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On the Cover

Vincent van Gogh (1853–1890)
The Potato Eaters (1885) (detail)
Oil on canvas (81.5 cm × 114.5 cm)
Van Gogh Museum Amsterdam
(Vincent van Gogh Foundation)

About the Cover p. 1001

Perspective

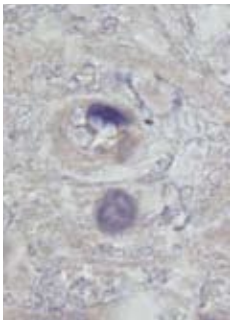
CME ACTIVITY

Synopsis

p. 867

Research

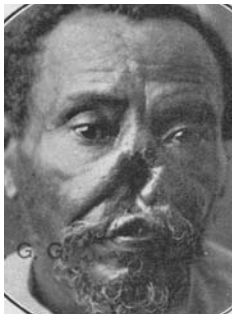
p. 882



Historical Review

EMERGING INFECTIOUS DISEASES
June 2009

Dispatch



p. 917

p. 990

Letters

Book Reviews

News and Notes

Online Report

Stockpiling Supplies for the Next Influenza Pandemic
www.cdc.gov/EID/content/15/6/e1.htm

Past, Present, and Possible Future Human Infection with Influenza Virus A Subtype H7

Jessica A. Belser, Carolyn B. Bridges, Jacqueline M. Katz, and Terrence M. Tumpey

CME ACTIVITY

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Learning Objectives

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

- Describe the transmission mechanism and attack rate for the H7 strain of influenza virus in humans
- Describe clinical manifestations of H7 virus infection in humans
- Identify reasons for increased prevalence of human infection with the H7 virus in future
- Describe differences in clinical presentation of infection with H5N1 and H7 viruses
- Identify the best strategy for protection against avian virus infection for humans

Editor

Carol Snarey, Copyeditor, *Emerging Infectious Diseases. Disclosure: Carol Snarey has disclosed no relevant financial relationships*.

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Influenza A subtype H7 viruses have resulted in >100 cases of human infection since 2002 in the Netherlands, Italy, Canada, the United States, and the United Kingdom. Clinical illness from subtype H7 infection ranges from conjunctivitis to mild upper respiratory illness to pneumonia. Although subtype H7 infections have resulted in a smaller proportion of hospitalizations and deaths in humans than those caused by subtype H5N1, some subtype H7 strains appear more adapted for human infection on the basis of their virus-binding properties and illness rates among exposed persons. Moreover, increased isolation of subtype H7 influenza viruses from poultry and the ability of this subtype to cause severe human disease underscore the need for continued surveillance and characterization of these viruses. We review the history of human infection caused by subtype H7. In addition, we discuss recently identified molecular correlates of subtype H7 virus pathogenesis and assess current measures to prevent future subtype H7 virus infection.

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Influenza A viruses belong to the family *Orthomyxoviridae* and possess 8 negative-sense RNA segments encoding 11 known proteins. Of these, the 2 viral surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), form the basis of multiple serologically distinct virus subtypes. Currently, 16 HA and 9 NA subtypes have been identified in wild water birds, the natural host for all influenza A viruses and the reservoir from which viruses emerge to infect domestic poultry and occasionally mammals. Most influenza viruses that infect wild or domestic birds cause no or limited illnesses and deaths and are characterized as being low pathogenicity avian influenza (LPAI) viruses. However, viruses within the H5 and H7 subtypes have the capacity to acquire genetic properties that confer high virulence and a

high proportion of deaths in chickens and other fowl after their introduction into domestic poultry; these viruses are characterized as highly pathogenic avian influenza (HPAI) viruses according to the intravenous pathogenicity index method described by the World Organization for Animal Health (*1*). LPAI viruses (H9N2) are also prevalent in poultry in many countries and are considered to have pandemic potential (*2*). Domesticated birds may serve as important intermediate hosts for the transmission of wild bird influenza viruses to humans, as may swine, as evidenced by recent human infections with swine influenza virus A (H1N1) on multiple continents. In April 2009, the World Health Organization (WHO) reported human illness caused by a new strain of swine influenza virus subtype H1N1; infections were soon confirmed in 7 countries. As of April 28, 2009, Mexico had reported the highest number of subtype H1N1 cases, with 26 confirmed human cases of infection and 7 deaths.

If an influenza virus with an HA against which the human population had little or no immunity crossed the species barrier and was efficiently transmitted among humans, a pandemic could result. Three pandemics occurred in the 20th century: in 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2). However, none of these pandemic strains possessed the HA cleavage site mutation characteristic of HPAI viruses (*3*). Thus, the HPAI phenotype is not required for an influenza virus to cause a pandemic. Three HA subtypes, H1–H3, subsequently established stable lineages in humans; 2 subtypes, H1N1 and H3N2, cause seasonal epidemics today, which result in ≈36,000 deaths in the United States annually (*4*). Although the severity of a pandemic virus cannot be known in advance, attack rates could reach 25%–35%. The resulting surge in the number of persons requiring medical or hospital treatment would undoubtedly overwhelm the healthcare system.

Within the past decade, HPAI and LPAI viruses have been found to be associated with human infection, primarily as a result of direct transmission from poultry to humans (*2*,*5*,*6*). However, none of these viruses have yet acquired the ability to be transmitted efficiently among humans. LPAI viruses of the H7 and H9 subtypes have caused mild respiratory or conjunctival infections in humans. However, some HPAI subtype H5 and H7 viruses, which cause a high proportion of deaths in experimentally infected chickens, have been associated with severe human disease and death (*5*,*6*). Due to an unprecedented geographic expansion of subtype H5N1 viruses since 2003 and continued sporadic human subtype H5N1 infections, much emphasis has been placed on the potential pandemic threat posed by subtype H5N1 viruses. In contrast, subtype H7 infection in humans has not been as extensively studied. In this perspective, we will discuss the epidemiology of subtype H7 in humans, current research that explores the pandemic potential of these viruses, and ongoing measures to prevent future human infection.

Prevalence of Subtype H7 Influenza Viruses in Poultry and Risk for Human Infection

Subtype H7 influenza viruses, like avian influenza viruses of all subtypes, fall into 2 geographically distinct genetic lineages, North American or Eurasian (*7*). Viruses within both lineages have been associated with human infection (Table). In recent years, poultry outbreaks caused by HPAI and LPAI viruses of the H7N1, H7N2, H7N3, H7N4, and H7N7 subtypes have resulted in the culling of >75 million birds (*18*). Notably, the geographic diversity of countries affected by the H7 subtype in poultry, which includes Pakistan, Australia, Ireland, Italy, Canada, Germany, Chile, the Netherlands, and the United States, readily demonstrates the global public health risk posed by viruses within this subtype (*18*).

Before 2003, reports of subtype H7 infection in humans were rare and primarily resulted from laboratory or occupational exposure. One exception was the first documented isolation of a fowl plague-like virus (FPV; HPAI viruses of the H7N7 subtype) from a human, which occurred in the United States in 1959, from the blood of a man with clinically diagnosed infectious hepatitis (*19,20*). In 1977, a laboratory technician became infected through accidentally splashing allantoic fluid containing FPV on her face, which resulted in conjunctival symptoms (*21*). During the winter of 1979–80, a virus antigenically similar to A/fowl plague/Dutch/27 (H7N7) caused the deaths of ≈500 seals on the New England coast**.** Subsequent

Table. Cases of human subtype H7 influenza A virus infection since 1996*

Location	Year	Subtype	IVPI	No. human infections	Symptoms	References
UK (England)	1996	H7N7	LPAI	1	Conjunctivitis	(*8,9*)
USA (Virginia)	2002	H7N2	LPAI	1†	Respiratory	(*10*)
USA (New York)	2003	H7N2	LPAI	1	Respiratory	(*11*)
Italy	2002–03	H7N3	LPAI	7†	Conjunctivitis, respiratory	(*12*)
The Netherlands	2003	H7N7	HPAI	89	Conjunctivitis, respiratory	(*6,13*)
Canada (British Columbia)	2004	H7N3	LPAI/HPAI	2	Conjunctivitis, respiratory	(*14,15*)
UK (Norfolk)	2006	H7N3	LPAI	1	Conjunctivitis	(*16*)
UK (Wales)	2007	H7N2	LPAI	4	Conjunctivitis, respiratory	(*17*)

*IVPI, intravenous pathogenicity index (*1*); LPAI, low pathogenicity avian influenza; HPAI, highly pathogenic avian influenza.
†Serologic evidence only.

study of the prototype virus A/seal/Massachusetts/1/80 (H7N7) resulted in the infection of a laboratory worker when an experimentally infected seal sneezed into the face and the right eye of the worker (*22,23*). Four persons who conducted necropsies of infected seals also contracted conjunctivitis within 2 days of known ocular exposure; although the virus was not isolated from the 4 field workers, clinical signs and duration of illness were consistent with subtype H7N7 virus infection (*22*).

The first reported case of direct transmission of a subtype H7 virus from an avian to a human host occurred in 1996, when conjunctivitis developed in a woman who kept pet ducks 1 day after she experienced a possible eye abrasion while cleaning her duck house (*8,9*). A conjunctival swab from this patient was found to be positive for an influenza virus A (H7N7), A/England/268/96, which was determined to be wholly avian in origin by sequence analysis (*9*). However, a rise in serum hemagglutination inhibition (HI) titer to virus postexposure was not detected in any of these early human infections. It is not known whether the absence of HI antibody detected in serum specimens from these infected persons was due to an actual lack of induction of serum antibodies after infection with these H7 subtypes or whether the relative insensitivity of the avian erythrocyte-based HI assay used at that time contributed to these findings. Nevertheless, these initial events clearly confirmed the ability for interspecies transmission of subtype H7 viruses to humans.

Recent Human Infections with Subtype H7 Influenza A Viruses

In contrast to these isolated instances of human infection with subtype H7 viruses, numerous outbreaks of LPAI and HPAI viruses of this type among poultry since 2000 have resulted in increased numbers of human exposure and infection (Table). This increase in detection may be a result of a combination of several factors: more human infections, improved PCR diagnostic testing, heightened awareness of the risk for avian influenza in humans caused by subtype H5N1, and increased surveillance and testing of humans exposed to avian influenza. The largest outbreak of subtype H7 infections in humans to date occurred in the spring of 2003, when an HPAI (H7N7) virus was detected in commercial poultry farms in the Netherlands and necessitated the culling of >30 million birds (*6*,*13*). All internal genes of this virus were of avian origin and were found to be related to low pathogenicity viruses detected during surveillance of ducks in the region in 2000 (*13*). Eighty-six persons involved in the culling operation and 3 of their family members who had not been in contact with infected poultry had virologically confirmed subtype H7 illness, which suggests that limited human-to-human transmission of the avian virus also had occurred (*6*). Among these persons, 78 had conjunctivitis, 5 had conjunctivitis and respiratory symptoms, 2 had respiratory symptoms only, and 1 died (*6*), a veterinarian who had visited several infected farms and in whom an acute respiratory distress syndrome and pneumonia developed. The virus isolated from a postmortem lung specimen of the patient with the fatal case, A/NL/219/2003, differed by 14 aa residues across 5 gene segments from a virus isolated from a chicken on the index farm, A/ck/NL/1/2003 (*6*). Serologic studies have provided further evidence of human infection during this outbreak (*24*). The number of human illnesses in this outbreak is in stark contrast to outbreaks of subtype H5N1 infection; most human cases of influenza virus A (H5N1) have occurred as isolated cases or small clusters of ≤3 cases with a maximum of 8 persons clinically ill (*5*).

In addition to the HPAI (H7N7) outbreak in the Netherlands, LPAI (H7N3) viruses caused outbreaks in poultry in northern Italy during 2002–03. Retrospective serologic analysis of workers involved in the outbreak response identified 7 of 185 persons who had close direct physical contact with poultry and were seropositive by microneutralization assay and Western blot analysis for subtype H7 influenza (*12*). One of these persons reported conjunctival symptoms during the outbreak. However, seroreactivity was not detected in workers involved in the earlier outbreak responses to LPAI and HPAI viruses (H7N1) that caused multiple poultry outbreaks in Italy from 1999–2001, which suggests either a different level of human exposure to subtype H7N1 viruses or differing abilities of subtype H7 viruses to transmit to humans (*12*).

HPAI and LPAI subtype H7 viruses have also caused poultry outbreaks and economic loss in the Americas. LPAI viruses (H7N2) have circulated in the northeastern United States live bird markets for over a decade and were the cause of a devastating outbreak predominantly on domestic turkey farms in 2002. One of 80 tested workers involved in the culling operations during this outbreak reported a temporally related respiratory illness and exhibited serum-neutralizing antibody responses consistent with a subtype H7N2 virus infection, providing the first evidence of possible human infection with a North American lineage LPAI virus (H7N2) (*10*). One year later, an immunocompromised New York resident with a fever and cough sought treatment at a hospital, and a subtype H7N2 virus, A/NY/107/2003 (NY/107), was subsequently isolated from a respiratory specimen (*11*). The HA gene of NY/107 virus exhibits 98% aa sequence identity with a representative virus from the 2002 outbreak in Virginia, A/tky/VA/4529/02 (*25*). The person recovered from the respiratory illness and demonstrated seroconversion to subtype H7N2 (NY/107) virus, but the source of his initial exposure to the avian virus remains unknown. LPAI virus (H7N2) was isolated from 133 of 4,675 poultry specimens from New York, and

1 of 3,406 specimens from New Jersey in early 2006, but this subtype has not been detected among domestic poultry in the United States since March 2006 (*26*).

HPAI subtype H7 viruses again caused human disease in North America, as observed in Spring 2004 during an outbreak of subtype H7N3 infection in poultry in British Columbia, Canada. The initial virus was an LPAI virus that subsequently became HPAI by acquisition of a 7-aa M1 gene sequence insertion at the HA cleavage site through a nonhomologous recombination event (*14*). Among workers associated with the outbreak response, 57 suspected human cases of subtype H7N3 infection were reported due to conjunctival or influenza-like illness symptoms (*15*). In 2 of these persons, who were involved in the culling of infected birds, conjunctivitis developed after direct ocular exposure to infected poultry after a breach in eye protection. Influenza virus A (H7N3) was isolated from a nasal specimen from 1 person with conjunctivitis and coryza, A/Canada/444/2004 (Can/444), and another from a conjunctival specimen from the other person who exhibited conjunctivitis and headache, A/Canada/504/2004 (Can/504); both persons recovered fully (*15*). Although both human isolates contained the 7-aa M1 gene sequence insertion, an intravenous pathogenicity index test determined that Can/504 was HPAI, whereas Can/444 was not (*14*). Notably, the emergence of HPAI from LPAI viruses by nonhomologous recombination has been reported with both North American and Eurasian lineage subtype H7 viruses, but not viruses within the H5 subtype (*14,27*).

Most recently, multiple H7 viruses have resulted in cases of human infection in the United Kingdom. In 2006, an LPAI virus (H7N3) first detected in a poultry flock in eastern England was isolated from a poultry worker with conjunctivitis (*16*). Four additional persons associated with the outbreak later presented with conjunctivitis or influenza-like illness, but all symptomatic persons were PCR negative for influenza (*16*). In 2007, poultry infected with LPAI (H7N2) were sold from a small market in the United Kingdom and resulted in 4 persons with confirmed cases of H7 human infection, 3 of whom were hospitalized for 3–7 days, and 19 additional symptomatic persons for whom PCR results were negative (*17*). Those exposed to the virus reported both conjunctivitis and influenza-like illness; one of the hospitalized patients had neurologic and gastrointestinal symptoms, but not respiratory disease (*28*). The increased frequency of human infection with H7 viruses in recent years, coupled with the continued detection of H7 influenza viruses in poultry in both Europe and North America, suggests that future human infections with viruses within this subtype are likely to occur.

Surprisingly, seroconversion for neutralizing antibody has rarely been observed among persons with virologically confirmed subtype H7 infection. For example, neutralizing antibody responses were not detected in persons confirmed to be infected with the HPAI virus (H7N7) in 2003 (*24*). Likewise, neutralizing antibody titers were not detected in convalescent-phase serum from any person exposed to infected birds during the 2004 subtype H7N3 outbreak in Canada, including those with confirmed cases with positive virus isolation (*15*,*29*). In contrast, infection with LPAI (H7N2) that resulted in respiratory illness in the United States did induce a detectable serum-neutralizing antibody response (*10*). Low antibody levels also were detected in a person who was infected with an LPAI virus (H7N3) (*30*). However, the optimal methods of detecting antibody and criteria for seropositivity to H7 virus in humans remain unclear; current criteria used are those established and adapted by WHO for H5N1 subtype human infection and extrapolated for the H7 subtype. In addition to evaluating potential avian subtype-specific differences in the detection of neutralizing antibodies, further study is needed to ascertain whether conjunctival avian virus infection routinely leads to detectable serum antibodies. Sensitive and specific methods of detecting mucosal antibody to influenza virus in ocular specimens are also needed.

Properties of Subtype H7 Influenza Viruses

Because of the sustained frequency of epornitics caused by influenza virus subtype H5N1 that have resulted in human infections during the past 5 years, viruses within this subtype are rightly considered a major pandemic threat. However, subtype H7 influenza viruses share many properties with viruses within the H5 subtype, and H7 outbreaks involving large numbers of infected persons have been documented. Thus, the pandemic potential of subtype H7 viruses should not be underestimated because viruses within this subtype have caused severe human infection and death, with limited human-to-human transmission (Figure) (*5*,*13*). Interestingly, although subtype H5N1 infection most frequently manifests as severe respiratory disease, human infection with subtype H7 viruses predominantly result in conjunctivital symptoms with occasional and generally mild respiratory illness. Despite the overall differences in human disease manifestations and severity, subtype H7 viruses can replicate efficiently in the respiratory tract of experimentally infected animals without the need for prior adaptation, and have the capacity to spread systemically, including to the central nervous system, in mammalian models (*31*,*32*).

North American lineage subtype H7 viruses, despite exhibiting reduced virulence in mammalian models as compared with subtype H7 viruses from the Eurasian lineage, nonetheless possess multiple features that underscore the public health threat posed by these viruses. This is especially apparent for the North American lineage LPAI viruses (H7N2), which circulated in the live bird markets of the

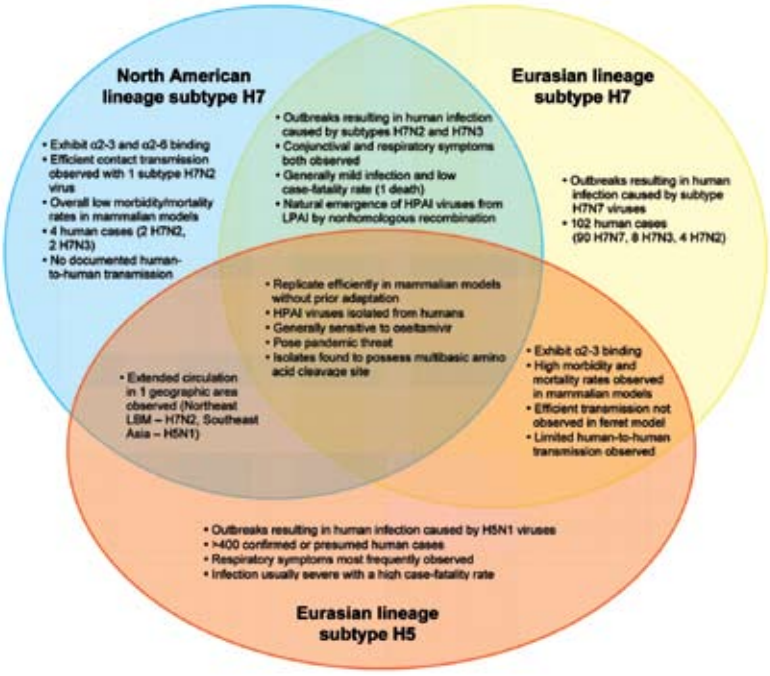

Figure. Public health impact of influenza virus A subtypes H7 and H5. HPAI, highly pathogenic avian influenza; LPAI, low pathogenicity avian influenza; LBM, live bird market.

northeastern United States for over a decade. These viruses possess a 24-nt deletion found in the HA and a 51-nt stalk deletion in the NA, which distinguishes them from other subtype H7 viruses found in domestic poultry in North America. Since these viruses were introduced in 1994, the HA cleavage site of circulating viruses acquired additional basic amino acids, a known correlate of pathogenicity for avian influenza viruses (*33*).

Recent work has also identified that contemporary North American lineage subtype H7 viruses, isolated in 2002–03, are partially adapted to recognize α2–6 linked sialic acids, which are the receptors preferred by human influenza viruses and found in the human upper respiratory tract (*34*). A critical determinant for viral transmission among humans believed to be the binding between the virus and sialic acid receptors located on cells in the upper airway. Therefore, if North American lineage subtype H7 viruses adapt further to enhance their ability to bind solely to α2–6 linked sialic acid receptors, these avian influenza viruses could have the potential to spread more efficiently from birds to humans and among humans. Although human-to-human transmission has not been documented among North American lineage subtype H7 influenza viruses, the discovery of an LPAI virus (H7N2) isolated from a human in 2003 that was transmissible by direct contact in ferrets identifies the potential of viruses within this lineage to acquire this property (*34*). In contrast, most avian influenza viruses tested in this manner fail to transmit. Human influenza viruses are thought to be transmitted primarily by respiratory droplets expelled during coughing or sneezing; no avian viruses of subtype H5 or H7 have yet demonstrated the ability to spread through respiratory droplets in the ferret transmission model.

In comparison with the generally mild infections observed with either HPAI or LPAI North American lineage subtype H7 viruses, HPAI (H7N7) European lineage viruses isolated from humans resemble subtype H5N1 viruses in their capacity for high virulence in mammalian models (*31*,*32*). Eurasian lineage subtype H5N1 viruses have been found to be more virulent in the mouse model than non-Eurasian lineage subtype H5N1 viruses; however, no molecular determinants have been associated with the hypothesis that Eurasian lineage avian influenza viruses are more capable of infecting mammals (*35*). Nevertheless, selected subtype H7 viruses within the Eurasian lineage possess molecular features (such as the E627K PB2 substitution) most frequently found in highly pathogenic subtype H5N1 viruses in poultry and, additionally, resemble highly pathogenic subtype H5N1 viruses with regard to the preservation of an avian receptor-binding preference and a general inability to transmit efficiently in the ferret model (*34*,*36*).

Despite >400 confirmed human cases of subtype H5 infection since 1997, all infections have resulted from viruses possessing the N1 NA subtype (*5*). In contrast, subtype H7 viruses with multiple NA subtypes have successfully transmitted from birds to humans, suggesting that multiple NA subtypes are compatible with the subtype H7 HA. Although subtype H5N1 viruses associated with disease in humans have predominantly been HPAI viruses from the Eurasian lineage, both lineages of subtype H7 viruses have been associated with disease in humans. The great diversity of subtype H7 viruses associated with dis-

ease in humans supports the need for active surveillance for illness among persons exposed to subtype H7 viruses, including farm workers, cullers, and the families of these workers, as well as healthcare providers who care for ill persons involved in subtype H7 outbreaks. The occurrence of conjunctival symptoms after infection with subtype H7 viruses, a clinical sign of illness not frequently associated with infection with other virus subtypes, further demonstrates the complexity of this virus subtype; research investigating the ocular tropism of selected influenza viruses is needed to better understand and protect humans from this possible route of virus entry.

Preventing Subtype H7 Virus Infection in Humans

Although effective vaccines offer the best protection against avian influenza viruses, technical limitations currently prevent the rapid generation and availability of a strain-specific vaccine against an emerging pandemic virus. The emergence of multiple antigenically distinct virus clades, resulting in a need for clade-specific vaccine candidates, has posed a substantial challenge for the design of subtype H5N1 virus vaccines (*5*). The generation of subtype H7 vaccine candidates faces similar challenges because antigenically distinct subtype H7 lineages have resulted in human disease, and the isolation of North American lineage subtypes H7N2, H7N3, and Eurasian lineage H7N7 and H7N3 viruses from humans in recent years identifies multiple distinct H7 subtypes that may warrant the development of appropriate vaccine candidates. Vaccination of poultry has been successful in controlling of subtype H7 influenza (*18*); vaccines for human use against both lineages of H7 influenza are under development and have been evaluated in preclinical studies (*25*,*32*,*37*).

