25.5 days. Long incubation periods for *Salmonella* spp. infections have been reported (*6*–*9*); reasons include exposure to a low dose of bacteria, specific populations (e.g., young children, child day care attendees), and method of food preparation (*6*–*9*). The age of persons in our investigation did not affect the length of the incubation period. The amount of food eaten was not collected during the interview; however, most persons in our investigation reported eating a wide variety of foods, and 1 reported eating small portions. All food was prepared during the week before the wedding and served cold. This length of time and the potential for temperature abuse could have increased the infectious dose and decreased the incubation period (*6*). In addition, the 1 person with travel-related infection was not exposed to these food items. We found no literature on the incubation period for *S. enterica* ser. Agbeni. The reason for the long incubation period in this investigation is unclear and could be due to host-specific factors, the implicated serotype, or the food source.

The 3-day time frame for exposures was not sufficient to identify appropriate exposures. Expanding the period for collecting exposure information about *Salmonella* spp. infections and the reporting and investigation of persons with *Salmonella* spp. identified in urine to public health authorities might be needed to help identify and solve outbreaks.

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Entamoeba bangladeshi nov. sp., Bangladesh

To the Editor: Diarrheal diseases have a major effect on global health, particularly the health of malnourished children (*1*). The enteric parasites *Entamoeba histolytica* and *E. moshkovskii* are potential causes of diarrheal disease in children (*2*). For the past 20 years, we have been studying *Entamoeba* infections in children from the urban slum of Mirpur in Dhaka, Bangladesh (*3*).

E. histolytica infections can be detected through fecal microscopy, culture, PCR, and antigen detection. Microscopy and culture have limited specificity because several species of *Entamoeba,* which vary in their pathogenic potential, have morphologically similar cysts and trophozoites (*4*). In 2010–2011,

during analysis of feces positive for *Entamoeba* organisms by microscopy or culture but negative for *E. histolytica*, *E. dispar*, and *E. moshkovskii* by PCR, a new species was identified, which we have named *Entamoeba bangladeshi* nov. sp. in recognition of the support of the Bangladesh community for this research.

Feces from both diarrheal and surveillance specimens were collected from a cohort of children living in Mirpur (*3*). A total of 2,039 fecal samples were examined microscopically (0.9% saline smear) and/or by fecal culture for amebic trophozoites and cysts (*4*). One hundred forty-nine (7%) of the samples were positive by microscopy and/or culture for an *Entamoeba* parasite with both cysts and trophozoites that closely resembled those of *E. histolytica*, *E. moshkovskii*, and *E. dispar*.

DNA was extracted directly from fecal samples by using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA from positive fecal cultures was isolated by using the cetyl-trimethylammonium bromide extraction method (*5*). PCR was conducted to detect *E. histolytica*, *E. dispar*, and *E. moshkovskii*, all of which are morphologically indistinguishable by microscopy and are endemic to Bangladesh (Table) (*6*–*9*). An antigen detection test (TechLab Inc., Blacksburg, VA, USA) was also used to identify fecal samples positive for *E. histolytica*.

Fecal samples (129) and cultures derived from fecal material (20) were tested by PCR. Forty-four fecal samples were positive for *E. histolytica*, 42 for *E. dispar*, and 7 for *E. moshkovskii*. PCR results for 48 samples were negative for all 3 parasites (mixed infections account for the total being >129); 5 cultures also were negative for all 3 parasites.

ENTAGEN-F and ENTAGEN-R primers, which exhibit a broad specificity for the small subunit ribosomal RNA (SSU rRNA) gene sequences of *Entamoeba*, were used in PCR to amplify DNA fragments from 43 of the samples that were negative by PCR for the 3 *Entamoeba* species; amplification conditions were adapted from Stensvold et al. (*10*). The amplified DNA was separated by electrophoresis by using a 2% agarose gel. Bands of the size predicted for the *Entamoeba* spp. SSU rRNA gene amplicon were detected in 15 samples (online Technical Appendix Table, wwwnc.cdc.gov/EID/pdfs/12-0122-Techapp.pdf). The PCR products were extracted by using the QIAquick Gel Extraction Kit (QIAGEN) and cloned by using the Zero Blunt TOPO Cloning Kit (Invitrogen, Carlsbad, CA, USA). The sequenced clones from 2 different isolates, 1 diarrheal and 1 surveillance specimen, were completely novel when compared with the SSU rRNA gene sequences from other organisms and did not match any previously sequenced *Entamoeba* species. These isolates represent a new species of *Entamoeba* (GenBank accession nos. JQ412861 and JQ412862), here named *E. bangladeshi* (online Technical Appendix)

We examined the phylogenetic relationship between *E. bangladeshi* and other *Entamoeba* parasites by using maximum-likelihood analysis as implemented in MEGA 5 (online Technical Appendix Figure, panel A). *E. bangladeshi*, although distinct, clearly grouped with the clade of *Entamoeba* infecting humans, including *E. histolytica*. *E. bangladeshi*, however, appeared more distantly related than the noninvasive *E. dispar*, but closer than *E. moshkovskii,* to *E. histolytica*.

To further characterize *E. bangladeshi,* we established it in xenic culture, and it displayed the ability to grow at 37°C and 25°C, a characteristic shared with *E. moshkovskii* and *E. ecuadoriensis* but that distinguishes it from *E. histolytica* and *E. dispar*. Cultured trophozoites were evaluated through light and transmission electron microscopy (online Technical Appendix Figure, panel B). By light microscopy, we detected no apparent differences between *E. bangladeshi* and *E. histolytica*. The physical resemblance between *E. histolytica* and *E. bangladeshi* is notable because direct microscopic examination of fecal samples is still used as a diagnostic tool in areas to which these species are endemic to detect *E. histolytica* parasites.

Table. Oligonucleotide primers used for screening and sequencing of *Entamoeba bangladeshi* nov. sp., Bangladesh*

Target organism	Primer name	Primer sequence, 5′ → 3′	Reference
Broad specificity *Entamoeba* sp.	Entagen-F	ACT TCA GGG GGA GTA TGG TCA C	(*6*)
	Entagen-R	CAA GAT GTC TAA GGG CAT CAC AG	(*6*)
E. histolytica	Eh-F	AAC AGT AAT AGT TTC TTT GGT TAG TAA AA	(*8*)
	Eh-R	CTT AGA ATG TCA TTT CTC AAT TCA T	(*8*)
	Eh-YYT Probe	**YYT-**ATT AGT ACA AAA TGG CCA ATT CAT TCA**-Dark Quencher**	(*8*)
E. moshkovskii	Em-1	CTC TTC ACG GGG AGT GCG	(*7*)
	Em-2	TCG TTA GTT TCA TTA CCT	(*7*)
	nEm-1	GAA TAA GGA TGG TAT GAC	(*7*)
	nEm-2	AAG TGG AGT TAA CCA CCT	(*7*)
E. dispar	E-1	TTT GTA TTA GTA CAA A	(*9*)
	E-2	GTA [A/G]TA TTG ATA TAC T	(*9*)
	Ed-1	AGT GGC CAA TTT ATG TAA GT	(*9*)
	Ed-2	TTT AGA AAC AAT GTT TCT TC	(*9*)

*__Boldface__ indicates the probe fluorophore and quencher.

Our findings add to the diversity of *Entamoeba* species found in humans. The incidence and effect of infection in infants by the newly recognized species *E. bangladeshi* await future epidemiologic studies.

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Autochthonous *Leishmania siamensis* in Horse, Florida, USA

To the Editor: *Leishmania siamensis*, a recently described species, was identified as the cause of autochthonous visceral leishmaniasis in 2 men in southern Thailand (*1*,*2*). Cutaneous leishmaniasis has been reported in horses in Europe and South America. Lesions in horses are solitary or multiple nodules that are often ulcerated and most commonly occur on the head, pinnae, legs, and neck. Other clinical signs are usually absent. In South America, biochemical characterization has identified *L. braziliensis* in horses (*3*). Leishmaniasis has been reported in horses in Puerto Rico (*4*), and equine leishmaniasis has been described, but no reports have been published, in the United States. *L. infantum* has been reported in equine cutaneous leishmaniasis in Europe (*5*). A report from central Europe recently identified an organism with 98% nucleotide identity over the ITS (internal transcribed spacer) 1 region to *L. siamensis* as the cause of cutaneous leishmaniasis in 4 horses (*6*). *L. siamensis* was also identified in a case of cutaneous bovine leishmaniasis in Switzerland (*7*).