Antiviral strategies that are effective against influenza viruses of multiple subtypes will be an important first line of defense in the event of a pandemic. Unfortunately, the emergence of antiviral-resistant subtype H5 and H7 influenza viruses has been documented. Viruses from the 2003 Netherlands outbreak were found to be sensitive to the NA inhibitors oseltamivir and zanamivir in vitro but resistant to the M2 ion-channel blocker amantadine both in vitro and in a mouse model (*13*,*38*). Amantadine-resistant variants have also been observed among subtype H7 viruses within the North American lineage (*39*). Together with the detection of subtype H5N1 viruses with reduced susceptibility to antiviral agents (*5*), these findings underscore the importance of surveillance for resistant viruses of avian influenza virus of multiple subtypes as well as the generation of novel antiviral strategies to combat influenza viruses of an unknown subtype.

In addition to pharmacologic interventions, the correct use of personal protective equipment during possible virus exposure should be emphasized. The frequency of conjunctival symptoms after subtype H7 virus exposure underscores the importance of protecting the ocular surface from possible abrasion and virus entry; eye protection is recommended for all persons during possible exposure to avian influenza viruses (*40*). Given the potential for human infection, active monitoring for illness and for adherence to appropriate use of personal protective equipment among all persons potentially exposed to subtype H7 viruses during outbreaks in poultry should be conducted, and testing should be readily available should illnesses occur.

Subtype H5N1 viruses are now endemic in countries in Asia and Africa, and subtype H7 viruses continue to circulate across Europe and North America, as demonstrated by the detection of subtype H7 influenza viruses in chickens in Arkansas and the United Kingdom, and swans in Rhode Island, during the summer of 2008. Future human infection with viruses of both subtypes will likely continue to occur. It is clear that the study of avian influenza viruses (H5N1) has greatly improved our understanding of avian viruses. Applying this knowledge toward the assessment of other HPAI and LPAI viruses with pandemic potential, such as those within the H7 subtype, will further improve our ability to respond to and reduce the severity of future pandemics, regardless of virus subtype.

Dr Belser is a microbiologist in the Influenza Division, Centers for Disease Control and Prevention. Her research has focused on the molecular determinants that confer virulence and transmissibility of influenza viruses, including subtype H7 viruses with pandemic potential.

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Diphyllobothriasis Associated with Eating Raw Pacific Salmon

Naoki Arizono, Minoru Yamada, Fukumi Nakamura-Uchiyama, and Kenji Ohnishi

The incidence of human infection with the broad tapeworm *Diphyllobothrium nihonkaiense* has been increasing in urban areas of Japan and in European countries. *D. nihonkaiense* is morphologically similar to but genetically distinct from *D. latum* and exploits anadromous wild Pacific salmon as its second intermediate host. Clinical signs in humans include diarrhea and discharge of the strobila, which can be as long as 12 m. The natural life history and the geographic range of the tapeworm remain to be elucidated, but recent studies have indicated that the brown bear in the northern territories of the Pacific coast region is its natural final host. A recent surge of clinical cases highlights a change in the epidemiologic trend of this tapeworm disease from one of rural populations to a disease of urban populations worldwide who eat seafood as part of a healthy diet.

Broad tapeworms such as *Diphyllobothrium latum* and *D. nihonkaiense* are exotic parasites that grow as long as 12 meters in the small intestine. By the mid-19th century, infection with the Japanese broad tapeworm was known to be contracted by eating salmon (Figure 1) and was considered to be infection with *D. latum* until 1986, when Yamane et al. revised the identification of the Japanese broad tapeworm and established the new species *D. nihonkaiense* (*1*). Both tapeworms exploit freshwater copepods as their first intermediate host. However, in contrast to *D. latum,* which uses freshwater fish such as perch, char, and pike as the second intermediate host, *D. nihonkaiense* uses anadromous fish, *Oncorhynchus* spp., such as *O. masou* (masu salmon), *O. gorbuscha* (pink salmon), and *O. keta* (chum salmon), which migrate across the northern Pacific Ocean to the Sea of Okhotsk and the Bering Sea (*2*,*3*). Recent studies have demonstrated complete mitochondrial genomes of *D. nihonkaiense* and *D. latum* (*4*,*5*). These genomes have not only rendered species diagnosis more reliable, but they have also provided a wealth of genetic markers that could be useful for investigating their population genetics, ecology, and epidemiology.

Diphyllobothriasis nihonkaiense was once endemic to coastal provinces of central and northern Japan, where salmon fisheries thrived. However, in the past several decades, regions with endemic diphyllobothriasis nihonkaiense have disappeared from Japan, yet the infection has been perpetuated among urban people who eat sushi and sashimi. Although the number of clinical cases of the infection in large cities has fluctuated some in the past 20 years, the incidence was particularly high in 2008. Moreover, clinical cases caused by *D. nihonkaiense* have been emerging even in European countries (*6*–*9*), suggesting that the globalization of this tapeworm disease is probably due to the worldwide expansion of commercial sales of fresh or frozen wild Pacific salmon. We outline the current situation of diphyllobothriasis nihonkaiense in Japan, together with its still-mysterious ecology and life cycle.

Recent Surge of Pacific Salmon–associated Diphyllobothriasis

We retrospectively examined annual case numbers of diphyllobothriasis nihonkaiense in 2 institutes; the Department of Medical Zoology of the Kyoto Prefectural University of Medicine in Kyoto (MZ) and the Department of Infectious Diseases of the Tokyo Metropolitan Bokutoh Hospital (BH) in Tokyo. MZ is the sole institute specializing in research and diagnosis of parasitic diseases in Kyoto city (population 1.4 million). BH is one of the major public hospitals in metropolitan Tokyo.

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Courtesy of the Tohoku University Medical Library.

Figure 1. Wood print depicting a man passing a strobila of a broad tapeworm. The caption (not shown) said, "The man ate masu salmon. After a time, a strange object emerged from the anus and was pulled out: it turned out to be 2–3 m long." From Shinsen Yamaino Soushi, by Daizennosuke Koan (1850).

From 1988 through 2008, a total of 149 cases of diphyllobothriasis have been recorded: 95 at MZ and 54 at BH. Diphyllobothriasis nihonkaiense was diagnosed by morphologic appearance and taxonomic characteristics of the strobila (body of the mature tapeworm) passed in feces of a person who had a history of eating salmon or a habit of eating sushi or sashimi, which are normally composed of sea fish, often salmon. DNA sequences of the tapeworm *cox*1 and/or *nad*3 genes were also analyzed from most (42) patient specimens obtained since 2004; results confirmed the identification of *D. nihonkaiense*. Molecularly confirmed *D. latum,* from humans or fish, has not been reported in Japan.

Annual incidence rates of the clinical cases at MZ and BH show an apparent surge in recent years (Figure 2). In a broad assumption that the case numbers at MZ represent all cases of this tapeworm infection in Kyoto, the average incidence in the past 20 years was 0.32 cases per 100,000 population per year, and that in 2008 was 1.0 case per 100,000 population. Incidence throughout Japan has not been estimated because a nationwide investigation has never been conducted. Nevertheless, these case numbers at MZ and BH suggest that *D. nihonkaiense* infection is equally as prevalent in Japan as *D. latum is* in some European countries (*10*).

Most patients regularly ate sushi and sashimi. Approximately half could recall that they ate raw or undercooked salmon in the past 6 months. Analyses of 149 cases at MZ and BH showed that the disease occurred during all seasons but that prevalence peaked in early summer (Figure 3). Every age group was affected, from 3 to 77 years. Most patients were 20–59 years of age, which probably reflects more frequent consumption of sushi and sashimi by persons in this age group than in other age groups (Figure 4). Twice as many men than women were affected.

Signs and Symptoms of Infection

The signs and symptoms caused by *D. nihonkaiense* differ little from those caused by *D. latum*. All 149 patients had consulted physicians after passing tapeworm strobila. Average length of the strobila was 83 cm (range 5–400); patients reported that the strobila tore somewhere along its length when they tried to pull it out. The patients also frequently reported abdominal pain or discomfort and several episodes of diarrhea before passing the strobila, but few complained of substantial weight loss. Of the 149 patients, 73 were treated at MZ, BH, or affiliated institutions. Treatment with anthelminthics (praziquantel for most; bithionol, paromomycin, or sodium amidotrizoate and meglumine amidotrizoate [Gastrografin; Bayer Schering Pharma AG, Berlin, Germany] for a few with older cases) showed that 69 (95%) of 73 patients were infected with 1 tapeworm, 2 were infected with 2 tapeworms, and 2 were infected with 3 tapeworms. The tapeworms obtained measured 50–1,200 cm (average 334 cm). The length of the strobila was not associated with the age or sex of the patient, suggesting that all age groups and both sexes are equally susceptible to this tapeworm.

Pernicious (megaloblastic) anemia has been reported in some patients infected with *D. latum* (*11*). Among the patients with *D. nihonkaiense* infection reported here, low hemoglobin concentration (<12 g/dL) was found in 2 of 43 patients examined. Mild eosinophilia (absolute count >600/μL) was also found in 4 of 37 patients examined. A causal relationship between the anemia or eosinophilia and diphyllobothriasis nihonkaiense for these patients was not determined because neither the type of anemia nor the outcome of anemia or eosinophilia after treatment was examined.

Wild Pacific Salmon and Risk for Diphyllobothriasis Nihonkaiense

Approximately half of the wild Pacific salmon sold in Japan are caught in the coastal areas of northern Japan, and

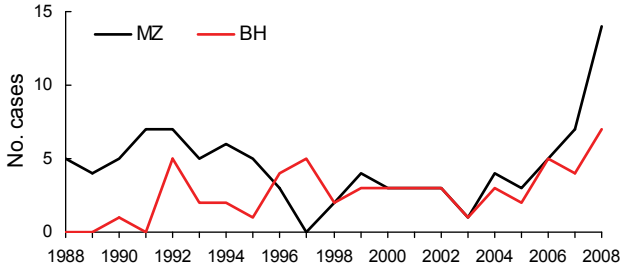

Figure 2. Diphyllobothriasis cases, Department of Medical Zoology of the Kyoto Prefectural University of Medicine (MZ) in Kyoto and Department of Infectious Diseases of the Tokyo Metropolitan Bokutoh Hospital (BH) in Tokyo, Japan, 1988–2008.

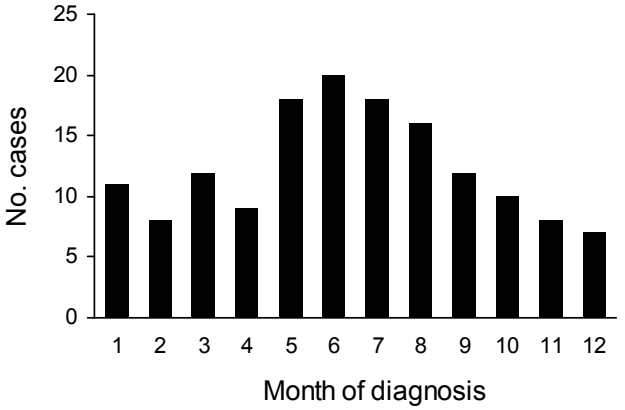

Figure 3. Seasonal occurrence of diphyllobothriasis nihonkaiense, 149 cases, Department of Medical Zoology of the Kyoto Prefectural University of Medicine in Kyoto and Department of Infectious Diseases of the Tokyo Metropolitan Bokutoh Hospital in Tokyo, Japan, 1988–2008.

the other half are imported from Far East Russia and the Pacific coast of North America. Salmon-harvesting rivers run through neither Kyoto nor Tokyo. Suzuki et al. (*12*) investigated plerocercoids (infective larvae) in wild Pacific salmon caught in waters off the coast of northern Japan and sent to Tokyo fish markets during March–July, 2000–2002. They showed that plerocercoids were found in 24 (51%) of 47, 10 (12%) of 82, and 5 (19%) of 27 samples of chum, masu, and pink salmon, respectively. Using PCR-based DNA sequence analysis targeting the *cox*1 and *nad*3 genes, they also showed that all plerocercoids recovered were identified as *D. nihonkaiense* and that 26 chum salmon caught during autumn lacked such infection. This finding implies that wild salmon caught in spring and early summer pose a higher risk for human infection than autumn-caught salmon, consistent with the observation that the incidence of human infection peaks in early summer (Figure 3).

Whether all salmon harvested in the coastal waters off Japan originated from rivers in Japan is unknown. Oshima and Wakai (*13*) investigated the characteristics of masu salmon harboring diphyllobothriid plerocercoids; rate of infection was 27%. They suggested that these masu salmon probably originated from rivers in Russia despite having been captured in the waters off the coast of Japan and unloaded at Japanese ports. To the contrary, an investigation of mature masu salmon captured in the rivers in Hokkaido showed a plerocercoid infection rate of 20%, although no plerocercoids were found in masu salmon juveniles that stayed in the rivers for 1.5 years before migrating to the sea (*14*).

Thus, although earlier exhaustive studies have indicated that the first intermediate host of *D. nihonkaiense* is the freshwater zooplanktonic copepod *Cyclops strenuus* (*15*), whether freshwater is the place of transmission of the parasite from the copepod to salmon remains controversial. Some researchers have been examining a hypothesis that Japanese masu salmon are infected with the plerocercoid not in freshwater but in the sea during their migration through the Sea of Okhotsk, possibly through another intermediate host that links the freshwater copepod and the wild salmon at sea (*14*). So far, no such intermediate host has been discovered.

Geographic Distribution of *D. nihonkaiense*

Until recently, diphyllobothriasis nihonkaiense had been reported almost exclusively in Japan. In northern communities bordering the Pacific, several additional diphyllobothriid species—*D. klebanovskii*, *D. ursi*, *D. latum*, *D. dendriticum*, and *D. dalliae*—have been implicated in human infections (*16–20*). In Far East Russia, *D. klebanovskii,* which also uses wild Pacific salmon as its second intermediate host, is the most common cause of human diphyllobothriasis (*16*,*17*). Recent molecular studies of the DNA sequences of the 18S rDNA, internal transcribed region 1, *cox*1, and *nad*3, clearly indicated the synonymy of *D. klebanovskii* to *D. nihonkaiense*, indicating that *D. nihonkaiense* is distributed not only in Japan but also in Far East Russia up to the Kamchatka Peninsula and that brown bears are its natural final host (*21*).

In 1980, on the Pacific coast of the United States, an outbreak of diphyllobothriasis was associated with consumption of Pacific salmon (*22*), but species identification of the tapeworm was not conducted. More recently, several clinical cases diagnosed by tapeworm DNA sequencing as *D. nihonkaiense* have emerged in Europe (*6–8*). These patients had eaten raw Pacific salmon, probably imported from the Pacific coast of North America. Another case, in a tourist to North America who had eaten raw sockeye salmon from British Columbia, was also diagnosed as caused by *D. nihonkaiense*. (*9*). These reports suggest a far broader geographic distribution *of D. nihonkaiense* than previously believed (Figure 5).

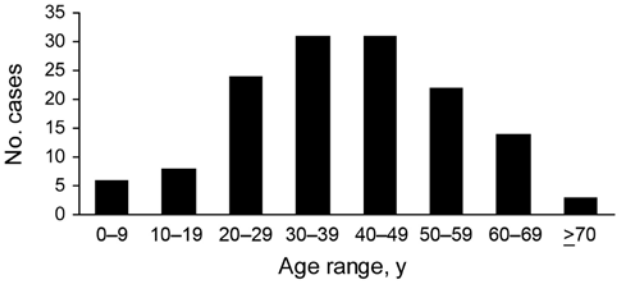

Figure 4. Age distribution of patients with diphyllobothriasis nihonkaiense, Department of Medical Zoology of the Kyoto Prefectural University of Medicine in Kyoto and Department of Infectious Diseases of the Tokyo Metropolitan Bokutoh Hospital in Tokyo, Japan, 1988–2008.

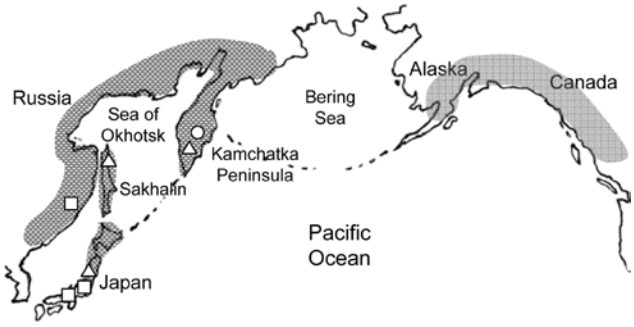

Figure 5. Possible distribution area of *Diphyllobothrium nihonkaiense*. Open circle, open square, and open triangle represent brown bears, humans, and Pacific salmon, respectively, from which *D. nihonkaiense* adult worms or plerocercoids were isolated and identified by DNA sequencing (DNA sequences refer to reference *21*). Patients in European countries are suspected to have eaten salmon imported from the Pacific coast of North America.

However, whether *D. nihonkaiense* in these regions consists of a biologically homogeneous population is still uncertain. The most enigmatic result of the molecular studies of *D. nihonkaiense cox*1 and *nad*3 genes is the presence of 2 deeply divergent lineages that are not defined by the localities of the samples examined so far (*21*). Thus, further studies are needed to look for an association between the host species and/or geographic localities and the 2 genotypes of *D. nihonkaiense*.

Other Diphyllobothriid Tapeworms in Salmon

D. nihonkaiense is not the sole tapeworm species carried by wild Pacific salmon. On the Pacific coast of North America, *D. ursi* has been isolated from brown bears, black bears, and humans (*18*,*19*,*23*,*24*). The plerocercoid of *D. ursi* is found predominantly in sockeye salmon (*O. nerka*) and occasionally in coho salmon (*O. kisutch*). A major difference between *D. ursi* and *D. nihonkaiense* (*D. klebanovskii*) is their plerocercoid stage: plerocercoids of *D. ursi* encyst on stomach serosa of salmon (*18*), and plerocercoids of *D. nihonkaiense* (*D. klebanovskii*) have been found mainly in the body musculature of chum, masu, and pink salmon (*1*–*3*). In some South American countries, cultivated Atlantic salmon (*Salmo salar*) have been implicated as the source of *D. latum* infection (*25*,*26*).

Conclusions

The epidemiology of diphyllobothriasis nihonkaiense has changed drastically from rural to urban areas because of the rapid expansion of the transport system for fresh and frozen fish to meet a demand for seafood in healthy diets. The uninterrupted occurrence of diphyllobothriasis nihonkaiense in urban areas implies that the *D. nihonkaiense* tapeworm perpetuates its natural life cycle successfully between salmon and its final host animals in northern territories of the Pacific Ocean; however, its definite natural life cycle remains to be elucidated. Freezing and storing at –20 °C for 7 days or –35 °C until solid and storing at –35 °C for 15 hours is sufficient to kill parasites, although these conditions may not be suitable for freezing particularly large fish, e.g., those thicker than 6 inches (*27*).

It seems that the general public in Japan is only vaguely aware of the possible risk for parasitic diseases associated with eating sushi and sashimi made from marine fish. Although some information on this health risk is provided through means such as health education programs open to the public or television programs, the emphasis is generally on the risk for anisakiasis, one of the most prevalent parasitic diseases among Japanese. Persons are generally underinformed, especially about the risk of diphyllobothriasis from eating raw salmon. Moreover, people like sushi and sashimi made of never-frozen fish far better than that made from frozen fish. Consumers and retailers should be made aware of the risk for tapeworm infection posed by eating raw or undercooked wild salmon.

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Geographic Clustering of Leishmaniasis in Northeastern Brazil[1]

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To determine whether disease outcomes and clades of *Leishmania braziliensis* genotypes are associated, we studied geographic clustering of clades and most severe disease outcomes for leishmaniasis during 1999–2003 in Corte de Pedra in northeastern Brazil. Highly significant differences were observed in distribution of mucosal leishmaniasis versus disseminated leishmaniasis (DL) ($p<0.0001$). Concordance was observed between distribution of these disease forms and clades of *L. braziliensis* genotypes shown to be associated with these disease forms. We also detected spread of DL over this region and an inverse correlation between frequency of recent DL diagnoses and distance to a previous DL case. These findings indicate that leishmaniasis outcomes are distributed differently within transmission foci and show that DL is rapidly spreading in northeastern Brazil.

Leishmaniasis accounts for ≈2 million disability-adjusted life years in ≈90 countries, most of which are in the developing world (*1*). The past 3 decades have witnessed accumulation of much knowledge about the host-parasite relationship, especially about host immune responses against *Leishmania* spp. The focus on immunity reflects in part the central role played by the immune system for pathogenesis of leishmaniasis (*2*,*3*) and the need for appropriate prophylaxis against this heterogeneous group of diseases that remain uncontrolled and are increasing in prevalence and incidence (*4*,*5*). Therefore, better understanding and control of this disease demand additional approaches, especially investigations that focus on the parasite, the host environment, and their relationship to clinical outcomes.

Differences in geographic distribution of distinct clinical forms of American tegumentary leishmaniasis (ATL) have long been recognized in Andean countries in South America. To a large extent, this phenomenon seems to be determined by the prevalence of various *Leishmania* spp. in diverse environments. For example, in Ecuador and Peru, the highlands harbor almost exclusively localized cutaneous leishmaniasis (CL) cases caused by several *Leishmania* spp., whereas mucosal leishmaniasis (ML) is mostly limited to the Amazon rain forest and caused by *L. braziliensis* (*6*,*7*). Conversely, observations such as those in the Peruvian lowlands, where *L. braziliensis* causes CL throughout the country but ML is almost exclusively found in Amazonian provinces (*7*), lend support to the hypothesis that strain variability within a species may influence the form and distribution of ATL. To understand whether geographic segregation of ATL outcomes occurs within a more confined geographic space (foci of ATL transmission), we compared how cases of ML and disseminated leishmaniasis (DL) were distributed during 1999–2003 in Corte de Pedra in northestern Brazil, where active transmission of parasites from a complex population of *L. braziliensis* to humans occurs.

Materials and Methods

Study Area

Corte de Pedra is composed of 20 municipalities in a rural area previously dominated by the Atlantic rain forest.

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Lutzomyia (*Nyssomyia*) *whitmany* and *Lu*. (*Nyssomyia*) *intermedia* sandflies that transmit *L. braziliensis* are endemic in the local fauna. This biome had not undergone any major changes during the period of the study. Residents in this area work mostly in agriculture, often in primary or secondary forests. There is little population migration in or out of this region. Study participants' mean time of residence at their addresses at the time of diagnosis and parasite sampling was 17 years; ≥90% of the study participants lived on farms.

Disease Definitions

CL was defined as a disease with <10 ulcerative skin lesions without evidence of mucosal involvement. DL was defined as a disease with ≥10 nodular, acneiform, or ulcerative lesions spread over the skin of ≥2 body areas. ML was defined as a disease with metastatic mucosal lesions affecting the nose, palate, pharynx, or larynx and not contiguous with primary cutaneous lesions. Patients who simultaneously satisfied the definitions for ML and DL were classified as patients with DL showing mucosal involvement (MDL). This classification distinguishes these patients from those with classic ML, which usually shows skin involvement compatible with CL. All patients had their diagnosis confirmed by detection of parasites in culture aspirates or by histopathologic analysis, and a delayed-type hypersensitivity reaction.

Patients with ATL

For geographic comparisons of disease distribution, participants with ATL were classified according to disease definitions into 3 groups: 30 patients with ML, 30 with DL, and 17 with MDL. Diagnoses were made during 1999–2003 in Corte de Pedra. Geographic coordinates of residence sites of these ATL patients were obtained by using a Brunton Multi-Navigator global positioning system apparatus (Brunton Company, Riverton, WY, USA), which has a range precision of 15 m. To characterize dynamics of DL spread within Corte de Pedra, we mapped the residences of 66 patients with DL with or without mucosal involvement. These patients received a diagnosis during 1993–2002 and represented ≈50% of all DL and MDL patients who came to the health post in Corte de Pedra during that period.