In August 2011, a 10-year-old, 505-kg Morgan horse mare in Florida, USA, with no history of travel outside the eastern United States was evaluated at the University of Florida for an ulcerated mass in the left pinna. When, 6 months earlier, the owner had noticed the mass, it was ≈1 cm in diameter, firm, raised, and covered with hair. Three months later, the ear was unchanged, and the mare was successfully impregnated. Over the subsequent 2 months, the mass began to grow and ulcerate. At that time, veterinary consultation was obtained and a biopsy performed. Histologic

study showed that the dermis was hyperplastic and diffusely infiltrated with neutrophils, macrophages, and lymphocytes (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/18/9/12-0184-Techapp.pdf). Numerous intracytoplasmic protozoal organisms with a small nucleoid and smaller kinetoplast most consistent with *Leishmania* sp. were observed in macrophages. No treatment was pursued. After 45 days, the mare was seen at the University of Florida because of progression of the lesions. The mass on the internal aspect of the pinna was 6 cm × 3 cm and ulcerated, and 3 new firm 1 cm–diameter nodules were observed on the outer pinna of the same ear. Multiple soft, less-defined, 1 cm to 3 cm–diameter nodules were observed along both sides of the neck, shoulders, and withers. No other abnormalities were observed on physical examination or thoracic and abdominal ultrasound, and lymph nodes were not enlarged. Ultrasound confirmed a ≈90 day viable pregnancy. Complete blood count and plasma chemistry were within normal limits.

Tissue aspirates were taken of the multiple ear lesions and of the nodules along the neck and shoulder. From the ulcerated lesion, marked mixed, predominantly neutrophilic inflammation was seen, and rare neutrophils and macrophages contained intracellular protozoal organisms consistent with *Leishmania* sp. amastigotes (online Technical Appendix Figure 2). These organisms were 4–5 μm in diameter and round with pale basophilic cytoplasm. They had an eccentrically placed, basophilic, oval nucleus and a small, basophilic, rod-shaped kinetoplast oriented perpendicular to the long axis of the oval nucleus. No organisms were seen in any other aspirates.

Fresh tissue was submitted for PCR, which has been determined suitable for detecting Old World leishmaniasis in dogs (*8*). Results were negative. Given the clear clinical, cytologic, and histologic evidence for cutaneous leishmaniasis, additional consensus PCR was performed as described (*9*), targeting the ITS1 region. Direct sequencing was performed by using the BigDye Terminator Kit (Applied Biosystems, Foster City, CA, USA) and analyzed on ABI 3130 automated DNA sequencers (Applied Biosystems) at the University of Florida Interdisciplinary Center for Biotechnology Research Sequencing Facilities (Gainesville, FL, USA). The resultant sequence was 310 bp after primers were edited out. Sequence alignment yielded a genotype with 99% identity to the first *L. siamensis* isolate (GenBank accession no. EF200012) and 100% identity to 2 more recently submitted sequences from human visceral leishmaniasis isolates from Thailand (GenBank accession nos. JQ001751 and JQ001752) (online Technical Appendix). The sequence was submitted to GenBank (accession no. JQ617283).

The mare delivered a stillborn foal at 350 days' gestation. Histopathology did not reveal any infectious organisms in the fetal tissues; however, the chorioallantois showed moderate villous atrophy, which was presumed to be the cause of fetal death. One month after foaling, the mare's cutaneous lesions were 90% resolved.

Because the mare in this report was born in the United States and had never left the country, this case appears to be autochthonous. Mode of transmission is unknown. Phlebotomine sand flies found in Florida include *Lutzomyia shannoni*, *Lu. cubensis*, *Lu. vexator*, and *Lu. cruciata*. *Lutzomyia* sp. are competent vectors of *Leishmania* spp. in other areas of the world. However, the vector for reported cases of *L. siamensis* in other regions has not been identified. Although leishmaniasis is infrequently diagnosed in any species in Florida, models have shown that with climate change, the range of sand flies and accompanying leishmaniasis in North America is expected to expand substantially (*10*). This report raises many avenues for further investigation: the prevalence of leishmaniasis in horses in the United States, understanding of the life cycle and vectors, and the zoonotic potential of this *Leishmania* species.

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Novel Vectors of Malaria Parasites in the Western Highlands of Kenya

To the Editor: The primary malaria control techniques, indoor application of residual insecticides and insecticide-treated bed nets, are used on the basis of previously assumed key characteristics of behaviors of vectors of malaria parasites, i.e., resting and feeding indoors (*1*). Any deviation from the typical activities of a species related to exophagy (feeding outdoors) and exophily (living and resting indoors) (*2*) or to population replacement, followed by increased outdoor biting or resting (*3*), may undermine malaria control efforts. Identification of mosquitoes that transmit malaria parasites has, for the most part, relied on the use of outdated morphologic keys (*4*,*5*) and, more recently, species-diagnostic PCR (*6*). Cryptic species or subpopulations that exhibit divergent behaviors (*7*) may be responsible for maintaining malaria parasite transmission, and without adequate discriminatory techniques, these vectors may be misidentified and their key behavioral differences overlooked.

We evaluated indoor and outdoor trapping methods for anopheline mosquitoes in Bigege village, in Kisii Central District in the highlands of western Kenya, which are prone to periodic malaria epidemics. During May–August 2010, we captured 422 female *Anopheles* spp. mosquitoes, primarily from indoor and outdoor light traps. Of these, we identified 161 (38.2%) as species previously described as vectors in the area (*An. gambiae* sensu latu, *An. funestus* s.l., or *An. coustani* [*8*]) by using the standard morphologic key for sub-Saharan species (*4*). We identified another 52 (12.3%) as species not associated with malaria parasite transmission (*1*), but 209 (49.5%) could not be definitively identified. We extracted DNA from 418 mosquitoes and analyzed it for sibling species of the *An. gambiae* complex by using a diagnostic PCR (*6*). Of the 418 DNA samples tested, 80 (19.1%) were identified as *An. arabiensis*; 2 specimens were identified as *An. gambiae* s.s. (0.4%) but the remaining 336 (80.3%) could not be identified by PCR because no amplification product was observed.

To identify these specimens further, we performed molecular characterization by sequencing the ribosomal second internal transcribed spacer (ITS2) and the mitochondrial CO1 loci. Of the 422 female *Anopheles* mosquito specimens, we sequenced DNA from 348, of which 74 (21.3%), 33 (9.5%), and 25 (7.2%) corresponded to GenBank sequences of *An. arabiensis*, *An. coustani*, and *An. funestus* mosquitoes, respectively. However, 216 (62.1%) could not be matched (<90% identity) to any of the 224 ITS2 or 164 CO1 published sequences of anopheline vectors or nonvectors. These 216 specimens could be grouped into several separate clades, distinct from known vectors in the area (Figure). Specimens were grouped by ITS2 sequence. These groups were ranked by abundance and arbitrarily named species A-J. Of the 348 sequenced DNA specimens, the most abundant group having identical but novel ITS2 and CO1 sequences (species A, n = 147, 42.2%) could not be matched definitively to a single species by using the morphologic key. The mosquitoes in this group were most frequently caught outdoors (132, 89.8%). For 64 of a total of 192 traps, collections were made every 2 hours between 6:30 PM and 6:30 AM for 64 nights. Of 30 specimens of species A from these collections, 22 (73.3%) were caught outdoors before 10:30 PM. Data we have collected on human sleeping patterns from this area suggest that a significant proportion of the population is still outdoors before 10:30 PM and therefore exposed to these vectors.