We analyzed clinical records of 102 patients with DL and 6,297 patients with ATL in the health post during 1993–2003. We also used geographic coordinates for another group of 21 patients (9 with *L. braziliensis* clade C isolates and 12 with clade A plus D isolates) whose isolated parasites had been used to define clades (i.e., subpopulations) of *L. braziliensis* genotypes circulating in Corte de Pedra, as determined by random amplified polymorphic DNA analysis (*8*). All ATL case-patients in this study were self-referred and diagnosed in 1 health post that treats ≈70% of patients with leishmaniasis in the region.

Geographic Distribution of Patients with ATL

High-resolution distribution of ATL cases was determined by acquisition of geographic coordinates of likely places of disease transmission by a global positioning system. Because leishmaniasis is believed to be transmitted mostly within plantations, where residents of the region live and work, patient residences were used as reference points for standardization purposes. Collected data were statistically compared as described below and plotted for visual inspection onto a high-definition satellite photograph of Corte de Pedra (ENGESAT, Curitiba, Brazil) by using ArcInfo version 8.3 software (Environmental Systems Research Institute Inc., Redlands, CA, USA).

Statistical Analyses

We studied distribution of ML and DL in Corte de Pedra by dividing the area into inner and coastal regions and compared frequencies of each of these forms of ATL in these 2 regions by using the χ^2 test. We also confirmed how patients with ML, DL, and MDL clustered by using the Cuzick and Edwards test in the geostatistical package Clusterseer version 2.2.4 (Terraseer Inc., Ann Arbor, MI, USA), which is sensitive for detection of geographic patterns. Times that patients resided at given places of residence were compared using Kruskal-Wallis 1-way analysis of variance. To analyze whether proximity to a DL patient was accompanied by an increased frequency of DL diagnosis among dwellers of the region, we used a geographic information system (ArcInfo version 8.3 software) to measure distances between the residence of each new patient during 1998–2002 (recent cases) and residences of all patients in the preceding 12 months (past cases). Resulting data were stratified into discrete distance intervals of 0–2,500, 2,501–5,000, 5,001–7,500, 7,501–10,000, and 10,001–12,500 m from patients with recent cases. Linear regression analysis was then used to compare the number of past cases to distances from patients with recent cases. A p value <0.05 was considered significant.

Results

A satellite view of Corte de Pedra with an arbitrary line dividing it into inner and coastal regions of approximately equal areas is shown in Figure 1. A total of 15 patients with DL were distributed in the coastal region and 15 patients with DL were distributed in the inner region ($p>0.05$, by χ^2 test). Patients with ML were rarely observed in the coastal area during the study period; 87% of patients with ML were observed in the inner region ($p<0.01$, by χ^2 test). To confirm the differences observed, we compared distribution of patients with ML and those with DL by using the Cuzick and Edwards test, which directly compares 2 sets of geographic events. Results were highly significant

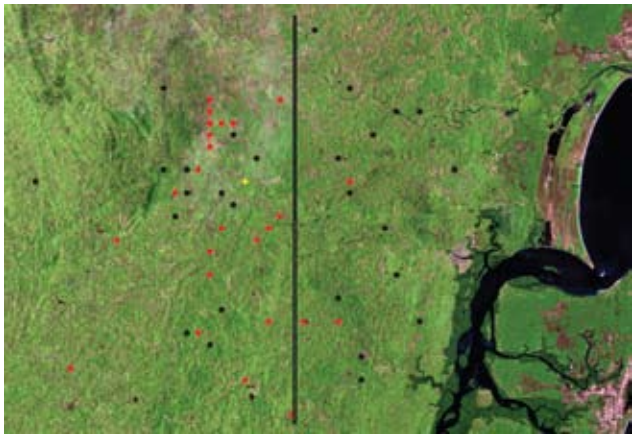

Figure 1. Satellite view of distribution of patients with disseminated leishmaniasis (DL; black circles) and patients with mucosal leishmaniasis (ML; red circles) in Corte de Pedra, Brazil, 1999–2003. Vertical line divides the region into inner (left) and coastal (right) areas of similar size. Total number of patients shown is smaller than the number of corresponding patients because of overlap of geographic coordinates for some patients. For details, see Materials and Methods. p = 0.00005, for data analyzed by using the Cuzick and Edwards test in Clusterseer version 2.2.4 (Terraseer Inc., Ann Arbor, MI, USA). The yellow mark indicates the health post.

(p = 0.00005), which indicated that these 2 types of leishmaniasis spread differently throughout Corte de Pedra.

Because we had detected different subpopulations (clades) of *L. braziliensis* genotypes defined by random amplified polymorphic DNA analysis in this area (*8*), we also determined whether distributions of patients with ML and those with DL overlapped distributions of some of those clades. Distributions of patients with ML and those with DL overlapped exclusively clades C and A plus D, respectively (p>0.05, by Cuzick and Edwards test). All other comparisons showed significant differences in distributions (p<0.03). Thus, different types of ATL caused by the same parasite species are distributed differently, even within a specific ATL-endemic region. Overlap between specific *L. braziliensis* subpopulations and patients with ML or DL also suggests that this phenomenon may be influenced by distribution of parasite genotypes in the region. However, this proposal needs to be tested by using a method that is capable of accurately identifying genotypes for a large panel of isolated parasites.

Observation of higher frequencies of ML in the inner region of Corte de Pedra suggested that extrinsic local factors not related to subpopulations of parasites might influence disease outcome. Because ≤40% of patients with DL also have mucosal involvement, we addressed this issue by comparing the distribution of patients with MDL with those with DL or ML. Patients with MDL showed a distribution pattern similar to that of patients with DL in this region (p = 0.8, by Cuzick and Edwards test; Figure 2) but different from that of patients with ML (p = 0.00003).

The broader distribution of DL compared with ML was surprising because only during the past decade has disseminated disease become more frequently diagnosed in Corte de Pedra. To better understand the dynamics of the spread of DL, we mapped the distribution of this disease in this region during 1993–2002. Our results show that up to 1996, DL, similar to ML, was concentrated mostly in the inner region of Corte de Pedra (Figure 3). However, the next 6 years showed progressive spread of DL to the coastal region until it reached an even distribution over the entire area (Figure 3). Two peaks in the incidence of DL

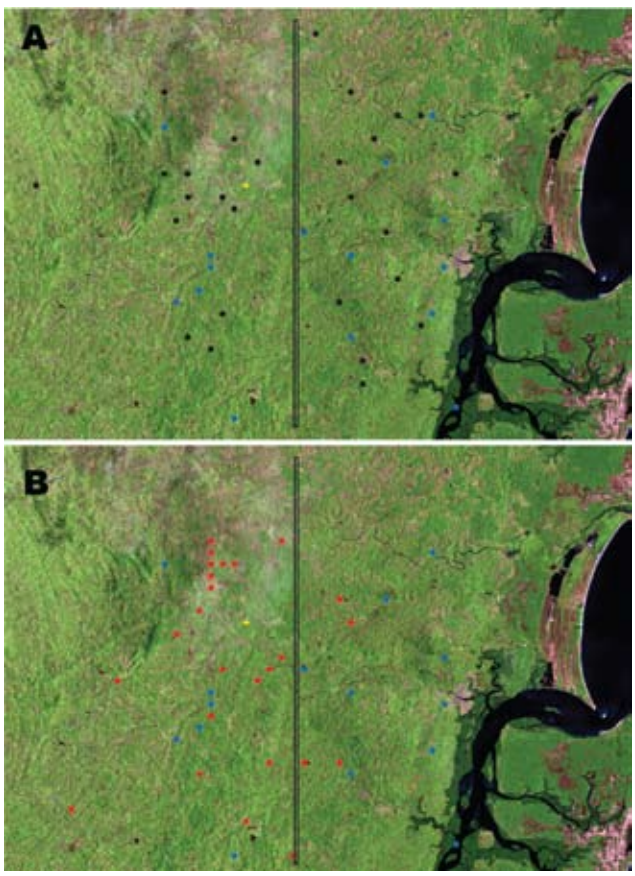

Figure 2. Satellite view of distribution of patients with disseminated leishmaniasis (DL) limited to the skin, patients with mucosal leishmaniasis (ML), and patients with DL showing mucosal involvement (MDL) in Corte de Pedra, Brazil, 1999–2003. A) Black circles indicate patients with DL, and blue circles indicate patients with MDL. B) Red circles indicate patients with ML, and blue circles indicate patients with MDL. Vertical line divides the region into inner (left) and coastal (right) areas of similar size. Total number of patients shown is smaller than the number of corresponding patients because of overlap of geographic coordinates for some patients. For details, see Materials and Methods. p = 0.8 in panel A and p = 0.00003 in panel B for data analyzed by using the Cuzick and Edwards test in Clusterseer version 2.2.4 (Terraseer Inc., Ann Arbor, MI, USA). The yellow mark indicates the health post.

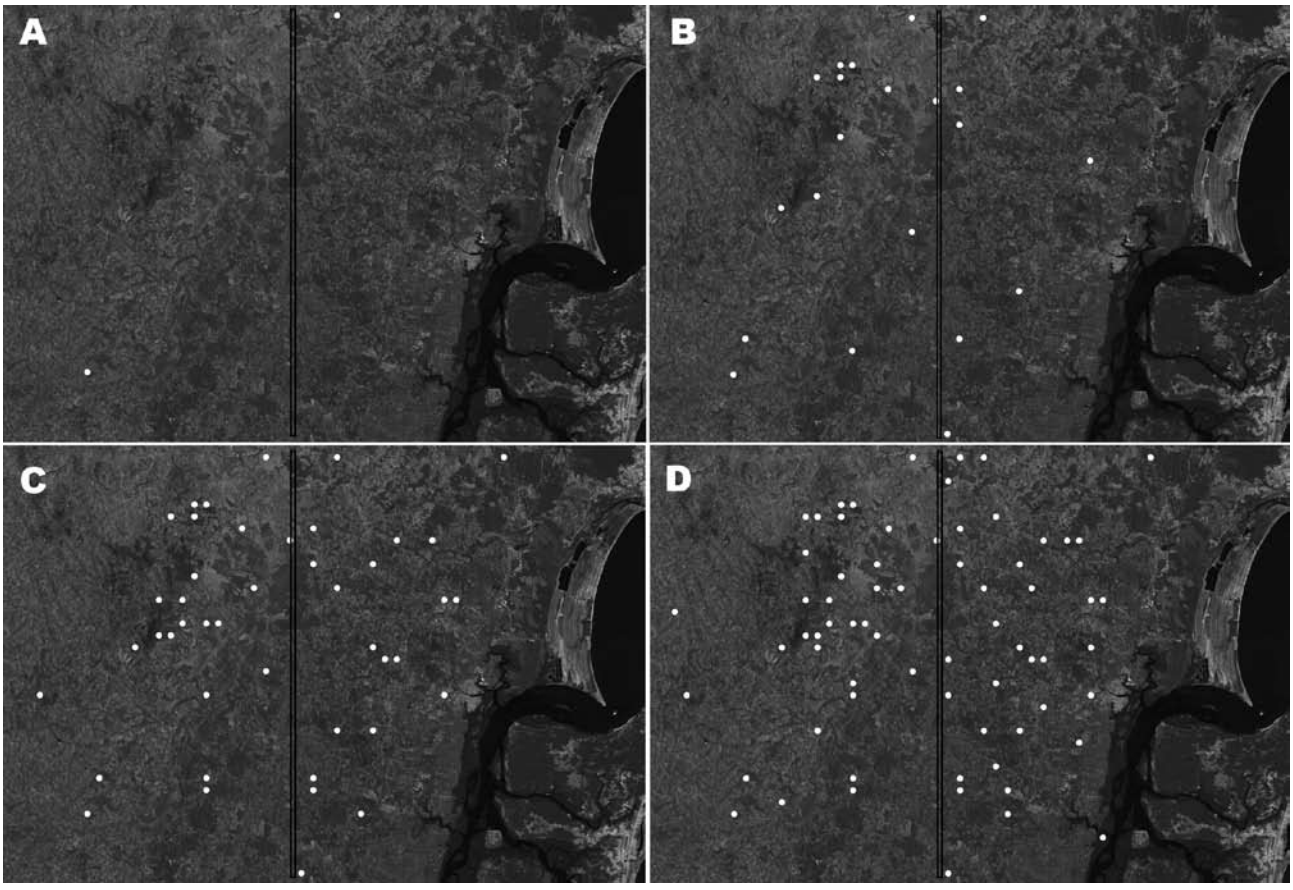

Figure 3. Satellite view of progressive spread of disseminated leishmaniasis in Corte de Pedra, Brazil, 1993–2002. Cumulative distributions of cases within affected areas are indicated by white circles. A) 1993, B) 1993–1996, C) 1993–1999, D) 1993–2002. The vertical line divides the region into inner (left) and coastal (right) areas of similar size.

paralleled the spread of DL in both regions of Corte de Pedra in the past decade (Figure 4). An increase was observed in the incidence of DL, with progressive spread into the inner region up until 1996, and another increase resulted in spread of this disease into the coastal region during 1998–2001. These 2 increases and the general increasing trend in incidence (Figure 4) also indicate that DL occurs in a pattern distinct from those of ML and CL. Linear regression (Figure 5) showed a significant inverse correlation between distance from a newly diagnosed case and frequency of diagnosis of DL patients in the preceding 12 months. These findings reinforce our previous suggestion that DL is an emerging disease (*9*) and that this form of leishmaniasis spreads in part through multiple outbreaks.

Discussion

A distinct geographic clustering of disease forms secondary to *L. braziliensis* infection was found in the study region. The overlap of ML and DL with genotypes of parasites associated with such outcomes in this area (clades C and A plus B, respectively) (*8*) suggests a cause–effect relationship. However, small sample sizes used to represent subpopulations of *L. braziliensis* genotypes found in Corte de Pedra and the low significance level obtained in comparisons prompt further studies before we can conclude whether the intraspecies parasite polymorphism affects disease distribution over an affected area. Our findings suggest a complex organization of the types of ATL within foci of active disease transmission.

The combination of human population movement in the study region and the usual long latency period of ML suggest cautious interpretation of our data. For ML, months to years may elapse between parasite acquisition and development of mucosal lesions. Thus, residences of patients with ML at the time of diagnosis may not reflect actual distributions of patients at the time of infection with the parasites. However, the likelihood of this possibility was precluded because participants' mean time of residence at their addresses at the time of ATL diagnosis was 17 years; mean times did not differ between the study groups.

Use of patients' homes as primary sources of geographic coordinates was another limitation intrinsic to

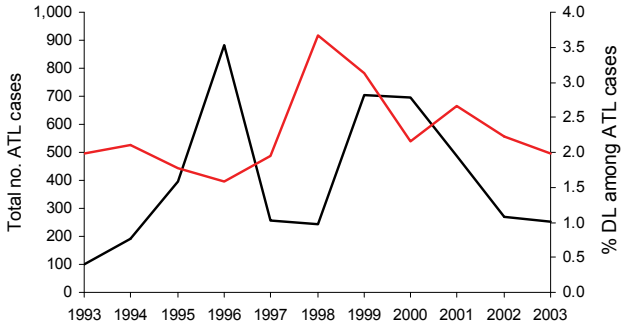

Figure 4. Distribution of American tegumentary leishmaniasis (ATL) (red line) and incidence of disseminated leishmaniasis/total ATL cases (black line) in Corte de Pedra, Brazil, 1993–2003.

the retrospective study design. A more accurate approach would be to delineate the personal activity spaces of each patient with ML or DL and then perform the geographic analyses. However, estimation of personal activity spaces is based on recollection of data gathered by questionnaires administered to the study participants regarding past information such as places of residence, history of migrations, work-related activities, and sites for these activities. Such information would be more reliable in a prospective study. Nonetheless, taking into account that residents of Corte de Pedra are mostly engaged in agriculture, often conducted within walking distance from their homes, and that population migration within this region is limited, we expect collection of geographic coordinates based on place of residence to be a fair approximation of actual places of infection with *Leishmania* spp. in most cases. More specifically, in our sample, >90% of patients had lived on farms for more than a decade.

Wide geographic differences in distribution of types of ATL have been reported for Ecuador and Peru (*6*,*7*). Our findings extend this observation to a smaller geographic setting that involves foci of endemic parasite transmission to humans. Distinct distributions of ML and DL over an area of only 10,000 km^2 support the complexity of *L. braziliensis* reported for this region (*8*). We hypothesize that some subpopulations of the parasite may be associated with disease manifestations and with factors that affect the transmission dynamics of *L. braziliensis* strains, such as various sandfly vectors present in the study area (*9*,*10*). However, other nonhuman hosts and reservoirs may also play a role.

African trypanosomiasis and schistosomiasis can illustrate the effects of parasite and vector heterogeneities on geographic distribution of these diseases. In eastern and southern Africa, infections with *Trypanosoma brucei rhodesiense* are characterized by an acute form of sleeping sickness. In western and central Africa, infections with *T. brucei gambiense* are characterized by a chronic form of this disease (*11*,*12*). Regions in which *Schistosoma hematobium* and *S. mansoni* are endemic are affected by the presence of snails of the genera *Biomphalaria* and *Bulinus*, respectively, in infested bodies of water. Although *S. hematobium*, which causes urinary schistosomiasis, was the predominant species in Egypt up to the 1930s (*13*), *S. mansoni*, which causes hepatointestinal disease, has progressively replaced *S. hematobium* in the Nile Delta and more recently in Upper Egypt (*14*). This change was paralleled by the concomitant replacement of *Bulinus truncatus* snails by *Biomphalaria alexandrina* snails in the affected areas, largely caused by human intervention and modification of the ecology for irrigation purposes (*15*–*17*).

Our findings also show that DL, which is a novel severe and difficult-to-treat form of leishmaniasis (*18*), is rapidly emerging and spreading within Brazil, 1 of the 5 countries with 90% of human cases of tegumentary leishmaniasis worldwide (*1*). The unique pattern of DL incidence (Figure 4), which shows 2 peaks, indicates that this form of leishmaniasis may occur as outbreaks. Conversely, the increased frequency of DL in persons living near persons with recent cases of this disease may be caused by other factors, such as uneven human population distribution and vector densities in the area and other potential environmental factors that affect parasite reservoirs. Human-to-vector-to-human transmission of parasites may also play a role in this form of American leishmaniasis. However, anthroponotic cycles are not considered to be predominant in ATL, except for a few reports suggesting that this mode of transmission may occur with *L. chagasi* within large urban areas in northeastern Brazil (*19*–*21*).

The 2 increases in the incidence of DL preceded similar increases in the total number of ATL cases by ≈2 years. One possible explanation would be that factors affecting

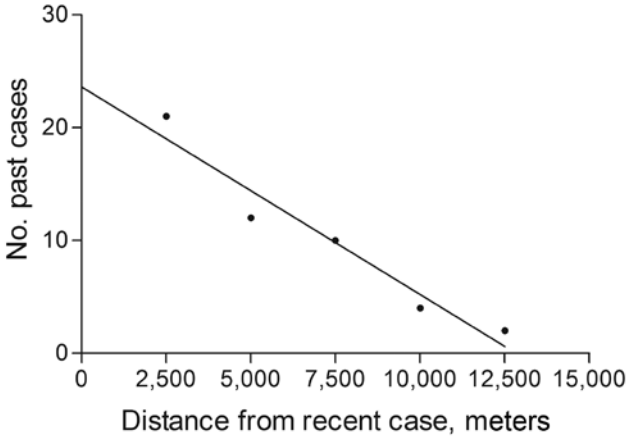

Figure 5. Linear regression comparing number of cases of disseminated leishmaniasis (past cases) diagnosed in the 12 months preceding a newly diagnosed case of DL (recent case) and distance to these recent cases, in increments of 2,500 meters in Corte de Pedra, Brazil, 1993–2003. p = 0.0061, r^2 = 0.94.

transmission of DL respond faster to changes in environmental parameters than those of CL and ML. The roles of climate and ecologic changes in leishmaniasis in regions near Corte de Pedra have been reported by Franke et al. (*22*). These authors reported a significant correlation between the southern oscillation of the El Niño phenomenon and the incidence of visceral leishmaniasis in the state of Bahia, Brazil. The major increase in the incidence of visceral leishmaniasis detected in that study occurred during 1995–1996, a period coincident with the first peak of DL shown in Figure 4. Although mechanisms responsible for the phenomena we describe remain elusive, we believe that information on clustering of disease types, increased frequency of DL among persons living near persons with recently diagnosed cases of the same disease, and predictive behavior of the incidence of DL relative to that of ATL may be used for better management and control of ATL.

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Lineage 2 West Nile Virus as Cause of Fatal Neurologic Disease in Horses, South Africa

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Serologic evidence suggests that West Nile virus (WNV) is widely distributed in horses in southern Africa. However, because few neurologic cases have been reported, endemic lineage 2 strains were postulated to be nonpathogenic in horses. Recent evidence suggests that highly neuroinvasive lineage 2 strains exist in humans and mice. To determine whether neurologic cases are being missed in southern Africa, we tested 80 serum or brain specimens from horses with unexplained fever (n = 48) and/or neurologic signs (n = 32) for WNV. From March 2007 through June 2008, using reverse transcription–PCR (RT-PCR) and immunoglobulin (Ig) M ELISA, we found WNV RNA or IgM in 7/32 horses with acute neurologic disease; 5 horses died or were euthanized. In 5/7 horses, no other pathogen was detected. DNA sequencing for all 5 RT-PCR–positive cases showed the virus belonged to lineage 2. WNV lineage 2 may cause neurologic disease in horses in southern Africa.

West Nile virus (WNV), a mosquito-born flavivirus of the family *Flaviviridae*, is widely distributed throughout Africa, the Middle East, Asia, parts of Europe, Australia, North and South America, and the Caribbean. The WNV transmission cycle involves birds as vertebrate hosts and ornithophilic mosquitoes as maintenance vectors (*1*). Isolates of WNV fall into 2 major genetic lineages: lineage 1 is found in North America, North Africa, Europe, and Australia; lineage 2 strains are endemic to southern Africa and Madagascar (*2*,*3*). Recently, additional lineages in central and eastern Europe (lineages 3 and 4) (*4*,*5*) and India (lineage 5) have been reported (*6*).

Humans and horses are incidental hosts for WNV (*7*). Although most infections are benign, ≈20% of infected persons have fever, rash, arthralgia, and myalgia, and for ≈1% of these, severe disease, including meningoencephalitis, encephalitis, and polio-like flaccid paralysis, may develop. Rare cases result in hepatitis, myocarditis, pancreatitis (*8*), and death (*1*). Signs in horses are ataxia, weakness, recumbency, and muscle fasciculation (*9*–*11*). Seroepidemiologic studies suggest that asymptomatic infections frequently occur in horses (*12*,*13*), but neurologic infections result in a high case-fatality rate (30%–40%) (*14*). In 2002 the largest outbreak of WNV encephalomyelitis in horses was recorded in the United States; 15,257 cases were reported from 40 states (*11*). This outbreak was followed in 2003 by the largest outbreak in humans in the Northern Hemisphere (9,832 cases) (*1*). The number of cases among horses was greatly reduced after the introduction of an inactivated vaccine for animals (*10*,*15*,*16*).

In the Karoo, a semidesert region in South Africa, in 1974, WNV caused one of the largest outbreaks ever recorded in humans, affecting tens of thousands of people. During this outbreak thousands of persons visited their local clinicians; however, no cases of neurologic disease were reported. In the 1980s, an epizootic involving WNV and Sindbis virus occurred in the Witwatersrand area of the Gauteng Province in South Africa; this epizootic

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affected hundreds of persons (*17*). Since then, the number of confirmed human cases has been ≈5–15 per year, although only a proportion of cases are subjected to laboratory investigation. In South Africa, severe disease has been recognized, including fatal hepatitis and several nonfatal encephalitis cases in humans as well as deaths in ostrich chicks, a foal, and a dog (*2*,*18*). Recently, a lineage 2 strain was isolated from encephalitic birds in central Europe, which suggests that lineage 2 strains can spread outside their known geographic range and may cause severe disease in birds in non–WNV-endemic countries (*19*).