Five of 293 mosquitoes tested had ELISA results positive for *Plasmodium falciparum* sporozoites. All 5 had no previously published ITS2 or CO1 sequences, nor could they be identified by morphologic features. All were collected outdoors. Four of the 5 were in the sequence A group (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/12-0283-Techapp.pdf), and 1 belonged to species I (Figure). The sporozoite

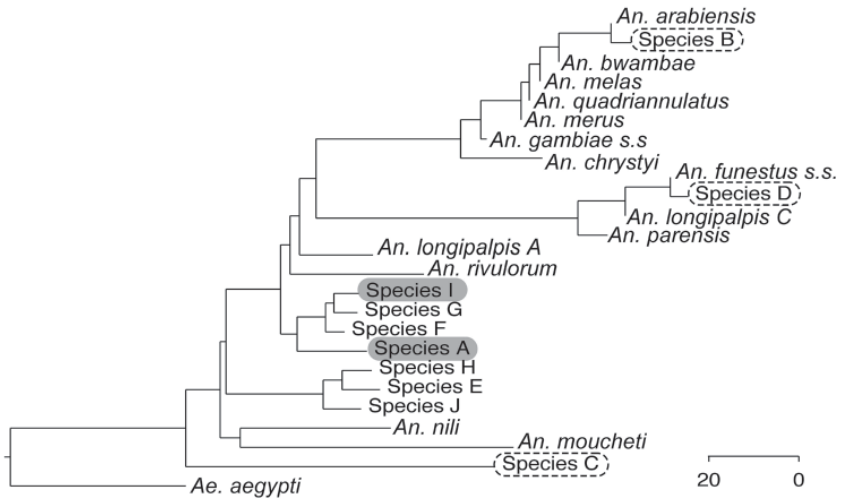

Figure. Phylogenetic tree of sequence group consensuses with National Center of Biotechnology Information reference sequences for *Anopheles* spp. mosquitoes caught in 2010 in Kisii District, Nyanza Highlands, western Kenya. Sequence groups of caught specimens arbitrarily named species A to J are ranked by abundance. Gray highlighting indicates study samples with sporozoites; dashed circles around text indicate study samples that match known African vectors. Scale bar represents nucleotide substitutions per 100 residues.

rate of 3% in species A was similar to that observed for other predominant anopheline vectors in the area (*8*).

Since the publication of the most widely available morphologic key (*4*), 15 new anopheline species have been discovered, for which test results for 1, *An. ovengensis* from Cameroon, were confirmed to be positive for sporozoites (*9*). The unidentified mosquitoes in the current study did not match the morphologic descriptions of any of the more recently identified species. These results demonstrate the presence of outdoor-active, early-biting potential malaria parasite vectors not previously described in western Kenya. The outdoor activity of these mosquitoes could lead to the failure of current indoor-based interventions to control this species, and this species could therefore contribute to malaria parasite transmission in the area. These findings highlight the value of the use of characteristics of local *Anopheles* spp. populations, including their behavior, based on morphologic features and DNA analysis, to accurately determine whether the species is contributing to malaria parasite transmission. This knowledge is essential for implementation of appropriate, and therefore successful, malaria control interventions.

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Paul Jacoulet (1902–1960) *Le Trésor* (*Corée*) Japan, 20th century Ink and paper (overall 23.5 cm × 30.2 cm; image 14.6 cm × 9.8 cm; card 14.6 cm × 9.8 cm) Pacific Asia Museum, Pasadena, California, USA, Gift of Eleanor L. Gilmore www.pacificasiamuseum.org

Riddle in Nine Syllables

Polyxeni Potter

"An elephant, a ponderous house / A melon strolling on two tendrils" is how Sylvia Plath described herself in "Metaphors." In addressing her physical condition, the poet was acknowledging the psychological toll of changes to the female body during pregnancy, which she termed "a riddle in nine syllables." The immensity of these changes has long preoccupied artists too, while the public health implications were left to physicians and others concerned with maternal and child health.

It was along these lines that artist Paul Jacoulet addressed the theme of maternity in *Le Trésor* (*Corée*) (The Treasure [Korea]), on this month's cover: maternity as a stage of life. Like Japanese master printer and painter of women Kitagawa Utamaro (1753–1806), a formative influence, Jacoulet was interested in mother and child as a special population, depicting them in their daily activities as persons with individuality and character.

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Jacoulet was born in Paris, a frail child, "like a damaged little worm," ill with chronic bronchial problems. His family moved to Japan when he was very young. He grew up in Tokyo firmly grounded in Japanese culture, multilingual, with early training in music, dance, drama, and calligraphy. But art prevailed. "Almost before I spoke, I was glad to have a pencil in my hand." He started to paint at age 11 under artist Seiki Kuroda (1883–1924), who taught Western art theories to the Japanese. Jacoulet grew up next door to *ukiyo-e* authority Yone Noguchi, father of sculptor Isamu Noguchi. Isamu's mother, American writer Léonie Gilmour, taught Jacoulet English.

An avid student of *ukiyo-e*, the woodblock print genre, Jacoulet quickly mastered and abandoned it for a style, uniquely his own, which combined exacting technique and Japanese brushwork with Western influences (Paul Gauguin, Édouard Manet, Henri Matisse) from his frequent trips to Paris and from a personal approach to line drawing and color use. Jacoulet was an innovator. He introduced embossing for added texture and enriched his prints with colored micas, crushed pearls, lacquers, and silver, gold,

and bronze metallic pigments for a shimmering sensuous effect.

"The woodcut colorprint is like music," Jacoulet believed, "Without harmony among painter, engraver, and printer, it is impossible to produce a fine picture." He engaged only distinguished carvers and printers and stamped their names on the margins of prints. He established the Jacoulet Institute of Prints and published nearly all his work himself, resisting massive production of copies. He brought only the best watermarked paper and boasted using as many as 300 blocks for one print. He produced thousands of drawings and water colors, many now lost; 166 color woodblock prints survive.

Instead of the usual young and beautiful theater performers and courtesans of traditional *ukiyo-e*, Jacoulet's work featured the aging and weak whose faces he observed and recorded in sketches and photographs during his many travels, widely throughout Japan, China, Mongolia, and the South Pacific Islands. Of special interest were the indigenous people of these areas and the Western residents of Japan, where he lived and worked most of his life. A confirmed naturalist, he collected specimens and painted disappearing wildlife and small villages overrun by modern civilization, becoming at times the only chronicler of island populations now extinct. In this way, his work was imbued effortlessly with the exoticism so sought after on the Western art scene.

"I am the greatest artist," Jacoulet wrote to collectors in the 1950s, seeking acceptance and recognition. His unconventional approach to painting and printing and unusual choices of subject matter did not make him popular. "I am anxious and rather down, very discouraged," he wrote to friends who tried to expose his work to a broader audience. By then, the once described "best looking young man in Tokyo," took to appearing in public with his face powdered and lips tinted with rouge, perhaps to correct a sallow complexion brought on by illness.

Le Trésor is from a series on Korean subjects. Jacoulet visited Korea frequently after the death of his father during War World II, when his mother moved there to live with her new husband, a Japanese physician. In addition to all manner of local characters, from scholars and the wealthy to common workers and beggars, he covered in this series of prints mothers with their children, a subject common in European as well as Japanese art. *Le Trésor* sold more than 300 copies.

The mother's face is common but clearly focused, the tassel on her headpiece undone, exaggerating her downward look and leading the viewer to the center of the picture. Her body envelops the child, fluid circular lines making a nest for the red bundle. This tender, private moment is sparingly drawn, accented only with the bold vest and decorated footwear of the child, whose little hand is reaching inside the mother's neckline.

Mother and child, one of the oldest and most frequent subjects in the history of art, draws on the universality of the complex psychological experience of having and being a mother. By the 19th century, religious and romanticized images of maternity gave way to a more down to earth approach, though the notion of mother as vessel without much control persisted. Like many artists of his generation, Jacoulet explored the uniqueness of the experience by capturing facets of maternity in the floating world, just as, in her own way, looking inward, Sylvia Plath examined changes in the context of a riddle.

Maternal and child health, its own riddle intertwined with pregnancy, features also in disease emergence because special populations, pregnant women among them, and their response to emergence are key to successful disease prevention and control. Jacoulet did not know and Sylvia Plath could only sense the physical hazards involved in being a vessel. The genetically foreign fetus challenges a woman's core defense against disease, the immune system, which has to make changes if the pregnancy is to succeed. These changes, not well understood, may alter susceptibility to and severity of certain infectious diseases (toxoplasmosis, listeriosis, malaria, measles) and could increase death rates from others, such as influenza and varicella. Hepatitis E virus infections continue to cause a disproportionate number of deaths among pregnant women in developing countries, despite the availability of vaccines. These and other still unknown health threats add meaning to the poet's lament. "I'm a mean, a stage, a cow in calf. / I've eaten a bag of green apples, / Boarded the train there's no getting off."