A recent serologic survey of thoroughbred horses has confirmed that WNV is widely distributed throughout South Africa; 11% of yearlings seroconverted over 1 year and up to 75% of their dams had been exposed (*13*). This study led to the postulation that endemic lineage 2 WNV strains were not a cause of neuroinvasive disease in horses because none of these horses had shown any clinical signs. Three seronegative horses inoculated with a recent WNV lineage 2 strain (SPU381/00) isolated from a person with benign disease did not develop clinical signs (*13*). However, the strain used in these experiments was subsequently shown to be of low neuroinvasiveness in mice, compared with certain other South African strains (*20*). Subclinical cases are also frequently reported in horses in the United States (*12*). Experimental infection of 12 horses with the highly neuroinvasive NY99 strain resulted in neuroinvasive disease in only 1 animal; the remaining animals all seroconverted, but clinical disease did not develop and virus could not be isolated from their organs (*21*).

Comparison of South African and North American strains of WNV has shown that differences in neuroinvasiveness are associated with specific genotypes, not with lineage, and that highly neuroinvasive strains exist in lineages 1 and 2 (*20*,*22*). To determine whether equine cases of WNV are being missed in South Africa, for 16 months we investigated horses with pyrexia or unexplained neurologic signs.

Materials and Methods

Clinical Cases

From March 2007 through June 2008, serum and/or postmortem brain specimens were collected from horses in South Africa with acute fever or neurologic disease; cases were detected by passive surveillance. Specimens were sent to the Department of Medical Virology, University of Pretoria, by the main veterinary diagnostic facilities in South Africa (Onderstepoort Veterinary Institute and the University of Pretoria Faculty of Veterinary Science, Onderstepoort) and by a group of private equine veterinarians from Gauteng and the Northern Cape provinces, who were invited to submit samples from horses with suspected cases. Specimens from horses with fatal and severe neurologic cases mostly came from the University of Pretoria (Pathology Department, Faculty of Veterinary Sciences) and the Onderstepoort Veterinary Institute; most were from horses with neurologic signs for which no alternative diagnoses were made. Specimens from horses with fever were collected by the private veterinarians around Gauteng from horses with less severe disease. Cases that resembled African horse sickness, i.e., pulmonary or cardiac disease, were not included.

Reverse Transcription–PCR Screening and DNA Sequencing

RNA was extracted with an RNeasy kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations. A nested real-time reverse transcription–PCR (RT-PCR) specific for the WNV NS5 gene that distinguishes between lineages 1 and 2 with WNV-specific FRET probes on the basis of dissociation curve analysis was used to screen specimens (*23*). A product of 214 bp could be seen on agarose gel. All positive RT-PCR products were confirmed by sequencing of the NS5 region (genome positions 9091–9191) and analyzed on an ABI 3130 sequencer as recommended by the supplier (Applied Biosystems, Foster City, CA, USA). RT-PCR amplification and sequence analysis of a 255-bp region of the E-protein (genome positions 1402–1656) was conducted as described before (*2*,*24*).

Phylogenetic Analysis

Sequences were aligned by using ClustalX version 1.83 (http://bips.u-strasbg.fr/fr/Documentation/ClustalX) with the multiple-sequence alignment option. Maximum-likelihood trees were generated by using PHYML (*25*). Bootstrap statistics for 1,000 replicates were calculated by neighbor-joining analysis with a maximum composite likelihood model and a gamma parameter of 2, using MEGA version 4 (*26*). Distances between sequences were calculated by using MEGA version 4 (*26*) with the P-distance analysis option.

Serologic Testing

Horse serum samples were tested for flavivirus-specific antibodies first by hemagglutination inhibition (HI) assay and next by a WNV-specific immunoglobulin (Ig) M-capture ELISA on HI-positive specimens as described in (*27*). IgM-positive specimens were confirmed by neutralization assays as described below.

IgM-Capture ELISA

The IgM-capture ELISA was conducted as described in (*28*) with virus-specific modifications. In brief, 100 μL/well of goat antihorse IgM μ-chain (Kirkegaard and Perry

Laboratories, Inc., Gaithersburg, MD, USA) diluted 1:500 in phosphate-buffered saline (PBS) without magnesium and calcium, pH 7.4, was adsorbed onto ELISA plates (MaxiSorp, Nunc, Denmark) overnight in a humidity chamber at 4°C. Plates were washed 3× with 0.1% Tween-PBS; the same washing procedure followed each subsequent stage of the assay. Plates were blocked with 200 μL/well of 10% skim milk (Merck, Darmstadt, Germany) in PBS and incubated at 37°C for 1 h. After washing, duplicate volumes of 100 μL of each test and control serum diluted 1:400 in 2% skim milk in PBS (diluting buffer) were added to wells in rows A–D:1–12 and to corresponding wells in rows E–G:1–12 and incubated at 37°C for 1 h. After washing, 100 μL/well WNV antigen and mock antigen, diluted 1:400 in diluting buffer, was added to rows A–D:1–12 and E–G:1–12, respectively. After incubation at 37°C for 1 h and washing, 100 μL/well of mouse anti-WNV antibody diluted 1:1,000 was added to each well and incubated at 37°C for 1 h. Production, inactivation, preservation, and safety testing of WNV antigen (strain H442/58), mock antigen, and hyperimmune mouse anti-H442/58 serum were conducted as described (*29*). After washing, 100 μL/well goat antimouse IgG (H + L chain) HRPO-conjugate (Zymed Laboratories, Inc, San Francisco, CA, USA) diluted 1:2,000 was added to each well and incubated at 37°C for 1 h. Plates were washed, and 100 μL/well ABTS (2,2′-azino diethyl-benzothiazoline-sulfonic acid) peroxidase substrate (Kirkegaard and Perry Laboratories, Inc.) was added to each well and the plate was incubated in the dark for 30 min at room temperature (22°–25°C). The stop reagent, 1% sodium dodecyl sulfate, was added, and optical densities (ODs) were measured at 405 nm. Specific activity of each serum sample (net OD) was calculated by subtracting the nonspecific OD in wells with mock antigen from the specific OD in wells with virus antigen. A threshold value for interpretation of results was determined as mean plus 3 standard deviations of duplicate net OD readings for negative control serum.

HI Assay

The HI assay was conducted as described previously (*27*), except that the sucrose-acetone-extracted H442/58 strain of WNV derived from mouse brain tissue (produced as described for the IgM-capture ELISA above) was used as an antigen. A serum sample was considered seropositive if it had a titer ≥1.3, equivalent to a serum dilution ≥1:20.

Serum Neutralization Test

The serum microneutralization procedure using African green monkey kidney (Vero cells) was conducted as described previously (*30*), except that the SPU 93/01 isolate of WNV recovered in southern Africa was used as a source of antigen. The titer was expressed as the reciprocal serum dilution that inhibited 100% of viral cytopathic effect. A serum sample was considered positive when it had a virus neutralization titer ≥1.0, equivalent to a serum dilution ≥1:10.

Tests for Differential Diagnoses

African horse sickness virus (AHSV), equine encephalosis virus (EEV), and equine herpesviruses (EHV) 1 and 4 were identified by using viral culture and antigen detection assays and/or complement fixation tests (*31*) on serum samples and using RT-PCR to detect AHSV and EHV (*32*). Rabies virus infections were identified by fluorescent antigen detection tests on brain tissue (*33*). Immunoperoxidase staining for EEV, EHV, AHSV, and flavivirus was performed, according to the method adapted from (*34*), on histopathologic sections of brain, spinal cord, spleen, liver, and lung after postmortem investigations.

Virus Culture

All specimens were inoculated onto Vero cell monolayers (18 hours old) in 25 cm^2 tissue culture flasks supplemented with Eagle Minimum Essential Medium containing 2% fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 1mg/mL L-glutamine (GIBCO BRL, Invitrogen, Carlsbad, CA, USA). Inoculated cultures were microscopically observed for cytopathic effects for 10 days.

Results

Screening of Specimens

A total of 80 serum or brain specimens from horses with unexplained fever (n = 48) and/or neurologic symptoms (n = 32) were tested for WNV over the 16-month period. Most specimens from horses with neurologic signs came from Onderstepoort Veterinary Institute and the Department of Medical Virology, University of Pretoria; horses were from across the country; the specimens from horses with fever were mostly from horses with less severe disease in Gauteng.

WNV infection was identified for 7 (21.8%) of 32 acute neurologic cases (Table 1). For 5 cases, acute WNV infection could be confirmed by the presence of RNA through RT-PCR (4 brain specimens, 1 serum sample) as well as virus isolation from 1 brain specimen. Two HI-positive cases could be confirmed as probable recent WNV infections by IgM ELISA and WNV-specific antibodies, which were confirmed by neutralization assays. None of the horses with fever had WNV, although EEV was isolated from several. Affected horses ranged from 4 months to 19 years and were thoroughbreds, Arabians, Lipizzaners, Welsh ponies, warmbloods, and mixed breeds. Cases were identified in Gauteng, the Northern Cape, and North-Western provinces and occurred in April 2007 and from March through

Table 1. Viral diagnostic findings, West Nile virus–infected horses, South Africa*

Case no.	Date sample received	Location	Specimen	Final diagnosis	Results of tests for other viruses
SAE12/07	2007 Apr 23	Johannesburg, Sandton, Gauteng	Plasma	WNV IgM+	AHSV–, EEV–
SAE89/08	2008 Mar 3	Colesburg North Cape	Plasma	WNV IgM+	AHSV–, EEV–
HS101/08	2008 Apr 15	Tiegerpoort Pretoria, Gauteng	Brain	WNV PCR+, DNA sequencing L2, virus isolae WNV PCR+	Rabies–, AHSV–, EEV–, EHV–, flavivirus antigen in lumbar spinal cord section and in some gray matter axons (Figure 1)
M123/08	2008 May 8	Midrand, Gauteng	Brain	WNV PCR+, DNA sequencing L2	AHSV–, EEV–, EHV–
HS125/08	2008 May 26	Pretoria, Hammans-kraal, Gauteng	Brain	WNV PCR+, DNA sequencing L2	AHSV from spleen PCR+; AHSV from lung IHC+, lymph node IHC–, liver IHC–
SAE126/08	2008 Mar 7	Midrand, Gauteng	Brain	WNV PCR+, DNA sequencing L2	AHSV RT-PCR+, AHSV type 7; IHC EEV–, AHSV+, AHSV IHC+ (lung liver, heart)
SAE134/09	2008 Jul 18	Potchef-stroom, North-Western Province	Serum	WNV PCR+, DNA sequencing L2	AHSV–, EEV–, EHV-1 weak sero+

*WNV, West Nile virus, Ig, immunoglobulin; AHSV, African horse sickness virus; EEV, equine encephalosis virus; EHV, equine herpesvirus; L2, lineage 2; –, negative; +, positive; RT-PCR, reverse transcription–PCR; IHC, immunohistochemistry.

June 2008. Of the 7 WNV-infected horses, 5 died or were euthanized for humane reasons (Table 2).

Co-infections

For 5 horses, 3 of which had fatal infections, no virus other than WNV was identified. Two horses that died (8 months of age and 6 years of age) had co-infections with AHSV. For both horses, WNV was detected by RT-PCR in the brain and AHSV was detected in the spleen or lungs but not in the brain. The 6-year-old horse had documented records of up-to-date AHSV vaccinations. All cases were negative by immunoperoxidase staining, virus isolation, and/or PCR for EHV and EEV.

Clinical Description of WNV

All confirmed WNV infections were identified in the group of horses with neurologic signs (Tables 1,2). Five horses with WNV died or had to be euthanized after becoming paralyzed. Signs included ataxia in all cases (7/7), weak hindlimbs and/or forelimbs and paresis (4/7), complete paralysis (2/7), seizures (2/7), chewing (1/7), partial blindness (2/7), jaundice and/or hepatitis (2/7), and miosis of the pupils (1/7). One horse (HS101/08) (Table 2) was recumbent from quadriplegia and displayed limb paddling, teeth grinding, and muscle twitching; signs progressed over 3 days to those similar to rabies, i.e., chewing fits, seizures, and coma before death, but fluorescent antigen detection results for rabies were negative. Fever was intermittent and not reported for all horses. The 2 WNV-infected horses that survived showed clinical signs for ≈21 days and had to be rested for several months, but each recovered fully.

Postmortem investigations were performed on 4 horses (HS125/08, SAE 126/08, M123/08, and HS101/08). All 4 had positive WNV results by real-time RT-PCR of brain tissue, and a WNV isolate was obtained from the brain of HS101/08. Virus isolation attempts on frozen specimens from the other 3 horses were not successful. AHSV was also isolated from the lungs and spleen of SAE126/08 and HS125/08. Immunohistochemical staining of organs, to identify AHSV, EEV, and EHV, confirmed the presence of AHSV in SAE126/08, but results were negative for all other horses. Detailed postmortem findings for the 2 horses that had no complications (HS101/08 and M123/08) are shown in Table 2. Immunohistochemical staining for flaviviruses demonstrated antigen in the lumbar spinal cord and in some gray matter axons of HS101/08 (Figure 1).

Sequence Confirmation and Phylogenetic Analysis

The 5 specimens that were positive for WNV by RT-PCR were subjected to sequence analysis and confirmed to be WNV by BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) search analysis. Phylogenetic comparison of the 189-bp NS5 gene region with representative sequences of all 5 currently recognized WNV lineages confirmed that all strains clustered with lineage 2 (Figure 2) and were closely related to lineage 2 strains isolated from humans in South Africa. The horse specimens differed by 0%–3% nucleotides from southern African lineage 2 strains in the NS5 region. Recent (2008) strains displayed the least variation from each other (0%–1.2%) and were most closely related to strains SPU116/89 and SA93/01, which had been isolated from a person with fatal hepatitis and nonfatal encephalitis, differing by 1.2% and 1.8%, respectively. Specimens from southern Africa had 1.2%–4.8% differences compared with the lineage 2 Hungary isolate of 2004. All lineage 2 strains from southern Africa differed by 18.6%–19.2% from the Madagascar strain and by 19.2%–25.7% with lineage 1 strains. Additional phylogenetic analysis of a more variable

region of the E-protein was performed on the 2008 isolate obtained from the brain of HS101/08 (Figure 3). HS101/08 had 0.07%–2% differences in the E-protein region compared with all other southern Africa lineage 2 strains and was the closest to SPU116/89; it differed by 6.9% from the prototype Uganda strain (B956), differed by 17.3% from a strain from Madagascar, and 23% from NY385/99 (lineage 1). The 2004 Hungary lineage 2 strains differed by only 1.6% from the southern Africa strains, which suggests that the Hungary strains may have originated from southern Africa. On the amino acid level, all southern Africa lineage 2 strains' E-protein regions were identical to each other and to the Hungary strain but differed by 7% from the Madagascar strain and by 23% from the lineage 1 NY385/99 strain.

Discussion

Lineage 2 WNV is known to be endemic to southern Africa; however, few cases of WNV disease have been reported in recent years and the role of lineage 2 as a human and horse pathogen has been disputed (*3*,*13*). Our previous investigations of the pathogenicity of lineage 2 strains in humans and mice have indicated the existence of lineage 2 strains that are highly pathogenic and neuroinvasive in mice (*2*,*20*). WNV is rarely considered in a differential diagnosis for neurologic disease of humans or horses in southern Africa. In the Northern Hemisphere, horses are highly susceptible and develop severe WNV disease and thus have been used as sentinels for human cases (*35*). Most indigenous birds in southern Africa do not display disease despite a high seroprevalence for WNV infection (*17*), which suggests that genetic resistance may exist in local birds.

We investigated 80 cases of unexplained disease in horses compatible with WNV clinical signs. It can be expected that not all cases were identified but rather that samples from only economically valuable horses were sent in for laboratory investigation when unexplained neurologic signs and fevers

Table 2. Clinical findings, West Nile virus–infected horses, South Africa*

Case no.	Age	Clinical findings	Outcome	Postmortem findings	Histopathologic findings
SAE12/07	5 y	Neurologic signs, hind and fore limb ataxia, pupil miosis, head held to left	Survived		
SAE89/08	1 y	Neurologic signs, hind and forelimb ataxia, fever, complete paralysis, anorexia, hepatitis	Died (euthanized after week of fluid therapy)	Postmortem not done	
HS101/08	8 y	Neurologic signs, severe ataxia especially hind limb, seizures and chewing, froth from mouth, fever, recumbency, paralysis	Died	Marked generalized subcutaneous edema involving trunk and proximal forelimbs, edema (periaortic, coronary grooves, neck, and hind quarters), partial pulmonary collapse, foam-filled trachea, mild serosanguinous hydrothorax, moderate hydropericardium, subpleural petechiae and ecchymoses, epicardial grooves and at bases of the mitral valves	Lesions in gray matter and meninges of lumbar spinal cord (pericentral canal gliosis, edema, gray matter gliosis with occasional neuronal degeneration or death), mild perivascular cuffing with mononuclear cells and scattered neutrophils, moderate vascular congestion, occasional perivascular petechiae, mild leptomeningitis (mostly round cells) and occasional mild spinal ganglioneuritis, segmental mural necrosis of the dural blood vessels and neutrophil invasion, less-marked lesions in rest of spinal cord and white matter of midbrain
M123/08	4 mo	Fever, neurologic signs, paralysis (Schiff-Sherington sign), rectal prolapse	Died	Severe perirenal and intermuscular edema, severe diffuse interlobular lung edema and mild serous hydropericardium	Marked white matter lesions in peripheral lateral and ventromedial spinal cord white matter, mild perivascular round cell cuffing in midbrain, patchy spongiosis and gliosis in brain and cerebellum white matter
HS125/08	8 mo	Neurologic signs, ataxia, mild colic, swollen head and neck	Died (shot by owner)	Severe lung edema and congestion, moderate serous hydropericardium, intermuscular edema	Subtle lesions on brain and cerebellum sections (e.g., gliosis and spongiosis in white matter and vascular leukostasis)
SAE126/08	6 y	Neurologic signs, head hanging, sick for week	Died suddenly		Spinal cord lesions
SAE134/09	19 y	Neurologic signs, partial blindness, hyperexcitability, seizures	Survived		

*AHSV, African horse sickness virus; IHC, immunohistochemistry.

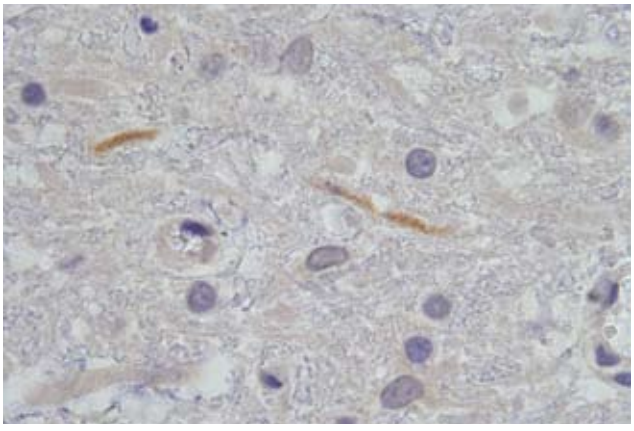

Figure 1. Histopathologic section of 2 lumbar spine gray matter dendrites that stained immunohistochemically positive with flavivirus antiserum on postmortem tissue of horse HS101/08. Magnification ×1,000.

were noted by the resident veterinarian. For 7 horses, WNV could be confirmed by RT-PCR and virus isolation (*5*) or as the probable cause of signs due to the presence of IgM confirmed by neutralization assays, suggesting a recent WNV infection. All 7 horses had substantial neurologic signs. The fact that 5 cases were fatal suggests that up to 7 (21.8%) of 32 cases of undetermined neurologic disease investigated in horses in South Africa over 16 months were caused by WNV. The high mortality rate (5 [71%] of 7 horses) and the clinical signs correlated with findings of neurologic disease in horses in the Northern Hemisphere.

Samples from 5 horses were positive by nested real-time RT-PCR (4 brain tissue specimens and 1 serum sample), and 2 were IgM positive. These findings indicate that these were useful diagnostic and surveillance tools.

Sequencing of cDNA confirmed that all WNV infections were caused by lineage 2 and were closely related to lineage 2 strains previously isolated from human case-patients in South Africa. The NS5 gene regions of the 2008 strains were most closely related to each other and to recent human isolates. The WNV SPU93/01 isolate was recovered from an immunocompetent adult who was hospitalized with encephalitis in Johannesburg in 2001; the SPU116/89 isolate was recovered from the liver of a human patient with fatal hepatitis. Analysis of horse isolate HS101/08 indicated that the 2008 strains were unique in the E-protein region and most closely related to SPU116/89, followed by SPU93/01. Each of these strains is highly neuroinvasive in mice (*18*,*20*).

All cases were identified in late summer to autumn, timing that coincides with AHSV and EEV outbreaks in South Africa (*36*,*37*). The extent of these cases caused by concurrent and cocirculating viruses may contribute to the underrecognition of WNV cases in horses in southern Africa. Some of the WNV cases reported here were submitted as suspected AHSV and EEV infections. AHSV is an insect-borne orbivirus that causes a noncontagious disease of equids and is associated with high death rates in sub-Saharan Africa. African horse sickness occurs in 4 forms: horse sickness fever (mild), cardiac (>50% mortality rate), mixed (75% mortality rate), and pulmonary (95% mortality rate). Signs of the cardiac form include subcutaneous edema, particularly of the head, neck, chest, and supraorbital fossae. The pulmonary form is peracute and may develop so rapidly that an animal can die without previous signs of illness. It is characterized by depression, fever, respiratory distress, severe dyspnea and coughing spasms, and severe sweating; terminally, quantities of frothy fluid may

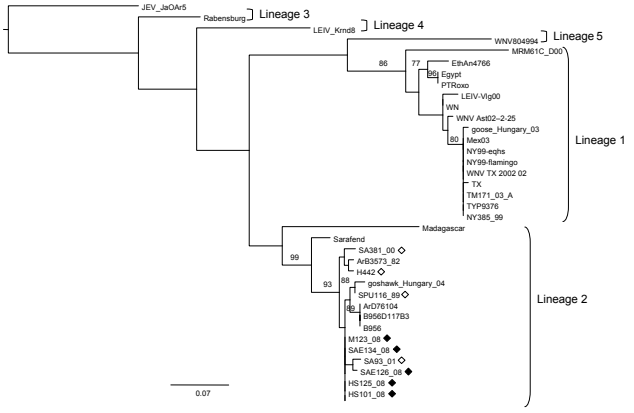

Figure 2. Maximum-likelihood comparison of the partial NS5 gene of West Nile virus (WNV) strains identified in horses in South Africa in 2008 with representative sequences of other WNV lineages. Bootstrap statistics are shown on the branches; only values >70% are included. Scale bar indicates 0.07 nt changes. Japanese encephalitis virus (JEV) was used as an outgroup. Black diamonds, WNV strains identified in horses in South Africa in the present study; white diamonds, WNV strains isolated from humans in South Africa in previous years. WNV strains and accession numbers and origin: HS123_08, FJ464376, South Africa; HS125_08, FJ464377, South Africa; HS101_08, FJ464378, South Africa; SAE126_08, FJ464379, South Africa; SAE134_08, FJ464380, South Africa; SA381_00, EF429199, South Africa; SA93_01, EF429198, South Africa; SPU116_89, EF429197, South Africa; goshawk_Hungary_04, DQ116961, Hungary; B956 polyprotein gene-1937, AY532665, Uganda; B956 117B3, M12294, Uganda; ArD76104, DQ318019, Senegal; H442, EF429200, South Africa; ArB3573_82, DQ318020, Central African Republic; Sarafend, AY688948, Uganda; Madagascar AnMg798, DQ176636, Madagascar; PTRoxo, AM404308, Portugal; Egypt101, AF260968, Egypt; EthAn4766, AY603654, Ethiopia; Kunjin MRM61C, D00256, Australia; WNV Italy 1998 equine, AF404757, Italy; WNV Ast02–2-25, DQ374653, Russia; LEIV-Vlg00–27924, AY278442, Russia; LEIV-Krnd88–190, AY277251, Russia; goose_Hungary_03, DQ118127, Hungary; NY385_99, DQ211652 NY, USA; NY99-eqhs, AF260967 NY, USA; NY99-flamingo382–99, AF196835 NY, USA; TYP9376 NY385_99, AY848697 NY, USA; WNV TX 2002 02, DQ164206 TX, USA; TM171_03, AY371271, Mexico; Mex03, AY660002, Mexico; TX 2002-HC, DQ176637 TX, USA; WNV804994, DQ256376, India; Rabensburg 97–103, AY765264, Czech Republic; JEV JaOAr5982, M18370, Japan.

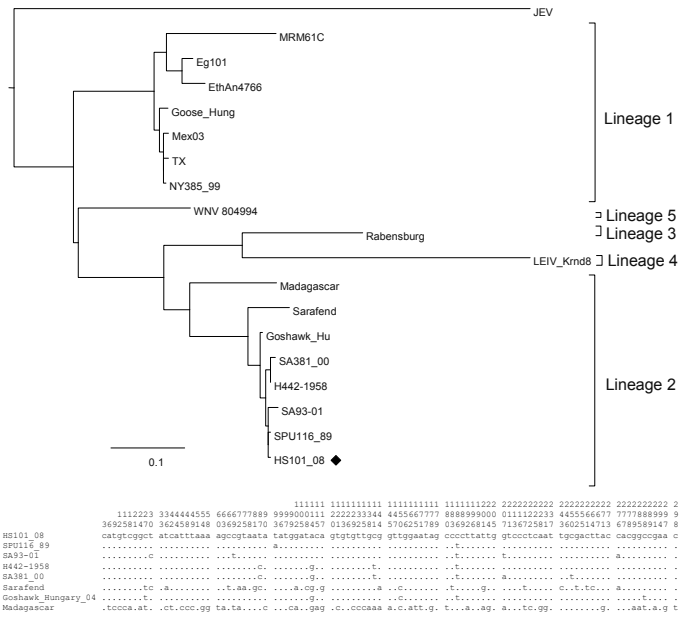

Figure 3. Maximum-likelihood analysis of the E-protein region of a West Nile virus isolate, HS101_08 (black diamond), recovered from horses in 2008 compared with isolates obtained from humans and animals from South Africa and other regions of the world. Nucleotide differences between lineage 2 strains included in the alignment are shown in the summarized alignment below the tree, indicating only unique nucleotides. Vertical numbers above the alignment indicate the position of each variable site on the gene fragment. Scale bar indicates nucleotide substitutions per site.

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                                                  111111 1111111111 1111111111 1111111222 2222222222 2222222222 2222222222 2
                    1112223 3344444555 6666777889 9999000111 2222233344 4455667777 8888999000 0111122233 4455566677 7777888999 9
                   3692581470 3624589148 0369258170 3679258457 0136925814 5706251789 0369268145 7136725817 3602514713 6789589147 8
HS101_08           catgtcggct atcatttaaa agccgtaata tatggataca gtgtgttgcg gttggaatag cccctattg gtccctcaat tgcgacttac cacggccgaa c
SPU116_89          ..........c .......... ...t..... .......... .......... .......... ..t..... t......... .......... a......... .
SA93-01            .........c .......... ...t..... .......... .......... .......... ..t..... t......... .......... a......... .
H442-1958          .......... .......... ........c. .......g.. .......t.. .......... ..t..... ..t..... .......... .......... .
SA381_00           .......... .......... ........c. .......g.. .......t.. .......... ..t..... a......... ...t..... .......... .
Sarafend           .......tc .a........ ...t.aa.gc. ....a.cg.g .........a ..c..... .t.....g.. ....t..... c..t.tc... a......... .
Goshawk_Hungary_04 ........t. .......... .......... .......g.. .......... ..c..... ..t..... .......... .......... .....t.... .
Madagascar         .tcccca.at. .ct.ccc.gg ta.ta....c ...ca..gag .c..cccaaa a.c.att.g. t...a..ag. a...tc.gg. .........g. ...aat.a.g t
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be discharged from the nares and periods of recumbency may occur (*37*). Annual vaccination with a live attenuated polyvalent AHSV vaccine provides protection against most of the 9 serotypes, although vaccine failures have been reported and outbreaks continue to occur. Vaccination complicates diagnosis by serologic testing, and virus may be isolated from horses after vaccination (*38*). EEV is a closely related orbivirus also widespread in southern Africa; it may cause fever, abortion, and neurologic involvement (*39*). Outbreaks of both occur annually and are associated with unusually high rainfall and increased vector (*Culicoides* midges) populations (*40*), which will also favor an increase in *Culex* mosquitoes, which transmit WNV. The 2007–2008 summer and late autumn seasons in South Africa were marked by unusually high rainfall, and the numbers of AHSV and EEV cases were also high (www.africanhorsesickness.co.za). Clinical signs of AHSV do not resemble those of WNV, and neurologic signs are not characteristic; but in the absence of laboratory testing, horses that die of unexplained causes may be dismissed as having been infected with AHSV. The identification of 2 AHSV–WNV co-infections in this study is therefore not surprising. In both cases, veterinarians and owners noted neurologic signs, which are not typical of AHSV. These co-infections may increase the disease severity for each virus and should be taken into consideration in areas where both diseases are endemic. In most (5/7) WNV cases identified in this study, no other pathogens were identified. All identified horses with WNV infection had neurologic involvement, which could be used to distinguish WNV–AHSV co-infections from conventional AHSV infections. None of the identified horses with EEV had neurologic signs, although EEV virus was isolated from several horses with fever.

Our findings should raise awareness that WNV lineage 2 can cause neurologic disease in both horses and humans in southern Africa. In the absence of bird deaths, detection of cases in horses may serve as an early warning system for WNV outbreaks among humans.

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Hantaviruses in Rodents and Humans, Inner Mongolia Autonomous Region, China

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Surveys were carried out in 2003–2006 to better understand the epidemiology of hantaviruses in the Inner Mongolia Autonomous Region of China (Inner Mongolia). Hemorrhagic fever with renal syndrome (HFRS) was first reported in this region in 1955 and has been an important public health problem here since then. During 1955–2006, 8,309 persons with HFRS were reported in Inner Mongolia (average incidence rate 0.89/100,000), and 261 (3.14%) died. Before the 1990s, all HFRS cases occurred in northeastern Inner Mongolia. Subsequently, HFRS cases were registered in central (1995) and western (1999) Inner Mongolia. In this study, hantaviral antigens were identified in striped field mice (*Apodemus agrarius*) from northeastern Inner Mongolia and in Norway rats (*Rattus norvegicus*) from middle and western Inner Mongolia. Phylogenetic analysis of hantaviral genome sequences suggests that HFRS has been caused mainly by Hantaan virus in northeastern Inner Mongolia and by Seoul virus in central and western Inner Mongolia.

Hantaviruses, members of the family *Bunyaviridae* and genus *Hantavirus*, can cause 2 human zoonoses: hemorrhagic fever with renal syndrome (HFRS), seen in Asia and Europe; and hantavirus pulmonary syndrome, seen in the Western Hemisphere (*1*). Rodents are a main virus reservoir and a source of human infection. Transmission of hantaviruses from rodents to humans generally occurs through inhalation of aerosolized excreta (*1,2*). In hantavirus-endemic areas, HFRS outbreaks have occurred among farmers and others who have close contact with excreta of infected rodents (*1–3*).

HFRS has been recognized as a notable public health problem in China (*4,5*). Currently, HFRS is endemic in 28 of 31 provinces in mainland China (*5,6*). HFRS cases have occurred mainly in China's northeastern, eastern, central, and southwestern parts, which are characterized by humid and semihumid zones, but HFRS has rarely occurred in northwestern China, which is in an arid zone (*2,4–7*). Although 7 sero/genotypes of hantaviruses have been identified in China (*8–11*), only Hantaan virus (HTNV), which is carried by striped field mice (*Apodemus agrarius*), and Seoul virus (SEOV), which is carried by Norway rats (*Rattus norvegicus*), are known to cause HFRS in China (*4,5,8*). The clinical disease caused by HTNV is more severe than that caused by SEOV.

The province of Inner Mongolia in China is located southeast of the Mongolia plateau. It is a frontier area of north China, extending 2,400 km from east to west and 1,700 km from north to south (Figure 1). The first HFRS outbreak was reported in 1955 in the town of Tulihe in the Hulunbeier District (*12*). HFRS cases for the next 40 years mainly occurred in northeastern Inner Mongolia. No cases were reported in the central and western parts until 1995, when outbreaks of HFRS occurred in the Huhehaote District and then in the Bayannaoer District. Previous epidemiologic investigations have suggested the presence of 2 pathogenic hantaviruses in Inner Mongolia: HNTV and SEOV (*8,12–14*). Recently, we found that Khabarovsk

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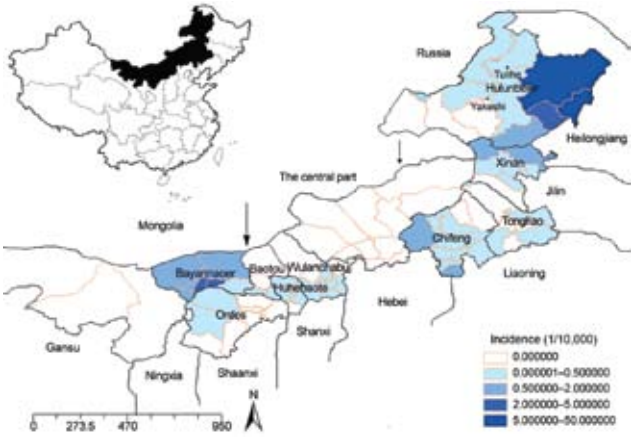

Figure 1. Geographic distribution and average annual incidence of hemorrhagic fever with renal syndrome by district in Inner Mongolia, China, 2001–2006. Arrows mark, from left to right, divisions between western, central, and eastern Inner Mongolia.

virus (KHAV) is circulating in voles (*Microtus maximowiczii*) in the area surrounding the town of Yakeshi in the Hulunbeier District (*10*). However, it is unknown whether the vole-associated hantaviruses, which include KHAV, are human pathogens.

In this study, we analyzed hantavirus disease in Inner Mongolia, including death resulting from it and its geographic distribution and dynamics. We also carried out epizootiologic surveys and defined causative agent(s) of hantavirus disease outbreaks in the western parts of the region. Our results suggested that HTNV, associated with striped field mice (*A. agrarius*), was mainly responsible for HFRS cases in northeastern Inner Mongolia; SEOV, associated with Norway rats (*R. norvegicus*), caused outbreaks in the central and western parts of Inner Mongolia.

Materials and Methods

Collection of Data for HFRS Cases

Since 1950, HFRS has been a class B notifiable disease in China; thus, annual numbers of human HFRS cases and their distribution have been archived. Records for HFRS cases during 1955–2006 were obtained from the Inner Mongolia Center for Disease Control and Prevention. Before 1982, HFRS cases were defined by a national standard of clinical criteria. As of 1982, cases were also confirmed by detecting antibodies against hantavirus in patients' serum samples.

Trapping of Rodents and Screening

From spring 2003 through autumn 2006, rodents were captured in fields and residential areas of 3 HFRS-endemic districts: Hulunbeier, Huhehaote, and Bayannaoer. To capture the rodents, snap-traps were set at 5-m intervals and baited with peanuts. Trapped animals were identified according to previously described criteria (*2,4*). Lung tissues from the animals were stored immediately at –196°C and then transported to our laboratory in Beijing for processing. Hantavirus-specific antigens in lungs were detected by indirect immunofluorescent antibody assay as described previously (*15*). Scattered granular fluorescence in the cytoplasm was considered a positive reaction.

Reverse Transcription–PCR and Sequencing

Total RNA was extracted from the lung tissue samples with the TRIzol reagent (Invitrogen, Beijing, China), according to the manufacturer's instructions. cDNAs were synthesized with avian myelobalastosis virus reverse transcriptase (Promega Biotech, Beijing, China) in the presence of primer P14 (Table 1) (*16*). Partial small (S) segment sequences (which encode the nucleocapsid protein [N]) of SEOV (nt 584–1019) were amplified with primers HV-SFO and HV-SRO for initial PCR (*17*) and with primers SEO-SF and SEOV-SR (*18*) for the second round of amplification, which yielded the 437-bp product (Table 1). For amplification of HTNV partial S segment sequences (nt 462–1025), primers HV-SFO and HV-SRO (*17*) were used for initial PCR, and primers HSF and HSR (*18*) were used for nested PCR, which amplifies the 564-bp product.

The PCR products were gel purified using QIAquick Gel Extraction kit (QIAGEN, Beijing, China), according to the manufacturer's instructions, and cloned into the pMD18-T vector (TaKaRa Biotechnology, Dalian, China).

Table 1. Specific primers used to detect hantavirus RNA in rodents, Inner Mongolia Autonomous Region, China, 2003–2006*

Type	Primer	Sequence (5′ → 3′)	Segment†	Reference
	P14	TAGTAGTAGACTCC	L, M, S	(*16*)
	HV-SFO	GGCCAGACAGCAGATTGG	S (+)	(*17*)
	HV-SRO	AGCTCAGGATCCATGTCATC	S (–)	(*17*)
HTNV	HSF	AACAAGAGGAAGGCAAACAAC	S (+)	(*18*)
	HSR	GCCCCAAGCTCAGCAATACC	S (–)	(*18*)
SEOV	SEO-SF	TGCCAAACGCCCAATCCA	S (+)	(*18*)
	SEO-SR	GCCATCCCTCCGACAAACAA	S (–)	(*18*)

*HTNV, Hantaan virus; SEOV, Seoul virus.
†The hantavirus genome consists of 3 segments defined as small (S), medium (M), and large (L). The segments encode the nucleocapsid protein, 2 envelope glycoproteins, and viral RNA-dependent RNA polymerase, respectively.

The ligated products were transformed into JM109 competent cells. DNA sequencing was performed with the ABI-PRISM Dye Termination Sequencing kit and ABI 373-A genetic analyzer (Applied Biosystems, Carlsbad, CA, USA). At least 2 cDNA clones were used to determine each viral sequence. If discrepancies occurred, a third cDNA clone was sequenced.

Phylogenetic Analysis

PHYLIP (version 3.65) (http://evolution.genetics.washington.edu/phylip.html) was used to construct phylogenetic trees by using the neighbor-joining and maximum-likelihood methods, with 1,000 bootstrap replicates. Alignments were prepared with ClustalW version 1.83 (www.ebi.ac.uk/Tools/clustalw2/index.html). Nucleotide identities were calculated by using the DNAStar program (DNASTAR, Madison, WI, USA). Hantavirus sequences used in the study were retrieved from GenBank (www.ncbi.nlm.nih.gov/Genbank) (Figure 2).

Results

Occurrence of HFRS in Inner Mongolia, 1955–2006

No HFRS cases were registered in Inner Mongolia before 1955, when 265 HFRS cases were reported in the town of Tulihe in the Hulunbeier District. From 1955 through 2006, Inner Mongolia had 8,309 reported cases of HFRS, an average incidence rate of 0.89/100,000 population, and 261 deaths caused by HFRS (3.14% of total cases).

At least 2 major HFRS epidemics have occurred in Inner Mongolia (Figure 3). The first peak was observed during 1955–1957, when 722 cases were registered. After this peak, the number of HFRS cases declined and was relatively stable for the next 24 years, when 168 more cases were registered. However, during this time, the reporting system was suboptimal, so the actual number of HFRS cases might have been higher. As of 1982, the number of cases increased to ≈60 per year, and this ascending phase continued throughout the 1980s. The second epidemic peaked in the 1990s and at the beginning of this century, when 661 HFRS cases, a record number, were reported for 2000.

Geographic Distribution and Dynamics of HFRS in Inner Mongolia

During 1955–1974, all HFRS cases were registered in the Hulunbeier District (Figure 3). In the late 1970s and in the 1980s, HFRS cases were also found in the neighboring districts of Hulunbeier, Chifeng, Xingan, and Tongliao (Table 2). HFRS cases were later reported in the central part of Inner Mongolia in 1995 (in Huhehaote, the capital of the region) and in the western district of Bayannaoer in 1999). To date, HFRS is prevalent in 9 districts with 56 counties affected (Table 2).

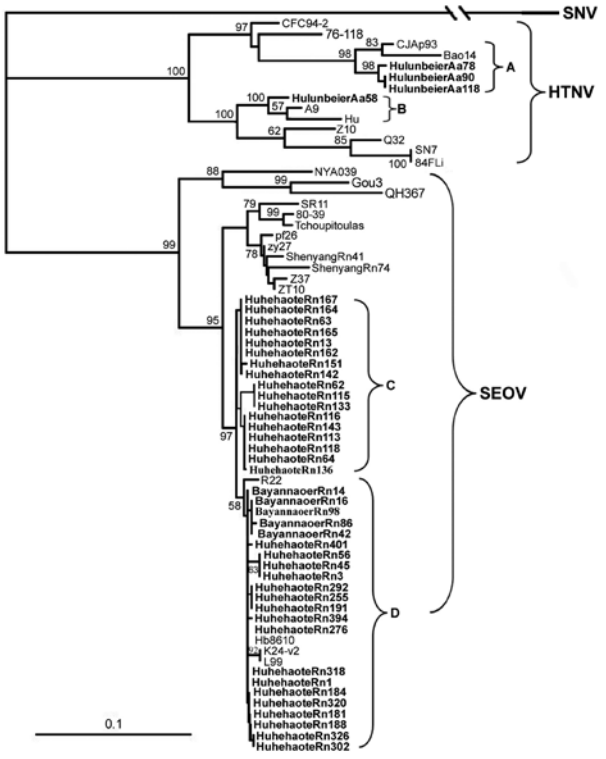

Figure 2. Phylogenetic tree of hantaviruses from rodents in Inner Mongolia, China, 2003–2006. The tree is based on partial sequences of the small (S) segment (nt 620–999 for Seoul virus [SEOV] and nt 614–993 for Hantaan virus [HTNV]). PHYLIP program package (3.65) (http://evolution.genetics.washington.edu/phylip.html) was used to construct the phylogenetic trees by using the neighbor-joining (NJ) and the maximum likelihood (ML) methods with 1,000 bootstrap replicates. The tree constructed by using the ML method had a similar topology as that constructed by using the NJ method (data not shown). Bootstrap values were calculated from 1,000 replicates; only the values >50% are shown at the branch nodes. The sequence of Sin Nombre virus (SNV) was used as the outgroup. Partial S segment sequences recovered from *A. agrarius* field mice trapped in the Hulunbeier District were designated as HulunbeierAa58, HulunbeierAa78, HulunbeierAa90, and HulunbeierAa118; partial S segment sequences from *R. norvegicus* rats trapped in the Huhehaote District were designated as HuhehaoteRn-; and those from *R. norvegicus* rats trapped in the Bayannaoer District were designated as BayannaoerRn14, BayannaoerRn42, BayannaoerRn86, BayannaoerRn98, and BayannaoerRn116. Sequences obtained in this study are shown in **boldface**. All nucleotide sequence data reported here are available in GenBank (accession nos. FJ514504–FJ514546). The GenBank accession nos. of the other partial S segment sequences are SNV/NM H10 (L25748), HTNV/76-118 (M14626), HTNV/CFC94-2 (X95077), HTNV/CJAp93 (EF208953), HTNV/Bao14 (AB127998), HTNV/A9 (AF329390), HTNV/Hu (AB027111), HTNV/Z10 (AF18498), HTNV/Q32 (AB027097), HTNV/SN7 (AF288657), HTNV/84Fli (AY017064); SEOV/NY039 (EF210131), SEOV/Gou3 (AF288651), SEOV/QH367 (DQ081717), SEOV/SR11 (M34881), SEOV/Tchoupitoulas (AF329389), SEOV/IR461(AF329388), SEOV/BJFT01 (DQ519033), SEOV/L99 (AF488708), SEOV/R22 (AF488707), SEOV/Bjhd01 (AY627049), SEOV/K24-V2 (AF288655), SEOV/Z37 (F187082), SEOV/ZT10 (AY766368), and SEOV/Hb8610 (AF288643). Scale bar indicates genetic distance.

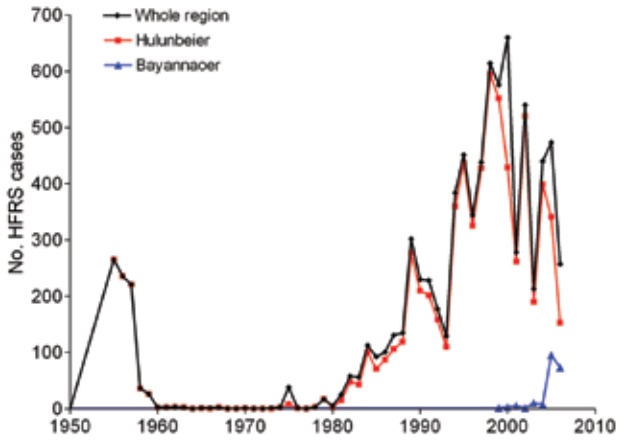

Figure 3. Annual number of cases of hemorrhagic fever with renal syndrome reported by year, Inner Mongolia, China, and selected districts, 1955–2006.

Although HFRS cases have been registered in 9 districts, most cases have been reported in the Hulunbeier District since the first outbreak, which occurred in Tulihe in 1955 (Figures 1 and 3). During 1955–2006, a total of 7,367 HFRS cases were reported in the Hulunbeier District, representing 88.7% of all cases registered in Inner Mongolia. Most of these cases (72.2%) occurred during the months of October–January.

In the Huhehaote District, the first HFRS case was reported in 1995. Later, HFRS occurred in the neighboring districts of Baotou and Wulanchabu. The HFRS incidence in these districts has been lower than in the districts of Bayannaoer, Chifeng, Hulunbeier, and Xingan, each of which reported fewer than 10 HFRS cases annually.

In the Bayannaoer District, no HFRS cases were reported before 1999; during 1999–2004, fewer than 10 cases were registered annually. Outbreaks of HFRS occurred in this district in 2005 and 2006, with 95 and 75 cases, respectively, being reported for these years, although this district initiated comprehensive control measures, including vaccination, after the first case occurred. Of 196 cases reported during 1999–2006, 3 people died. In contrast to the HFRS cases in the Hulunbeier District, more than 60% of cases in the Bayannaoer District occurred during March–June.

Screening of Rodents for Hantaviral Antigens in Lung Tissues

From spring 2003 through autumn 2006, 1,466 rodents belonging to 11 species were trapped in the fields and residential areas of Inner Mongolia (Table 3). Of that total, 529 were trapped in the Hulunbeier District, 15 (13 *A. agrarius* and 2 *A. peninsulae* field mice) of which were found by indirect immunofluorescent antibody assay to be positive for hantavirus antigens. Of the 594 rodents trapped in the Huhehaote District, 38 *R. norvegicus* rats were positive. In the Bayannaoer District, 343 rodents were trapped, among which 8 *R. norvegicus* rats and 8 *Meriones meridianus* gerbils were found to be positive. The identification of hantavirus antigens in *M. meridianus* gerbils suggests that this rodent species is a new carrier and may carry additional unidentified hantavirus(es).

Phylogenetic Analyses

To establish molecular epidemiologic links between hantaviruses in rodents and HFRS outbreaks in central, western, and northeastern Inner Mongolia, we recovered partial S segment sequences (nt 462–1025 for HTNV; nt 584–1019 for SEOV) from the rodent tissue samples and subjected them to genetic analysis. Four of 13 hantaviral antigen–positive mice (*A. agrarius*) from the Hulunbeiere District, 34 of 38 hantaviral antigen–positive rats (*R. norvegicus)* from the Huhehaote District, and 5 of 8 hantaviral antigen–positive rats (*R. norvegicus)* from the Bayannaoer District were found positive by reverse transcription–PCR. The hantaviral sequences recovered from these rodents were designated respectively as HulunbeierAa-, HuhehaoteRn-, and BayannaoerRn- (Figure 2). Unfortunately, our attempts to amplify partial S segment sequences from antigen-positive *M. meriidanus* gerbils were unsuccessful. In addition, KHAV–specific partial S segment sequences were amplified earlier from 5 voles of species *M. maximowiczii* (*10*).