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

Spread of Influenza Virus (H5N1) Clade 2.3.2.1 to Bulgaria in Common Buzzards

Methicillin-Resistant *Staphylococcus aureus* Sequence Type 239-III, Ohio, 2007–2009

WU and KI Polyomaviruses in Respiratory Samples from Allogeneic Hematopoietic Cell Transplant Recipients

Dengue Outbreak Investigations in a High-Income Urban Setting, Kaohsiung City, Taiwan, 2003–2009

Nontuberculous Mycobacteria in Household Plumbing and Chronic Rhinosinusitis

Wild Birds and Urban Ecology of Ticks and Tick-borne Pathogens, Chicago, Illinois, 2005–2010

Constant Transmission Properties of Variant Creutzfeldt-Jakob Disease Cases from 5 Countries

Epidemiology of Foodborne Norovirus Outbreaks, United States, 2001–2008

Emerging Autochthonous and Dormant *Cryptococcus gattii* Infections in Europe

Echinococcus multilocularis in Urban Coyotes, Alberta, Canada

Orthobunyaviruses Antibodies in Humans, Yucatan Peninsula, Mexico

Tetanus as Cause of Mass Die-off of Captive Japanese Macaques, Japan, 2008

Hepatitis E Virus Seroprevalence among Adults, Germany

Scarlet Fever Epidemic, Hong Kong, 2011

Factors Associated with Visceral Leishmaniasis in Rural Bihar, India

Circulation of Influenza A(H1N1)pdm09 Virus in Pigs, Reunion Island

Human Infection with *Candidatus* Neoehrlichia mikurensis, China

Powassan Virus Encephalitis, Minnesota

Complete list of articles in the October issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

September 5–8, 2012
Incidence, Severity, and Impact Conference
Munich, Germany
http://www.isirv.org

September 9–14, 2012
XVIIIth International Pathogenic Neisseria Conference (IPNC) 2012
Maritim Hotel, Würzburg, Germany
http://www.ipnc2012.de

October 17–21, 2012
IDWeek Annual Meeting
San Diego, CA, USA
http://www.IDWeek.org

October 23–24, 2012
Emerging Viruses: Disease Models and Strategies for Vaccine Development
A symposium in honor of CJ Peters, MD
Galveston, TX, USA
http://www.utmb.edu/wrce

October 24-26, 2012
European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE)
Edinburgh, Scotland, UK
http://www.escaide.eu

October 27–31, 2012
APHA 140th Annual Meeting & Expo
San Francisco, CA, USA
http://www.apha.org/meetings/AnnualMeeting

November 11–15, 2012
ASTMH 61st Annual Meeting
Atlanta Marriot Marquis
Atlanta, GA, USA
http://www.astmh.org

Announcements

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Announcements may be posted on the journal Web page only, depending on the event date.

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Article Title

Effectiveness and Timing of Vaccination during School Measles Outbreak

CME Questions

1. You are on the health advisory board of your local elementary school district. Two students in one fourth-grade class were diagnosed with measles today. The overall rate of vaccination against measles in the school is 90%.

What should you consider regarding vaccination and infection with measles?

A. The estimated basic reproduction number R_0 for measles is in the range of 12 to 40
B. The case-fatality rate in industrialized countries is approximately 10%
C. Vaccination rates in Europe generally exceed 95%
D. Vaccinating during the incubation period of measles has no salutary effect

2. You estimate that the R_0 for measles is actually 16. Based on the results of the current study, what is the maximum amount of time that can pass before an outbreak response vaccination (ORV) is initiated in order to limit measles infection to no more than 20 children?

A. 2–4 days
B. 6–10 days
C. 11–15 days
D. 12–24 days

3. Based on the results of the current study, what is the maximum amount of time that can pass before an ORV is initiated in order to affect the size of the measles outbreak at all?