As expected, partial S segment sequences recovered from *A. agrarius* mice were more closely related to HTNV (sequence identities 79.2%–98.9%) than to other known hantaviruses. On the phylogenetic tree, 4 strains recovered from *A. agrarius* mice belonged to 2 lineages (A and B) (Figure 2). The sequences HulunbeierAa78, HulunbeierAa90, and HulunbeierAa118 showed a closer evolutionary relationship to strain Bao14 isolated from *A. agrarius* mice trapped in the neighboring province of Heilongjiang (*8*)

Table 2. Number of cases and geographic distribution of hemorrhagic fever with renal syndrome in Inner Mongolia, China, by decade or partial decade, 1955–2006

Years	No. cases	Districts (no. affected counties)
1955–1960	786	Hulunbeier (2)
1961–1970	12	Hulunbeier (5), Chifeng (1)
1971–1980	67	Hulunbeier (7), Chifeng (1), Xingan (1), Tongliao (1)
1981–1990	1,242	Hulunbeier (9), Chifeng (2), Xingan (2), Tongliao (1)
1991–2000	4,002	Hulunbeier (9), Chifeng (6), Xingan (5), Tongliao (4), Huhehaote (3), Baotou (1), Bayannaoer (2), Wulanchabu (2)
2001–2006	2,197	Hulunbeier (9), Chifeng (10), Xingan (6), Tongliao (4), Huhehaote (9), Baotou (4), Bayannaoer (7), Wulanchabu (6), Ordos (1)

Table 3. Prevalence of hantavirus(es) in rodents by species, district, and ecological location, Inner Mongolia, China, 2003–2006*

	Hulunbeier District		Huhehaote District		Bayannaoer District	
Species	Field/ grassland	Residential area	Field/desert grassland	Residential area	Field/desert grassland	Residential area
Apodemus agrarius	132/9/3	81/4/1	0/0/0	0/0/0	0/0/0	0/0/0
Apodemus peninsulae	25/2/0	11/0/0	0/0/0	0/0/0	0/0/0	0/0/0
Rattus norvegicus	12/0/0	23/0/0	79/5/4	418/33/30	0/0/0	132/8/5
Mus musculus	0/0/0	7/0/0	0/0/0	21/0/0	0/0/0	19/0/0
Microtus maximowiczii	38/3/3	26/2/2	0/0/0	0/0/0	0/0/0	0/0/0
Myodes rufocanus	18/0/0	3/0/0	0/0/0	0/0/0	0/0/0	0/0/0
Myodes rutilus	89/0/0	54/0/0	0/0/0	0/0/0	0/0/0	0/0/0
Cricetulus barabensis	7/0/0	3/0/0	33/0/0	0/0/0	41/0/0	0/0/0
Meriones meridianus	0/0/0	0/0/0	43/0/0	0/0/0	140/8/0	0/0/0
Allactage sibirica	0/0/0	0/0/0	0/0/0	0/0/0	6/0/0	0/0/0
Dipus sagitta	0/0/0	0/0/0	0/0/0	0/0/0	5/0/0	0/0/0
Total	321/14/6	208/6//3	155/5/4	439/33/30	192/8/0	151/8/5

*Data show no. of rodents captured/no. positive for hantavirus antigen/no. PCR positive.

and to strain CJAp93 isolated from *A. peninsulae* mice captured in Jilin Province (*19*). The sequence HulunbeierAa58 clustered together with strain A9 isolated from *A. agrarius* mice from Jiangsu Province and strain Hu isolated from a person in the Hubei Province (*8*).

All sequences recovered from *R. norvegicus* rats showed higher identity to SEOV (80.2%–99.5%) than to HTNV or other hantavirus types. These sequences were closely related to each other, with 92.5%–99.9% sequence identity. The partial S sequences from *R. norvegicus* rats from the Huhehaote District formed 2 clusters (marked C and D, Figure 2). Although the C cluster included only sequences from the Huhehaote District, the D cluster included sequences from both the Huhehaote and Bayannaoer Districts and also included sequences from the Chinese strains K24 (from Zhejiang Province), Hb8610 (from Shanxi Province), L99 (from Jiangxi Province), and R22 (from Henan Province) (*8*). All sequences recovered from *R. norvegicus* rats from the Bayannaoer District belonged to cluster D, suggesting that SEOV variants causing the HFRS outbreak in the Bayannaoer District are genetically very close to those from the Huhehaote District.

Discussion

In this study, we describe the incidence, geographic distribution, and dynamics of HFRS in Inner Mongolia from 1955 through 2006. HFRS had been a serious concern in the region for the past 20 years. Habitat differences, host distribution, rodent serosurveys, and phylogenetic analysis suggest that HFRS in the northeastern region has been caused mainly by HTNV, and the HFRS outbreaks occurring in the central and western parts have been caused mainly by SEOV.

The occurrence and epidemics of HFRS are influenced by both natural (e.g., ecological) and occupational factors (*2,3,7,20,21*). Many hantavirus infections have occurred in persons of low socioeconomic status because of poor housing conditions (*1,2,5*). In China, the highest HFRS incidence occurred in the humid and semihumid areas, where annual precipitation levels are 400–800 mm, and no cases have been reported from the arid areas, where the precipitation is <200 mm (*6,7*). Notably, most HFRS cases occurred in rural areas (*5,7*). The Hulunbeier District is situated in northeast Inner Mongolia and belongs to humid areas. The largest coniferous forest in north China is situated there. Consequently, the *A. agrarius* mouse is a species that most frequently carries the hantavirus antigen in this area (Table 3), a finding consistent with earlier epidemiologic investigations (*12–14*). On the other hand, housing conditions for most farmers in the Hulunbeier District are poorer than in neighboring areas because of the relatively slow development of the local economy in this district. Thus, the local natural conditions, which support a high density of rodents and a high prevalence of hantavirus(es), as well as the generally low socioeconomic status of the Hulunbeier District, could contribute to the high numbers of HFRS cases in this district.

Huhehaote and Bayannaoer Districts are located in the central and western parts of Inner Mongolia, respectively. Both belong to a semi-arid or arid zone. Until the 1990s, epizootiologic surveys had not shown the presence of hantaviruses circulating in rodents and infecting humans in these districts (*14*). The first HFRS cases were reported in the Huhehaote District in 1995 and in the Bayannaoer District in 1999. In the Bayannaoer District, a relatively large HFRS outbreak occurred in 2005 and 2006, with 95 and 75 cases reported, respectively. The ecology of central and western Inner Mongolia differs from that of the northeastern part. In the central and western parts, *A. agrarius* mice are absent, and *R. norvegicus* rats are abundant. Consequently, a high number of human hantavirus infections are registered. Since the 1980s, the incidence of HFRS has been

high in the provinces of Hebei and Shanxi (*2,5,6,22–24*), which share borders with central Inner Mongolia (Figure 1). Particularly, the annual number of HFRS cases reported in Hebei Province was more than 4,000 during 1990–2002 (*6*). *R. norvegicus* rats were the predominant reservoir; hence, SEOV was prevalent in the provinces of Hebei and Shanxi (*2,5,22–24*).

Our phylogenetic analysis showed that SEOV sequences isolated from the rats trapped in Huhehaote and Bayannaoer Districts were closely related to those of strains HB8610 and K24 from Shanxi and Zhejiang Provinces, respectively, and also to sequences of 2 other strains from China (Figure 2). This genetic lineage of SEOV seems to be widely distributed in China (*8,15,18,25,26*). Norway rats, the carriers of SEOV, are more invasive than the hosts of other hantaviruses and have dispersed throughout much of the world through various modes of transportation. As a result, SEOV is the only cosmopolitan hantavirus known so far (*27*). Genetic variants of SEOV currently circulating in rats in central Inner Mongolia could come from the provinces of Shanxi or Hebei and then spread westward into the Bayannaoer District due to increased transportation of goods and human migration that followed the rapid economic development in China over the past decades.

Of host species, *A. agrarius* mice carried hantavirus antigens most frequently in the Hulunbeier District, whereas *R. norvegicus* rats were the predominant carriers in the central and western parts of Inner Mongolia (Table 3), where *A. agrarius* mice have never been found because of this species' desert ecosystem (*14,28,29*). Phylogenetic analysis confirmed the presence of HTNV in *A. agrarius* mice in the Hulunbeier District and SEOV in *R. norvegicus* rats in the Huhehaote and Bayannaoer Districts. In addition, the peak of HFRS associated with *A. agrarius* mice occurred in the winter, whereas HFRS associated with *R. norvegicus* rats occurred mainly in the spring (*2,4*). Thus, our results showed that HFRS in the northeastern part was caused mainly by HTNV, although we demonstrated that KHAV is also circulating in *M. maximowiczii* voles in the Hulunbeier District (*10*), and SEOV was responsible for the HFRS outbreaks in the Huhehaote and Bayannaoer Districts.

The annual incidence of HFRS has gradually decreased in China during the past 7 years (*5*). Our data suggest that the HFRS outbreak in the central and western parts of Inner Mongolia was probably caused by SEOV variants. HFRS has occurred in the areas such as the Bayannaoer District, where the disease was not previously reported, even during the 1980s and 1990s when the overall incidence was high (*5*). Whether these newly appearing HFRS cases result from improved surveillance or reflect a recent spread of the virus to these areas remains to be seen. This study reinforces the need for vigilance in preventing HFRS caused by HTNV and SEOV (and perhaps other hantaviruses) in China. This vigilance should include regular surveillance of local rodent populations for evidence of hantavirus infection.

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Case-based Surveillance of Influenza Hospitalizations during 2004–2008, Colorado, USA

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Colorado became the first state to make laboratory-confirmed influenza-associated hospitalizations a case-based reportable condition in 2004. We summarized surveillance for influenza hospitalizations in Colorado during the first 4 recorded influenza seasons (2004–2008). We highlight the similarities and differences among influenza seasons; no 2 seasons were entirely the same. The 2005–06 influenza season had 2 distinct waves of activity (types A and B), the 2006–07 season was substantially later and milder, and 2007–08 had substantially greater influenza B activity. The case-based surveillance for influenza hospitalizations provides information regarding the time course of seasonal influenza activity, reported case numbers and population-based rates by age group and influenza virus type, and a measure of relative severity. Influenza hospitalization surveillance provides more information about seasonal influenza activity than any other surveillance measure (e.g., surveillance for influenza-like illness) currently in widespread use among states. More states should consider implementing case-based surveillance for influenza hospitalizations.

Each year, in the United States, influenza infections cause an estimated 36,000 deaths (*1*) and ≥200,000 hospitalizations (*2*). The Centers for Disease Control and Prevention (CDC) monitors seasonal influenza activity to document the timing and geographic spread of influenza infection, track influenza related illness in the community, monitor the proportion of deaths caused by pneumonia and influenza, determine which influenza viruses are circulating, and identify emerging virus changes (*3*). Influenza surveillance can also indicate the relative severity of a given influenza season compared with previous seasons.

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Similarly, state health departments monitor seasonal influenza activity within their jurisdictions and contribute data to CDC. There is little standardization, however, among these surveillance systems. All 50 states monitor influenza-like illness (ILI) and report these data weekly to CDC (L. Brammer, pers. comm.), but other measures of influenza activity are conducted only by subsets of states.

In 2004, Colorado became the first state to make influenza-associated hospitalizations a reportable condition and part of routine influenza surveillance (*4*). We summarized the first 4 seasons (2004–2008) of case-based surveillance for influenza hospitalizations in Colorado.

Methods

Notifiable conditions in Colorado have specified time frames for reporting, either within 24 hours or 7 days of diagnosis. Influenza-associated hospitalizations must be reported within 7 days. The list of notifiable conditions, which includes specified time frames for reporting, is sent to acute care hospitals annually. These conditions are reported primarily by hospital infection control practitioners and may be reported through the Colorado State Health Department's web-based electronic disease reporting system (CEDRS) or by fax or phone. Colorado has 59 nonfederal acute care hospitals licensed for ≥25 beds; hospitals licensed for <25 beds (as well as some licensed at 25 beds) are critical access hospitals in rural areas, which infrequently diagnose notifiable conditions.

Data from hospitalized patients with influenza reported to CEDRS for the 2004–2008 influenza seasons are analyzed in this report. Colorado defines an influenza-associated hospitalization as a hospital admission accompanied by a report of an appropriate positive laboratory test result for influenza (*4*). Acceptable and available laboratory tests in Colorado are viral culture, reverse transcription–PCR (RT-

PCR), direct immunofluorescent antibody (DFA) staining, and rapid diagnostic tests.

Our analysis defined each influenza season as October 1 through May 31 of the subsequent year. Week 1 (first calendar week) was defined as the week containing January 1 and ending with Saturday of that week. The last week (week 52) corresponded to the last full calendar week of the year that did not contain January 1 of the subsequent year. Specimen collection date was used as a surrogate for date of diagnosis (typically the same date for rapid influenza testing and DFA) or, if not available, the report date. Timeliness of reporting was calculated as the difference between specimen collection date and entry date in CEDRS.

For all 4 influenza seasons, hospitalizations reported early in the season on the basis of rapid tests were not included as cases until adequate virologic evidence of circulating influenza virus was demonstrated by testing at the state laboratory. Hospitals were requested to submit repeat specimens that tested positive by rapid diagnostic tests to the state laboratory for confirmatory testing by PCR (viral cultures were additionally performed in 2004–05). After approximately half of specimens referred in a given week were confirmed by RT-PCR, hospitals were informed that they no longer needed to refer specimens to the state laboratory; reported hospitalized cases based on positive rapid tests were included. The dates for including reported hospitalized cases based on rapid test results occurred during weeks 51, 50, 6, and 48, respectively, for the 2004–05 through 2007–08 seasons, respectively.

Outpatient surveillance for ILI has been a longstanding component of influenza surveillance at the state and national levels. CDC has suggested that states recruit a minimum of 1 healthcare provider per 250,000 population to report weekly the total number of patients seen and the number of those patients with ILI. During the influenza seasons included in this analysis, Colorado's volunteer provider–to-population ratio ranged from ≈1 provider per 165,000 persons (2004–05) to 1 provider per 244,000 persons (2007–08 season). The time series from Colorado ILI data (*5*) was qualitatively (timing and relative magnitude of peak) compared with that from reported hospitalized influenza cases to provide a measure of validity.

Characteristics of reported influenza hospitalizations for each season were summarized by percentages and number of reported influenza hospitalizations. Population estimates for 2004–2007 obtained from the Colorado Department of Local Affairs were used to calculate seasonal age-specific rates of influenza-associated hospitalization. Population estimates for the first calendar year of each season (e.g., 2004 for the 2004–05 influenza season) were used to calculate each season's rates. Data analysis was conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Influenza hospitalizations were reported from a mean of 44 (range 38–47, 75%) acute care hospitals (licensed for ≥25 beds during the 4 influenza seasons. The 2006–07 season was notable for having the lowest number of reporting hospitals; however, many of these reported substantially fewer cases compared with the 3 other seasons. Overall, 90% (range 86%–92%) of influenza hospitalizations were reported within 7 days and 68% (range 64%–71%) were reported within 3 days of diagnosis.

The total number of reported influenza hospitalizations varied somewhat across the 4 seasons as did the distribution of selected characteristics of the cases (Table 1). Similar numbers of influenza cases were reported during the 2004–05 (n = 978) and 2007–08 (n = 1,004) seasons; slightly lower numbers were reported during the 2005–06 (n = 848) season. In contrast, only 367 influenza hospitalizations were reported during the 2006–07 influenza season. Similarly, moderate proportions of influenza B hospitalizations were reported during the 2004–05 and 2005–06 seasons (13% for each); a low proportion (3.3%) of influenza B was reported during 2006–07. An unusually high proportion of influenza B (34.2%) was reported among influenza hospitalizations during 2007–08.

For 3 of the 4 seasons, the greatest numbers and percentages of influenza hospitalizations were among persons ≥80 years of age (Table 1). In contrast, the 2006–07 season was noteworthy for lower proportions of cases in the 50–64 y, 65–79 y, and ≥80 y age groups, and a higher proportion in the 18–49 y age group. By region, overall distributions of cases were fairly similar across seasons.

Distribution of test types was similar across the 4 seasons. Rapid diagnostic tests were the most frequently reported test type (mean 87.3%, range 85.1%– 88.3%) followed by viral culture (mean 5.75%, range 4.8%–7.4%) and DFA (mean 5.7%, range 4.3%–6.9%). PCR was the reported test type for <1.5% of cases in any given season.

The time series of reported influenza hospitalizations for each of the 4 seasons (Figure 1) showed that the 2004–05 and the 2007–08 seasons peaked at almost the same time (weeks 7 and 8, respectively) and with similar magnitude. However, influenza hospitalizations began to increase several weeks earlier during the 2007–08 season with a less steep upslope. In contrast, the 2005–06 season appeared to have 2 peaks of similar magnitude during weeks 5 and 9, and the 2006–07 season exhibited the latest onset (weeks 3–4) and peak (week 11) and the lowest magnitude. By week 15, the time series for all 4 seasons converged at low levels of reported hospitalizations.

Compared with the time series from surveillance for ILI, the timing of influenza hospitalization peaks was quite similar (Table 2). ILI and influenza hospitalizations peaked the same week during the 2004–05 and 2007–08 seasons.

Table 1. Characteristics of patients hospitalized with influenza, Colorado, USA, 2004–08 influenza seasons*

Characteristics	Influenza season (October 1–May 31), no. (%) patients			
	2004–05	2005–06	2006–07	2007–08
Total recorded cases	978	848	367	1,004
Influenza type				
A	777 (79.45)	699 (82.43)	345 (94.01)	629 (62.65)
B	127 (12.99)	110 (12.97)	12 (3.27)	343 (34.16)
Unknown	74 (7.57)	39 (4.60)	10 (2.72)	32 (3.19)
Age				
<6 mo	64 (6.54)	81 (9.55)	39 (10.63)	79 (7.87)
6–23 mo	72 (7.36)	103 (12.15)	46 (12.53)	78 (7.77)
2–4 y	56 (5.73)	59 (6.96)	27 (7.36)	65 (6.47)
5–17 y	56 (5.73)	72 (8.49)	29 (7.90)	74 (7.37)
18–49 y	140 (14.31)	86 (10.14)	78 (21.25)	180 (17.93)
50–64 y	149 (15.24)	103 (12.15)	39 (10.63)	142 (14.14)
65–79 y	201 (20.55)	169 (19.93)	56 (15.26)	179 (17.83)
≥80 y	240 (24.54)	175 (20.64)	53 (14.44)	207 (20.62)
Gender				
M	488 (49.90)	405 (47.76)	186 (50.68)	461 (45.92)
F	485 (49.59)	443 (52.24)	180 (49.05)	517 (51.49)
Unknown	5 (0.51)	0 (0.00)	1 (0.27)	26 (2.59)
Region*				
Western Slope	57 (5.83)	100 (11.79)	31 (8.45)	94 (9.36)
Northern Front Range	122 (12.47)	108 (12.74)	48 (13.08)	121 (12.05)
Denver Metro	550 (56.24)	383 (45.17)	210 (57.22)	520 (51.79)
South Central	36 (3.68)	32 (3.77)	8 (2.18)	14 (1.39)
San Luis Valley	7 (0.72)	8 (0.94)	12 (3.27)	15 (1.49)
Southern Front Range	174 (17.79)	177 (20.87)	48 (13.08)	205 (20.42)
Eastern Plains	32 (3.27)	40 (4.72)	10 (2.72)	35 (3.49)

*Colorado regions can be further divided into counties: Western Slope: Archuleta, Delta, Dolores, Eagle, Garfield, Grand, Gunnison, Hinsdale, Jackson, La Plata, Mesa, Moffat, Montezuma, Montrose, Ouray, Pitkin, Rio Blanco, Routt, San Juan, San Miguel, Summit; Northern Front Range: Larimer, Weld; Denver Metro: Adams, Arapahoe, Boulder, Broomfield, Denver, Douglas, Jefferson; South Central: Chaffee, Clear Creek, Custer, Fremont, Gilpin, Huerfano, Lake, Las Animas, Park, Teller; San Luis Valley: Alamosa, Conejos, Costilla, Mineral, Rio Grande, Saguache; Southern Front Range: El Paso, Pueblo, and Eastern Plains: Baca, Bent, Cheyenne, Crowley, Elbert, Kiowa, Kit Carson, Lincoln, Logan, Morgan, Otero, Phillips, Prowers, Sedgwick, Washington, Yuma.

The 2005–06 season showed 3 peaks for ILI and influenza hospitalizations (the first was a minor peak; Figure 1); the corresponding time differences were 0, 1, and 2 weeks. During the 2006–07 season, ILI peaked 2 weeks earlier than influenza hospitalizations. The relative magnitudes of peak ILI also corresponded to the relative magnitudes of reported influenza hospitalizations; the lowest magnitude for each occurred during the 2006–07 season.

The time series of hospitalizations stratified by influenza type showed distinctly different patterns among seasons. During the 2004–05 season, influenza A and B peaked at week 7, although the influenza B proportion was relatively small. The 2005–06 season was notable for distinctly separate time courses for influenza A and B (i.e., 2 waves of activity) with influenza A hospitalizations peaking 5 weeks before that for influenza B (Figure 2). The 2006–07 season

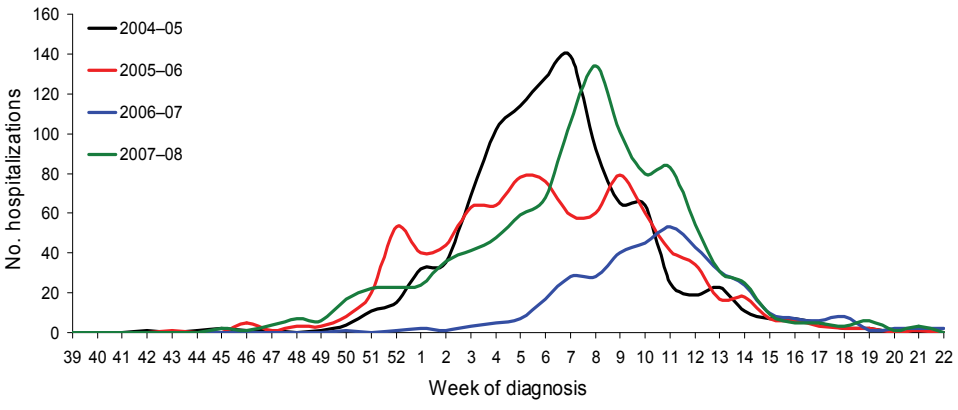

Figure 1. Hospitalized influenza patients in Colorado, USA, by week of diagnosis and influenza season.

Table 2. Timing of peak activity for influenza hospitalizations and influenza-like illness, Colorado, USA, 2004–08

Category	Influenza season (October 1–May 31)			
	2004–05	2005–06	2006–07	2007–08
Hospitalizations, wk	7	5, 9*	11	8
Influenza-like illness, wk	7	52, 4, 11	9	8

*Smaller initial peak during wk 52.

was mild with minimal influenza B activity. The 2007–08 season was notable for a high proportion of influenza B activity, and the time courses for influenza A and B hospitalizations were superimposed with both peaking at week 8.

When stratified by geographic region, the time series of influenza hospitalizations showed peaks that clustered within 2 to 3 weeks for 3 of the 4 seasons. During 2005–06, however, the Western Slope geographic region (west of the Continental Divide) showed a distinct early peak 10 weeks before the Denver metropolitan area; other regions peaked at varying weeks between peak in Western Slope and peak for Denver (Figure 3).

Age group-specific rates of influenza hospitalizations for 3 of the 4 influenza seasons showed characteristic U-shaped distributions, with rates highest for infants <6 months of age and adults ≥80 years of age (Table 3). Because the 2006–07 season was uncharacteristically mild, lower rates were seen for all age groups, especially for persons >65 years of age, resulting in more of a J-shaped distribution. Children 6–23 months of age, for whom influenza vaccination has been recommended since 2004, had the third highest age group–specific rate of hospitalization during 3 of the 4 seasons (second highest rate during 2006–07). There was no apparent declining trend across the 4 seasons in rates of hospitalizations in this age group; in fact, rates were similar during 2004–05 and 2007–08; fluctuation during the 2 intervening seasons was wide.

When stratified by influenza type, age group–specific rates for influenza B hospitalizations were greatest for those <6 months and 6–23 months of age during all but the 2007–08 season. In contrast, the 2007–08 season was noteworthy for unusually high rates of influenza B, especially for persons ≥80 years of age, but also for persons 60–79 years of age. For the ≥80 years age group, rates of influenza A and B hospitalizations were almost the same, whereas, for infants <6 months of age and children 6–23 months of age, rates of influenza A were approximately 3.5–4× higher than those for influenza B (Figure 4).

Discussion

This summary of surveillance data from case-based reporting of influenza hospitalizations highlights the similarities and differences among influenza seasons. Each of several characteristics was fairly similar for 3 of the 4 seasons presented (not necessarily the same 3), including the numbers and time course of hospitalizations and age distribution of cases and rates. In contrast, the amount and timing of influenza B activity demonstrated substantial variability. On the basis of the combination of characteristics available from reporting of influenza hospitalizations though, no 2 seasons were entirely the same; 2005–06 had 2 distinct waves of activity (types A and B), 2006–07 was substantially later and milder, and 2007–08 had substantially greater influenza B activity.

Surveillance for influenza hospitalizations during the past 4 seasons in Colorado has substantially added to the state health department's ability to monitor and describe seasonal influenza. Implementation and maintenance of this surveillance activity has been easily absorbed by the existing influenza surveillance coordinator position and has required no additional resources. Approximately 20% of the surveillance coordinator's weekly time is devoted to managing and tabulating influenza hospitalization surveillance data. Essential to successful implementation has been acceptability by hospital infection control practitioners statewide, who report almost all of the notifiable diseases diagnosed in hospitals in Colorado.

This surveillance component provides more information about seasonal influenza activity than any other surveillance measure (e.g., ILI) currently in widespread use among states. Influenza hospitalization surveillance pro-

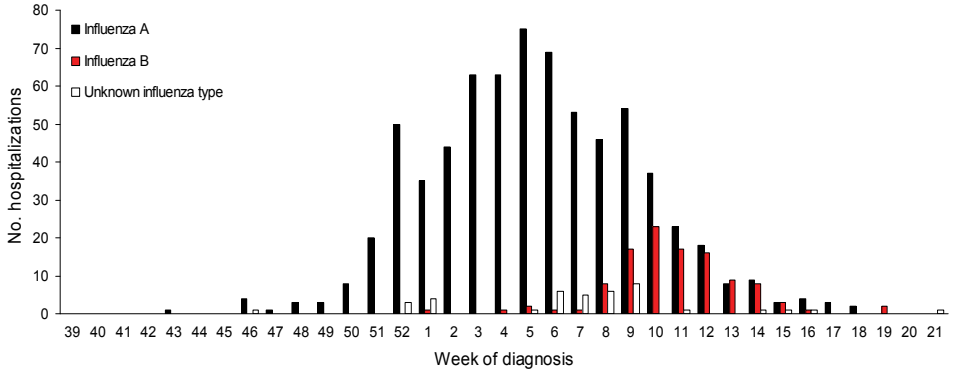

Figure 2. Hospitalized influenza patients in Colorado, USA, by week of diagnosis and influenza type, 2005–06 season.

Table 3. Rates of influenza hospitalizations per 100,000 population, by age group, Colorado, USA, 2004–08

Age group	Influenza season (October 1–May 31), no. cases/100,000			
	2004–05	2005–06	2006–07	2007–08
<6 mo	185.6	234.6	111.8	225.4
6–23 mo	104.3	148.9	66.0	110.5
2–4 y	27.9	28.4	12.8	30.3
5–17 y	6.7	8.6	3.4	8.5
18–49 y	6.2	3.8	3.4	7.8
50–64 y	19.4	12.8	4.6	16.1
65–79 y	59.4	49.0	15.9	49.5
≥80 y	214.4	153.6	45.7	174.5
Overall rate (all age groups)	21.2	18.1	7.7	20.6

vides information regarding the time course (start, peak, end) of seasonal influenza activity, including influenza A and B; the reported case numbers and population-based rates of seasonal influenza by influenza virus type, age group, gender, and geographic region; and a measure of the relative severity of an influenza season compared with previous seasons. Some states conduct surveillance for the numbers of pneumonia and influenza hospital admissions, on a syndromic basis, (*6,7*) but this may not necessarily be population based and only provides information on time course and relative severity of influenza activity without the additional information available from laboratory-confirmed, case-based reporting.

The relative rates of influenza A and B, especially at the extremes of age, during 2007–08 were unique among the seasons summarized. Influenza A rates were 3.5–4× higher than influenza B rates for young infants and young children 6 –23 months of age, whereas, for persons ≥80 years of age, and to a lesser extent persons 65–79 years of age, influenza A and B rates were similar. Since infants <6 months old are not approved to receive influenza vaccine and will not have acquired their own immunity from previous influenza seasons, their rates of hospitalization related to influenza A versus B most closely reflected the epidemiology of circulating influenza viruses, on the basis of prevalence and relative virulence. Low rates of vaccination among infants 6–23 months of age (*8*), for whom influenza vaccination has been recommended since 2004, produce similar relative rates of influenza A and B as for infants <6 months old. In contrast, a high proportion of older adults receive seasonal influenza vaccine (*9*), and influenza type-specific rates of hospitalization in older age groups might reflect protection conferred by vaccination with the current season's vaccine and possibly cross-protection from previous influenza infection or immunization (*10,11*). The 2007–08 influenza vaccine was suboptimally matched to circulating viruses, and estimated vaccine efficacy against the predominant influenza A strain was 58% compared with zero vaccine efficacy against circulating influenza B virus (*12*). Thus, the observed rates of influenza A and B hospitalizations for the older age groups during 2007–08 might reflect partial protection from a moderately effective vaccine against the predominant circulating influenza A virus and no protection from an ineffective vaccine against the predominant circulating influenza B virus.

The validity of influenza hospitalization surveillance as an indicator of seasonal influenza activity was supported by comparison with Colorado ILI surveillance data, which showed good agreement. ILI surveillance can be somewhat challenging to maintain at the state level due to its reliance on providers willing to report data regularly (weekly) for which they are not compensated (hence the range of ratios for participating provider to population during the 4 seasons included). Although ILI surveillance is a longstanding

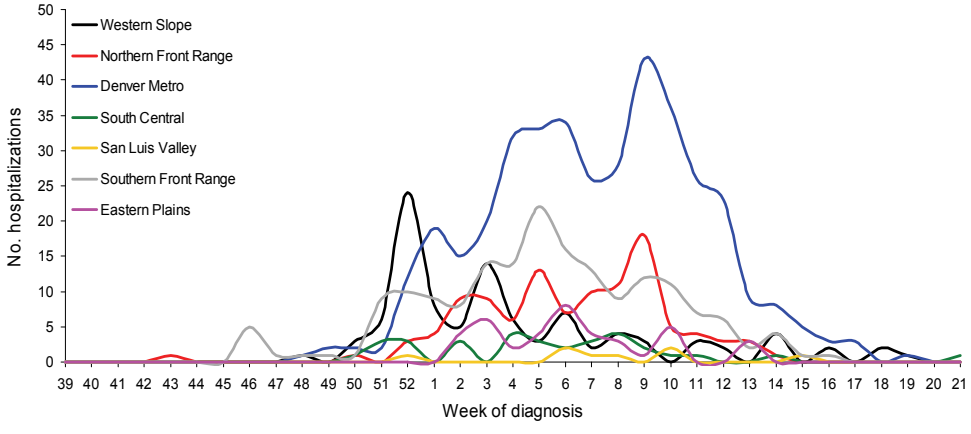

Figure 3. Hospitalized influenza patients in Colorado, USA, by week of diagnosis and region, 2005–06 season.

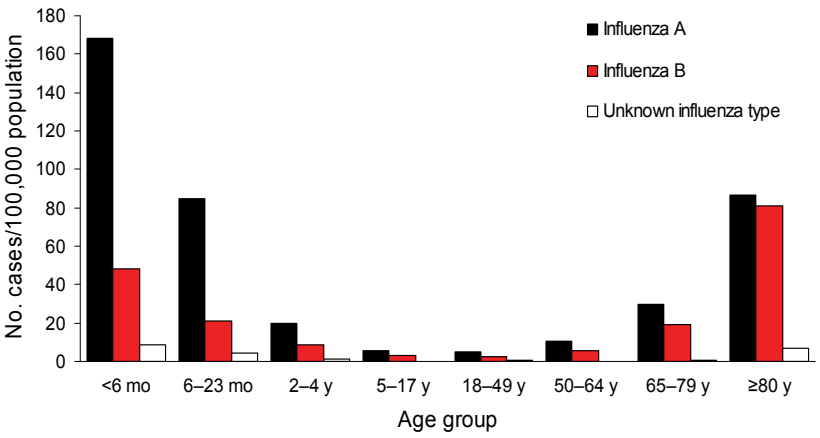

Figure 4. Rates of reported influenza hospitalizations in Colorado, USA, by age group and influenza type, 2007–08 season.

component of seasonal influenza surveillance and has been heavily promoted by CDC, reports of formal evaluation of this surveillance activity are lacking. Colorado hospitalized influenza surveillance data also showed fair agreement with national summary indicators (timing and relative severity) of seasonal influenza for the 4 seasons included. However, this is not the most appropriate comparison because national surveillance data are an aggregate of regional influenza outbreaks that typically vary in time course and intensity.

There are several limitations to these data resulting from surveillance for influenza-related hospitalizations. First, rapid influenza tests, which were the tests used for >85% of the hospitalizations in this report, have suboptimal performance characteristics. The sensitivity of rapid influenza tests is only moderate, more so among adults than children (*13,14*). This will result in underestimation of the true rates of influenza hospitalization, to a greater degree for adults than for children, because adults may be admitted for influenza-related complications a number of days after influenza infection when virus is less readily detectible. The positive predictive value of influenza rapid tests is low (and probability of a false-positive test result is high) when the prevalence of circulating influenza virus is low (*15*), which occurs during the early and late parts of the influenza season. Use of influenza rapid tests can result in false identification of the start of seasonal influenza activity based on reported hospitalizations and extend the left tail of the time series curve. To address this issue, the Colorado state health department does not include early season hospitalized influenza cases in official case counts or surveillance data summaries until the prevalence of circulating influenza virus is documented to be adequate based on virologic surveillance by RT-PCR at the state health department laboratory (see Methods).

Second, testing practices can affect ascertainment of hospitalized influenza cases. This is likely more of an issue for adults among whom exacerbation of underlying co-morbidities by influenza might not result in testing for influenza at the time of hospital admission. In 1 study involving retrospective medical records review, a low proportion of adults with a discharge diagnosis of pneumonia had been tested for influenza (*16*).

Third, passive public health surveillance of reportable diseases is known to be incomplete (*17–19*). Reporting of influenza hospitalizations as part of passive, case-based notifiable disease reporting is no exception. Some data on completeness of reporting of hospitalized influenza cases from the Denver metropolitan area (approximately half the state's population) was available from review of statewide hospital discharge data combined with retrospective medical records review as part of a special multisite enhanced influenza surveillance project (*20*). During the 2006–07 season, completeness of reporting of adult hospitalized patients with positive test results in the medical record was estimated to be 65% (*16*) and 70% for pediatric cases (Colorado Department of Public Health, unpub. data). For the 2007–08 season, estimated completeness of reporting was 75% for adult cases and 66% for pediatric cases (Colorado Department of Public Health, unpub. data). Thus, it seems unlikely that variable completeness of reporting between pediatric and adult cases or across seasons is the main factor contributing to variation in the relative age group–specific rates of hospitalized patients with influenza. Multiple other factors that might contribute to the observed epidemiologic pattern include virulence of seasonal circulating viruses, host immunity from previous seasons, and protection afforded by each season's vaccine.

Despite these limitations that undoubtedly resulted in underascertainment of influenza-related hospitalizations, to a greater extent for adults than children, surveillance for influenza hospitalizations can contribute useful information for public health monitoring of seasonal influenza activity. The numbers of cases and rates derived from passive reporting of hospitalized influenza cases should be viewed as a minimum estimate, especially for adults. Incomplete case ascertainment and reporting should have little effect on

monitoring the time course of hospitalizations for patients with influenza. As is true for passive surveillance systems in general, assessing the relative severity of a given influenza season should still be valid as long as surveillance methods and system performance (i.e., completeness of reporting) remain relatively unchanged over time.

In conclusion, case-based surveillance for laboratory-confirmed influenza in hospitalized patients provides multiple useful population-based measures of seasonal influenza activity that focus on more severe illness attributed to influenza. Influenza hospitalization surveillance can also contribute to better characterization of the epidemiology of influenza across seasons. If more state health departments implemented case-based surveillance for influenza hospitalizations, the aggregated data could comprise a useful contribution to national influenza surveillance. Surveillance for influenza hospitalizations might also contribute to state surveillance capacity in preparation for an influenza pandemic as well as help target vaccination programs for seasonal influenza.

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Ms Proff is pursuing a master's degree in public health with a concentration in epidemiology from the University of Colorado School of Public Health, Denver. This study was performed as part of her master's program. Her primary research interests are infectious diseases.

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Tuberculosis Disparity between US-born Blacks and Whites, Houston, Texas, USA[1]

Jose A. Serpa, Larry D. Teeter, James M. Musser, and Edward A. Graviss

Tuberculosis (TB) rates in the United States are disproportionately high for certain ethnic minorities. Using univariate and multivariate analyses, we compared data for 1,318 US-born blacks with 565 US-born non-Hispanic whites who participated in the Houston TB Initiative (1995–2004). All available *Mycobacterium tuberculosis* isolates underwent susceptibility and genotype testing (insertion sequence *6110* restriction fragment length polymorphism, spoligotyping, and genetic grouping). TB in blacks was associated with younger age, inner city residence, HIV seropositivity, and drug resistance. TB cases clustered in 82% and 77% of blacks and whites, respectively (p = 0.46). Three clusters had >100 patients each, including 1 cluster with a predominance of blacks. Size of TB clusters was unexpectedly large, underscoring the ongoing transmission of TB in Houston, particularly among blacks.

After the unprecedented resurgence in tuberculosis (TB) in the United States during 1985–1992, the annual incidence rate of TB steadily decreased from 1993 to 2007. However, this decline recently decelerated, raising concerns that progress toward eliminating TB is slowing. In 2007, a total of 13,293 TB cases (4.4 cases per 100,000 population) were reported in the United States, representing a 4.2% decline in incidence from 2006 (*1*). Despite this decline, TB incidence remains higher among certain racial/ethnic minorities, i.e., blacks, Hispanics, and Asians, than for non-Hispanic whites. For instance, US-born young blacks probably account for the largest number of secondary TB cases in the country (*2*). In addition, TB rates for non-Hispanic blacks continue to be 8 times greater than for non-Hispanic whites (*3*). Hispanics and non-Hispanic blacks also accounted for nearly three quarters (73.9%) of 11,480 TB cases in children reported during 1993–2001, of which more than half occurred in children <5 years of age (*4*).

These findings clearly suggest recent ongoing transmission of *Mycobacterium tuberculosis* in these ethnic groups rather than reactivation of latent infection. Recently, molecular epidemiologic studies, which estimate the proportion of clustered TB cases, have been used to support this hypothesis (*5*). For instance, a molecular epidemiologic study of TB in San Francisco during 1991–1992 found *M. tuberculosis* clustering in 191 (41%) of 471 patients studied that were distributed in 44 clusters. Hispanic ethnicity or black race, birth in the United States, and an AIDS diagnosis independently predicted clustering (*6*). Similarly, other population-based studies in New York City and in Tarrant and Harris counties in Texas found clustering of 36%–52% (*7*–*9*).

Molecular studies of clustering and identification of risk factors that contribute to these racial/ethnic disparities are crucial for efforts to eliminate TB in the United States. Although differences in socioeconomic status and access to the healthcare system among non-Hispanic blacks have been postulated as the main barriers to TB diagnosis and treatment in this group, further information is needed (*3*,*10*). We studied the traditional and molecular epidemiology of TB in US-born blacks enrolled in the Houston Tuberculosis Initiative (HTI) during 1995–2004.

Materials and Methods

Study Population

The study population comprised patients recruited through HTI, a population-based active surveillance and

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molecular epidemiologic study that enrolls persons with TB reported to the City of Houston Department of Health and Human Services and Harris County Public Health and Environmental Services. In this study, we examined data from TB patients enrolled by HTI who had been reported during October 1995–September 2004.

Study participants were interviewed by trained interviewers using a standardized questionnaire designed to gather details of participant demographics, living situation, transportation, travel and social contacts, tobacco and alcohol use, illicit drug use, sexual behavior, history of incarceration, and personal medical history. Clinical information was supplemented through record review of all available inpatient, outpatient, and public health medical records.

Molecular Characterization of *M. tuberculosis*

We analyzed *M. tuberculosis* isolates with 3 molecular typing methods. First, isolates were characterized by an internationally standardized protocol of insertion sequence [IS] *6110* restriction fragment length polymorphism (RFLP) profiling (*11*) and analyzed with BioImage Whole Band Analysis version 3.2 software (BioImage, Ann Arbor, MI, USA). Second, for isolates with <5 IS*6110* copies, the spacer oligonucleotide type (spoligotype) was determined by using a commercially available kit (Isogen Bioscience BV, Maarsen, the Netherlands) according to the manufacturer's instructions. A member of the Beijing family was defined as having an octal spoligotype pattern of 000000000003771 (previously designated as S1) (*12*). Third, isolates were assigned to a principal genetic group on the basis of polymorphisms at codon 463 of the *katG* gene, which encodes catalase peroxidase, and at codon 95 of the *gyrA* gene, which encodes the A subunit of DNA gyrase (*13*). Susceptibility testing was performed by using BACTEC 460 radiometric culture system (Becton Dickinson, Sparks, MD, USA), at the hospital or reference laboratories supplying isolates to the HTI (*14*).

Definitions

We assigned race/ethnicity on the basis of self-report. *M. tuberculosis* isolates were defined as clustering if they satisfied 1 of the following criteria: 1) ≥2 isolates from the same principal genetic group with identical IS*6110* banding profiles containing ≥5 copies of IS*6110* or 2) isolates with identical IS*6110* banding profiles containing ≤4 copies of IS*6110* and sharing the same spoligotype and principal genetic group. We evaluated clusters H03 and H33 as a single cluster because they differ in only 1 band by IS*6110* RFLP.

We defined drug-resistant TB as an isolate resistant to at least 1 drug, including isoniazid, rifampin, ethambutol, pyrazinamide, or streptomycin. Multidrug-resistant TB was defined by resistance to isoniazid and rifampin, with or without resistance to other agents.

Data Analysis

Questionnaire data were entered into a longitudinal database (initially Epi Info version 6.02b, Centers for Disease Control and Prevention, Atlanta, GA, USA; and subsequently Microsoft Access, Microsoft, Redmond, WA, USA) for storage and analysis. Statistical analyses were conducted with STATA version 8.0 SE (StataCorp., College Station, TX, USA). Descriptive analysis was initially conducted, and selected demographic, social, and clinical factors were studied by univariate logistic regression to test for differences between US-born blacks and US-born whites. We used US-born whites, as opposed to all others, as the comparison group because of the heterogeneity in the latter group, particularly in non–US-born Hispanics and Asians. Variables with $p<0.2$ in the univariate analysis were considered in the elaboration of a multivariate logistic regression model to identify with factors independently associated with black race in TB patients. Covariates were not included in the model if they substantially decreased the sample size because of missing data. In our final model, we used the variable injection drug use instead of drug use because of colinearity and a strong association with the former variable. Covariates that were no longer significant after adjustment for other significant variables in the full model were dropped from the final parsimonious model.

Ethical Approval

Individual participants gave written informed consent before enrollment in the study. We obtained parental consent and patient assent for participants 13 to <18 years of age, and a parent served as a proxy for the interview of participants <13 years of age. Proxy permission and interview also were used for participants who had died or were not mentally capable of giving informed consent. The study was approved by the Institutional Review Board of Baylor College of Medicine, Houston, Texas, and affiliated hospitals, and the University of Texas Health Science Center–Houston, Committee for the Protection of Human Subjects.

Results

Study Population

A total of 4,312 persons with TB were reported in Harris County during October 1995–September 2004. Of those, 3,662 (85%) agreed to participate in the study and were interviewed by trained HTI personnel. The study population comprised 1,318 (36.0%) US-born blacks, 1,220 (33.3%) Hispanics, 545 (14.9%) US-born non-Hispanic whites, 463 (12.6%) Asians, 85 (2.3%) foreign-born blacks, 20 (0.5%) foreign-born non-Hispanic whites and 11 (0.3%) others. Of participants interviewed, 3,064 (84%) had a positive culture for *M. tuberculosis*; 2,806 (92%) of those isolates

underwent molecular characterization. Interviewed participants were more likely to be younger, black, HIV-seropositive, and *M. tuberculosis* culture–positive than were persons who refused to participate or could not be located ($p<0.01$ for first 3 comparisons and $p = 0.03$ for the last comparison).

Rates of TB during the Study Period

Although the overall US incidence of TB decreased from 8.0 to 4.9 cases per 100,000 persons during 1996–2004, TB incidence in Harris County has remained fairly stable since 2000 after an initial decrease in 1996–1999 (Figure 1). Blacks consistently had the highest TB incidence in Harris County. In 2004, blacks accounted for approximately 56% of all TB cases among US-born participants despite representing only 18% of the Harris County population (Texas State Data Center and Office of the State Demographer, http://txsdc.utsa.edu/tpepp/txpopest.php).

US-born Blacks versus US-born Whites

US-born blacks were more likely than US-born whites to be younger, unmarried, and unemployed; have less education; reside in the inner city, earn less income; use public transportation; have renal and extrapulmonary disease; be HIV seropositive, *M. tuberculosis* culture–positive, and part of a cluster; and have drug-resistant *M. tuberculosis* (Table 1).

The 614 HIV-infected TB patients in our study reflected a 28% rate of co-infection for US-born blacks and 20% for US-born whites ($p<0.001$). Rates of HIV co-infection were markedly lower for Hispanics and Asians (9.1% and 1.7%, respectively). Pulmonary disease was the predominant clinical presentation in the study population; however, extrapulmonary disease was reported more frequently for US-born blacks than for US-born whites ($p<0.01$).

Resistance to at least 1 anti-TB agent was recorded for 80 (7.3%) isolates from US-born blacks, compared with 22 (4.5%) isolates from US-born whites ($p = 0.04$). Multidrug-resistant TB represented only 1% of all cases. Mortality rate (i.e., death within 180 days after TB diagnosis) was ≈10% and was similar for US-born blacks and US-born whites.

Clustering of Cases within Groups

Among all 4,312 cases reported during the study period, 3,578 (including enrolled and nonenrolled participants) had at least 1 positive *M. tuberculosis* culture. Isolates from 3,227 (90%) were genotyped, including 1,984 (61.5%) that matched at least 1 other isolate. A total of 242 clusters were identified, and cluster size varied from 2 to 172 patients (mean 57.2, median 27).

Isolates of 1,765 (63%) of the 2,807 enrolled persons were clustered, including 822 (82%) of 1,007 isolates from US-born blacks and 349 (77%) of 448 isolates from US-born whites ($p = 0.05$). Rates of clustering were significantly lower for Hispanics and Asians (52% and 28%, respectively; $p<0.01$), probably underscoring a higher proportion of reactivation of latent infection in these patients. Cluster size for US-born blacks was larger (mean 69.3, median 45) than for US-born whites (mean 46.7, median 14; $p<0.001$). TB strains belonging to the Beijing family represented 26% and 29% of isolates from US-born blacks and US-born whites, respectively ($p = 0.25$). Two (H03/H33 and L16) of the 9 largest clusters containing at least 30 patients had more US-born blacks than US-born whites (Table 2, Figure 2).