A. 25 days
B. 35 days
C. 50 days
D. 65 days

4. Based on the results of the current study, when can you expect the peak incidence of measles if an outbreak develops?

A. 1 to 3 weeks
B. 4 to 7 weeks
C. 6 to 14 weeks
D. 20 to 25 weeks

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

Evaluation of Diagnostic and Therapeutic Approaches for Suspected Influenza A(H1N1)pdm09 Infection, 2009–2010

CME Questions

1. You are seeing a 17-year-old young woman with a history of malaise, low-grade fever, sore throat, and frontal headache for 2 days. You suspect that this patient might have influenza. Based on the results of the current study, what should you consider regarding influenza testing?

A. 99% of patients with a positive test for influenza met CDC-defined criteria for influenza-like illness (ILI)
B. Children had higher rates of positive testing as outpatients compared with adults
C. All tested patients had some indication suggesting influenza infection
D. The rates of positive testing exceeded 50% among both children and adults in the outpatient setting

2. You decide to initiate treatment with oseltamivir as an outpatient. What does the current study demonstrate regarding the use of oseltamivir in the outpatient setting?

A. Adults were more likely than children to receive oseltamivir
B. Nearly half of patients with a positive rapid influenza diagnostic test (RIDT) were not treated according to CDC guidelines
C. Half of patients receiving oseltamivir had a negative RIDT
D. One quarter of patients received oseltamivir within 48 hours of the onset of symptoms

3. The patient returns to your clinic 2 days later with worsening cough, dyspnea, and fever. You decide to admit her to the hospital. What does the current study demonstrate regarding the management of influenza in the inpatient setting?

A. Over 90% of inpatients received oseltamivir
B. Over 90% of inpatients who received oseltamivir had positive influenza testing
C. Most patients prescribed oseltamivir received an incorrect dose or duration of therapy
D. The most common error in prescribing oseltamivir was extending the duration of therapy

4. What else should you consider regarding the management of influenza in the setting of lower respiratory tract infection (LRTI) in the current study?

A. All patients with cases of LRTI were admitted to the hospital
B. Most patients presented within 2 days of symptom onset
C. Two thirds of patients failed to receive appropriate treatment
D. The principal reason that patients failed to receive oseltamivir was the belief of physicians that the drug would not work

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.				
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1	2	3	4	5

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Article Title

Control of Fluoroquinolone Resistance through Successful Regulation, Australia

CME Questions

1. You are a public health official advising a health management organization regarding use of fluoroquinolones. Based on the review by Dr. Cheng and colleagues, which of the following statements about restrictions in Australia regarding use of fluoroquinolones is most likely correct?

A. Quinolones are permitted in steer but not in cows
B. Ciprofloxacin is listed as an option in lower urinary tract infection only in patients with high fever
C. Moxifloxacin is listed as an option for empiric management of most cases of outpatient community-acquired pneumonia
D. Australia's subsidized outpatient pharmaceutical scheme limits usage of relatively expensive drugs unless prescribed by doctors for indications listed in the Pharmaceutical Benefits Scheme

2. Based on the review by Dr. Cheng and colleagues, which of the following statements about development of fluoroquinolone resistance in Australia compared to that in other countries is most likely correct?

A. In *Echerichia coli*, quinolone resistance rates in disease-causing isolates have plateaued at a moderate level in Australia
B. Quinolone resistance in locally acquired *Campylobacter* spp. is about the same in Australia as in most other countries
C. An increase in quinolone resistance in pneumococci in North America was noted following the introduction of levofloxacin into respiratory infection guidelines and widespread usage
D. Quinolone resistance in isolates of *Campylobacter* spp., *Salmonella* spp., and *E. coli* from a variety of food-producing animals and products is not uncommon in Australia due to importation of meat

3. Based on the review by Dr. Cheng and colleagues, which of the following statements about potential harms of restricting fluoroquinolone use, and strategies to eliminate those harms, would most likely be correct?

A. Quinolones can no longer be prescribed at all in Australia
B. Most recommended empirical regimens use quinolones, and appropriate empirical therapy would therefore be delayed
C. No strategies are currently available for quinolone therapy in severely ill, hospitalized patients
D. Quinolones can still be prescribed in Australia when they are necessary or the indicated preferred treatment

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.				
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4. The activity was presented objectively and free of commercial bias.				
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1	2	3	4	5

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Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

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Figures. Submit figures in black and white. If you wish to have color figures online, submit both in black and white and in color with corresponding legends. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpeg or .tif files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

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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.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

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 (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. 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.

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