Multivariate Logistic Regression Analysis

In our final model (Table 3), factors independently associated with black race among TB patients included younger age, fewer years of education, inner city residence, use of public transportation, prison history, renal disease, and HIV seropositivity. US-born blacks were significantly less likely than U.S.-born whites to report current homelessness, smoking, alcohol abuse, injection drug use, and same-sex behavior. Although male sex, not being married, *M. tuberculosis* culture positivity, employment, and extrapulmonary disease were significant in the univariate analysis, they were no longer significant in the full model and therefore were dropped from the final parsimonious model. Because including certain covariates significantly decreased the sample size (and the representativeness of the dataset), we developed a second model (data not shown) by adding drug resistance and clustering to the final model. In this model, drug resistance remained significantly higher for US-born blacks than for US-born whites (odds ratio [OR] 1.76; 95% confidence interval [CI] 1.02–3.04, $p = 0.04$); however, clustering did not remain significant (OR 1.13, 95% CI 0.82–1.54, $p = 0.46$).

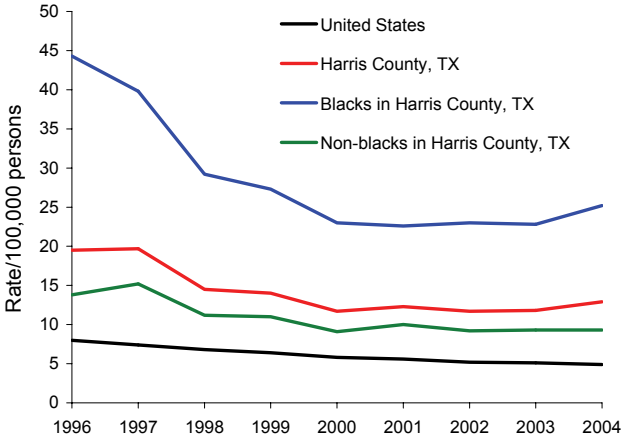

Figure 1. Tuberculosis rates, Houston Tuberculosis Initiative, Texas, 1996–2004.

Table 1. Univariate analysis of selected epidemiologic and clinical characteristics of participants in the Houston Tuberculosis Initiative, Texas, 1995–2004*

Variable	US-born black	US-born white	Hispanic	Asian	US-born black vs. white, OR (p value)
Demographics					
Age, y, mean (median)	42.5 (43.0)	48.2 (47.0)	36.9 (36.0)	45.1 (43.0)	0.98 (<0.001)
Male sex	938 (71.2)	429 (78.7)	783 (64.2)	257 (55.5)	0.67 (0.001)
Not married	1,091 (82.8)	412 (75.6)	742 (60.8)	202 (43.6)	1.56 (<0.001)
Years of education, mean (median)	10.6 12.0	11.7 12.0	254 (20.8)	260 (56.3)	0.90 (<0.001)
Employed	453 (34.4)	214 (39.3)	501 (41.1)	209 (45.1)	0.81 (0.045)
Inner city residence†	538 (40.9)	198 (36.7)	384 (31.5)	40 (8.6)	1.19 (0.094)
Household size, mean (median)	5.2 (3.0)	4.5 (2.0)	4.7 (4.0)	5.1 (4.0)	1.00 (0.454)
Social history					
Income <$10K/year	554 (51.7)	161 (36.0)	466 (46.0)	78 (18.1)	1.90 (<0.001)
Use of public transportation	664 (50.4)	177 (32.5)	363 (29.8)	40 (8.6)	2.11 (<0.001)
Current homelessness	129 (9.8)	77 (14.2)	32 (2.6)	4 (0.9)	0.66 (0.007)
Stay in a shelter‡	132 (10.0)	65 (11.9)	17 (1.4)	5 (1.1)	0.82 (0.222)
Current smoking	745 (56.7)	388 (71.5)	279 (22.9)	80 (17.3)	0.52 (<0.001)
Alcohol abuse§	601 (45.8)	311 (57.4)	251 (20.6)	50 (10.8)	0.63 (<0.001)
Drug use‡	351 (26.7)	129 (23.7)	110 (9.0)	4 (0.9)	1.17 0.179)
Injection drug use‡	25 (1.91)	25 (4.6)	9 (0.74)	1 (0.22)	0.40 (0.002)
Same-sex sexual behavior	129 (9.9)	91 (17.0)	69 (5.7)	2 (0.4)	0.54 (<0.001)
Commercial sex	168 (13.1)	67 (12.6)	52 (4.3)	2 (0.4)	1.05 (0.758)
Prison history	281 (21.4)	92 (16.9)	49 (4.0)	5 (1.1)	1.34 (0.026)
Medical history					
Known TB exposure	502 (38.1)	222 (40.7)	375 (30.7)	86 (18.6)	0.88 (0.222)
Previous TB	112 (8.5)	55 (10.1)	56 (4.6)	41 (8.9)	0.83 (0.274)
Asthma	85 (6.4)	28 (5.1)	40 (3.3)	12 (2.6)	1.27 (0.282)
Diabetes	168 (12.7)	61 (11.2)	224 (18.4)	62 (13.4)	1.16 (0.353)
Renal disease	76 (5.8)	15 (2.8)	34 (2.79)	21 (4.54)	2.16 (0.007)
HIV seropositivity	364 (27.6)	109 (20.0)	111 (9.1)	8 (1.7)	1.52 (0.001)
Diagnosis and prognosis					
Pulmonary TB	1,067 (81.0)	496 (91)	966 (79.2)	341 (73.7)	0.42 (<0.001)
Extrapulmonary TB	400 (30.3)	97 (17.8)	354 (29.0)	153 (33.0)	2.01 (<0.001)
Cavitation	458 (45.1)	213 (46.1)	405 (45.0)	111 (37.1)	0.96 (0.714)
Smear-positive pulmonary TB	577 (54.2)	257 (51.9)	473 (49.2)	132 (38.7)	1.10 (0.405)
M. tuberculosis culture positivity	1,102 (83.7)	486 (89.2)	979 (80.2)	402 (86.8)	0.62 (0.002)
TB clustering	822 (81.6)	349 (77.2)	466 (51.8)	100 (27.9)	1.31 (0.050)
Drug resistance	80 (7.3)	22 (4.5)	105 (10.7)	79 (19.7)	1.65 (0.043)
Multidrug resistance	3 (0.3)	1 (0.2)	15 (1.5)	9 (0.9)	1.32 (0.808)
Death¶	143 (10.8)	62 (11.4)	80 (6.6)	28 (6.0)	0.95 (0.741)

*Stratification based on race does not include information about foreign-born blacks. Data presented as no. (%) participants except as indicated. OR, odds ratio; TB, tuberculosis; *M. tuberculosis*, *Mycobacterium tuberculosis*.
†Defined according to specific zip codes.
‡Within 6 months before TB diagnosis.
§Drinks alcohol daily or nearly daily.
¶Within 180 days after TB diagnosis.

Discussion

Although the incidence rate of TB in the United States during 2007 was the lowest recorded since national reporting began in 1953, the decline has slowed from an average of 7.1% per year (1993–2000) to an average of 3.8% per year (2001–2007) (*1*). Our population-based study of TB in a large US metropolitan city emphasized the epidemiologic, molecular, and clinical characteristics of US-born blacks. During the 9-year study period, the overall incidence of TB in Harris County, Texas, decreased dramatically; however, the incidence among blacks remained fairly stable (22.6–25.2 cases per 100,000) over the past 5 years of the study. This incidence is 4–5× the US national TB incidence rate (*1*). Numerous factors have been postulated to explain the disproportionately high TB rate for blacks, including socioeconomic characteristics, biasing presence of comorbidities, and genetics (*15*,*16*).

Our evaluation of socioeconomic characteristics demonstrated that younger age, fewer years of education, use of public transportation, and inner city residence were independently associated with black race among TB patients. These associations support the concept that TB remains predominantly a disease of disadvantaged and marginalized persons (*17*,*18*). Contrary to findings recently reported

Table 2. Characteristics of the 9 largest *Mycobacterium tuberculosis* clusters, Houston Tuberculosis Initiative, Texas, 1995–2004*

Cluster	IS*6110* copy no.	CDC spoligotype designation	Genetic group	Participant data				
				US-born black, no. (%)	US-born white, no. (%)	Hispanic, no. (%)	Asian, no. (%)	US-born black vs. white, OR (p value)
H01	12	7760 3777 7760 771	3	96 (9.5)	29 (6.4)	22 (2.44)	4 (1.11)	1.54 (0.051)
H03/H33	20/21	0000 0000 0003 771	1	109 (10.8)	31 (6.9)	8 (0.89)	2 (0.56)	1.65 (0.018)
H02	13	0000 0000 0003 771	1	77 (7.75)	28 (6.2)	20 (2.22)	1 (0.28)	1.25 (0.322)
H15	9	0000 0000 0003 771	1	25 (2.48)	7 (1.5)	6 (0.67)	0	1.62 (0.264)
H07	10	0000 0000 0003 771	1	46 (4.6)	22 (4.9)	11 (1.22)	0	0.94 (0.802)
H16	9	7777 0375 7760 771	2	28 (2.8)	8 (1.8)	5 (0.56)	0	1.59 (0.254)
H04	6	7777 7677 7760 771	2	12,(1.2)	50 (11.1)	10 (1.11)	1 (0.28)	0.10 (<0.001)
L08	2	7777 7677 7760 601	2	55 (5.5)	23 (5.1)	28 (3.11)	2 (0.56)	1.08 (0.770)
L16	3	7777 7677 7760 601	2	26 (2.6)	2 (0.4)	4 (0.44)	0 (0.00)	5.96 (0.015)

*IS, insertion sequence; OR, odds ratio.

(*18*), our population-based study showed that US-born whites with TB were more likely than US-born blacks with TB to be homeless. In addition, certain clinical conditions such as renal disease and HIV seropositivity were more likely in US-born blacks with TB.

M. tuberculosis isolates from US-born blacks were more likely to be resistant than were those from US-born whites, which was in accordance with previously reported findings (*19*). However, foreign-born persons, particularly Hispanics and Asians, still have the most drug-resistant TB in Houston (*20*). Among our study population, death from any cause within 6 months after TB diagnosis reported was ≈10%. We found no difference in TB-associated mortality rates between US-born blacks and whites.

TB strains clustered in a high proportion of both US-born blacks and US-born whites (81.6% and 77.2%, respectively; p = 0.46). We previously demonstrated that ethnicity was not a significant covariate for strain clustering after adjustments for factors related to socioeconomic status (*21*). Isolates belonging to the Beijing genotype family clustered in 26% and 29% of US-born blacks and US-born whites, respectively (p = 0.25). This genetically highly conserved family, reported worldwide but particularly prevalent in Asia and the territories of the former Soviet Union (*22*,*23*), is frequently associated with large TB outbreaks, increased virulence, and multidrug resistance (*24*,*25*).

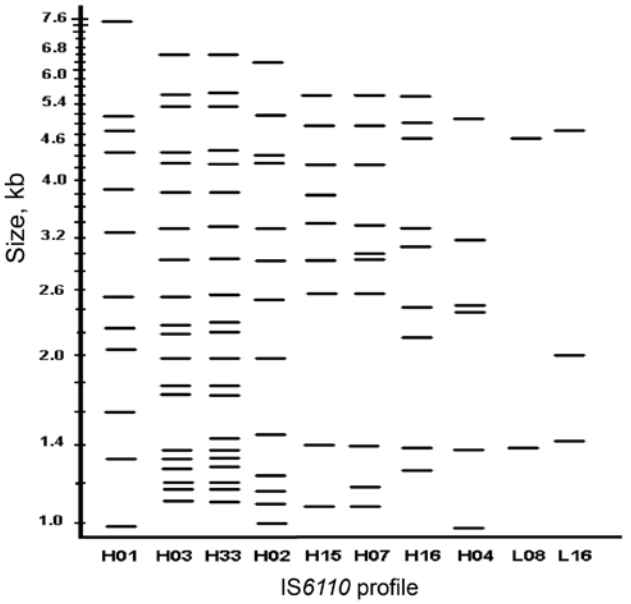

Figure 2. Insertion sequence (IS)*6110* profiles of the 10 largest *Mycobacterium tuberculosis* clusters, Houston Tuberculosis Initiative, Texas, 1995–2004.

One of the most striking findings of our study is the markedly large size of clusters. Previous US-based studies had reported cluster sizes not exceeding 30–78 patients each (*6*,*8*,*9*). We reported 9 clusters with >30 patients each and 3 clusters with >100 patients each, including 1 with a predominance of blacks. This may be due to the longer surveillance period used here, as well as to endemic spread of highly transmissible strains, including several of the Beijing family. We estimated that at least 54% (1,984 – 242 [of 3,227]) of TB cases in Houston resulted from recent infection that had progressed to active disease during the 9-year study period (*6*). We believe this estimate is conservative because a strict definition of identical IS*6110* RFLP bands

Table 3. Factors associated with black race in multivariate analysis in US-born TB patients, Houston Tuberculosis Initiative, Texas, 1995–2004*

Risk factor	OR	95% CI	p value
Age	0.98†	0.97–0.99	<0.001
Years of education	0.93†	0.89–0.96	<0.001
Inner city residence‡	1.44	1.13–1.85	0.003
Use of public transportation	2.44	1.91–3.11	<0.001
Current homelessness	0.48	0.33–0.68	<0.001
Current smoking	0.54	0.42–0.70	<0.001
Alcohol abuse§	0.63	0.50–0.80	0.001
Injection drug use¶	0.30	0.16–0.57	<0.001
Same-sex sexual behavior	0.28	0.19–0.41	<0.001
Prison history	1.42	1.06–1.91	0.020
Renal disease	2.61	1.38–4.92	0.003
HIV seropositivity	1.89	1.37–2.61	<0.001

*TB, tuberculosis; OR, odds ratio; CI, confidence interval.
†ORs per additional year at risk.
‡Defined according to specific zip codes.
§Drinks alcohol daily or nearly daily.
¶Within 6 months before TB diagnosis.

was used for clustering, and several RFLP patterns in our study were similar but with ±1 band (Figure 2).

Several TB genotypes remain endemic to Houston, particularly in minorities such as US-born blacks. A high proportion of endemic strains of *M. tuberculosis* remain actively transmitted within this population. Further research on the dynamics of the disease, including possible delays in care-seeking behavior or diagnosis in this racial group, is of paramount importance to achieve the national goal for TB elimination.

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Changes in Fluoroquinolone-Resistant *Streptococcus pneumoniae* after 7-Valent Conjugate Vaccination, Spain

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Among 4,215 *Streptococcus pneumoniae* isolates obtained in Spain during 2006, 98 (2.3%) were ciprofloxacin resistant (3.6% from adults and 0.14% from children). In comparison with findings from a 2002 study, global resistance remained stable. Low-level resistance (30 isolates with MIC 4–8 μg/mL) was caused by a reserpine-sensitive efflux phenotype (n = 4) or single topoisomerase IV (*parC* [n = 24] or *parE* [n = 1]) changes. One isolate did not show reserpine-sensitive efflux or mutations. High-level resistance (68 isolates with MIC ≥16 μg/mL) was caused by changes in gyrase (*gyrA*) and *parC* or *parE*. New changes in *parC* (S80P) and *gyrA* (S81V, E85G) were shown to be involved in resistance by genetic transformation. Although 49 genotypes were observed, clones Spain^{9V}-ST156 and Sweden^{15A}-ST63 accounted for 34.7% of drug-resistant isolates. In comparison with findings from the 2002 study, clones Spain^{14}-ST17, Spain^{23F}-ST81, and ST88^{19F} decreased and 4 new genotypes (ST97^{10A}, ST570^{16}, ST433^{22}, and ST717^{33}) appeared in 2006.

The bacterium *Streptococcus pneumoniae* is a serious cause of illness and death and a major etiologic agent of community-acquired pneumonia, meningitis, and acute otitis media. Pneumococcal resistance to antimicrobial drugs (including β-lactams, macrolides, tetracycline, and cotrimoxazole) has become a worldwide problem (*1*); new fluoroquinolones are being used as therapeutic alternatives for treatment of adult patients with community-acquired pneumonia (*2*). Resistance to fluoroquinolones in *S. pneumoniae* can be acquired by point mutations, intraspecific recombination (*3*) or interspecific recombination with the *S. mitis* group (*3–7*). Resistance is caused mainly by amino acid changes in quinolone resistance–determining regions (QRDRs) of the subunits of DNA topoisomerase IV (topo IV; $parC_2$ and $parE_2$) and DNA gyrase ($gyrA_2$ and $gyrB_2$) enzymes that control DNA topology. In addition, fluoroquinolone efflux also contributes to resistance (*8*). Genetic and biochemical studies have shown that for most fluoroquinolones, such as ciprofloxacin and levofloxacin, topo IV and gyrase are primary and secondary targets, respectively (*9–13*). However, gyrase is the primary target for moxifloxacin (*14*).

Although current prevalence of fluoroquinolone resistance in pneumococci is <5% (*15–17*), surveillance is necessary. Introduction of the 7-valent conjugate pneumococcal vaccine (PCV7), which includes serotypes such as 6B, 9V, 14, and 23F that are often associated with resistance to fluoroquinolones and other antimicrobial drugs, has resulted in changes in the epidemiology of invasive pneumococcal disease (*18–20*). Since the introduction of PCV7 in Spain in late 2001, ≈47% of children have been vaccinated (*21*).

In this study, we investigated the prevalence of fluoroquinolone-resistant pneumococci in Spain during 2006. Mutations in the QRDRs of *parC*, *parE*, and *gyrA* were identified, and the presence of reserpine-sensitive fluoro-

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quinolone efflux was determined. In addition, resistance associations with other antimicrobial drugs and characteristics of drug-resistant clones were determined. To better evaluate changes in the epidemiology of resistance after the introduction of PCV7 in children, we compared our results with those of a similar study that tested isolates from 2002.

Methods

Bacterial Isolates, Serotyping, Susceptibility Testing, and Genetic Transformation

We studied 4,215 *S. pneumoniae* isolates from 2 hospitals (in Barcelona and San Sebastián), and a sample from 110 hospitals throughout Spain (Spanish Reference Laboratory, Madrid). Of the isolates, 2,682 were from adults, 1,400 from children, and 133 from persons whose ages were unknown. A total of 2,101 (49.9%) isolates were obtained from blood or other sterile sites; 1,055 (25%) from the lower respiratory tract; 960 (22.8%) from the upper respiratory tract, otic and conjunctival sites; and 99 (2.3%) from other sites. Isolates were confirmed as *S. pneumoniae* by standard methods, and serotypes were determined by the Quellung reaction. Ciprofloxacin susceptibility was determined by broth microdilution tests (Sensititer; Trek Diagnostics Inc., East Grinstead, UK) and by agar dilution according to the Clinical and Laboratory Standards Institute guidelines (*22*). Reserpine-sensitive fluoroquinolone efflux phenotype was determined as described (*23*). We performed genetic transformation as described (*24*) by using *S. pneumoniae* strains R6 and T1 (*25*) as receptors. For selection of transformants, we used media plates containing 1 μg/mL (R6 derivatives) or 8 μg/mL (T1 derivatives) of ciprofloxacin.

Pulsed-Field Gel Electrophoresis and Multilocus Sequence Typing

Pulsed-field gel electrophoresis (PFGE) patterns were determined by using *Sma*I and *Apa*I as described (*24*) and compared with 26 representative clones of the Pneumococcal Molecular Epidemiology Network (*26*). Isolates with patterns varying by ≤3 bands were considered to represent the same PFGE type (*27*). Multilocus sequence typing was performed as described (*28*) with representative isolates of PFGE types shared by ≥3 isolates (www.mlst.net). We analyzed selected strains representative of dominant clones from the 2002 study by multilocus sequence typing.

PCR Amplification and DNA Sequence Determination

Oligonucleotides parE398 (*29*) and parC152 (*10*) were used to amplify *parE* and *parC* QRDRs. All isolates yielded fragments of 1.6 kb, with the exception of ciprofloxacin-resistant (CipR) isolates CipR17, CipR39, CipR74, and CipR76, which yielded fragments of ≈5, 5, 5, and 7 kb, respectively. These PCR fragments were sequenced as described (*24*). Oligonucleotides gyrA44 and gyrA170 (*29*) were used to amplify and sequence *gyrA* QRDRs. Oligonucleotides antUP and antDOWN (*4*) were used to detect the *ant* gene.

Results

Among the 4,215 isolates studied, 98 were CipR. Of these isolates, 30 (30.6%) showed low-level resistance (LL-CipR, MICs 4–8 μg/mL) and 68 (69.4%) high-level resistance (HL-CipR, MICs 16–128 μg/mL) (Table 1). By age group, the prevalence of CipR was 0.14% (2/1,400) among isolates from pediatric patients (<15 years of age) and 3.6% (96/2,682) among isolates from adult patients. Resistance was higher among noninvasive pneumococci (3.3%, 70/2,114) than among invasive isolates (1.3%, 28/2,101, $p<0.001$). The highest rate of Cip resistance was found for isolates from adults >64 years of age (Table 1). All HL-CipR isolates were from adult patients; most (53/68, 77.9%) were isolated from sputum. CipR isolates showed high rates of resistance to antimicrobial drugs. However, these rates were lower than those found in the 2002 study (Table 1).

The *parC*, *parE*, and *gyrA* QRDRs of the 98 CipR isolates were characterized. Most CipR isolates (93/98) showed low nucleotide sequence variations (≤1%) in their QRDRs, but 5 isolates showed high variations (>4%). Four of them were in *parC*, *parE*, and *gyrA*, and only 1 was in *gyrA*. These results suggest an interspecific recombinant origin for these genes. In accordance, all isolates with recombinant *parE* and *parC* genes carried the *ant* gene, typical of the *S. mitis* group (*4*), as shown by PCR amplification.

Twenty-one of the 98 isolates had efflux for Cip; 3 of them also had efflux for levofloxacin (Tables 2, 3), and none had efflux for moxifloxacin. Efflux was equally distributed among LL-CipR and HL-CipR isolates. The contribution of the efflux mechanism to resistance in those isolates is unclear. Mutations not previously described that produced changes in parC (D78N, S80P, D83E), parE (I476F), and gyrA (G79A, S81V, E85G, V101I) were found in 8 isolates. To test the contribution of these changes to resistance, transformation experiments using strains R6 or T1 (as R6, parC S79F) as receptors of *parC* or *gyrA* QRDRs, respectively, were performed. The QRDRs of several independent transformants were sequenced to confirm the presence of the same mutation in the donor DNA and MICs of these transformants were determined. Although no transformation was achieved with PCR products carrying parC D78N or parE I476F, transformation to increased resistance was observed with products carrying parC S80P, gyrA S81V, and gyrA E85G changes (Table 2).

Three of these changes were accompanied by other changes known to be involved in resistance: gyrA G79A with S81F; parC D83E with S79F, and gyrA V101I with

Table 1. Comparison of 2 surveillance studies on ciprofloxacin-resistant *Streptococcus pneumoniae* isolates in Spain, 2002 and 2006*

Characteristic	No. ciproflaxin resistant/no. isolates (%) 2002	2006	p value
Ciproflaxin resistance			
Global	75/2,882 (2.6)	98/4,215 (2.3)	NS
Low-level (MICs 4–8 μg/mL)	14/75 (18.7)	30/98 (30.6)	NS
High-level (MICs ≥16 μg/mL)	61/75 (81.3)	68/98 (69.4)	NS
In persons <15 years of age	0/978 (0)	2/1,446 (0.14)	NS
In persons 15–64 years of age	22/1,166 (1.9)	34/1,455 (2.3)	NS
In persons >64 years of age	53/738 (7.2)	62/1,314 (4.7)	0.02
PCV7 serotypes	49/75 (65.3)	35/98 (35.7)	<0.001
Other antimicrobial drug resistance	No. resistant/no. ciproflaxin-resistant isolates (%)		
Penicillin MIC ≥0.12 μg/mL	55/75 (73.3)	44/98 (44.9)	<0.001
Erythromycin MIC ≥0.5 μg/mL	53/75 (70.7)	53/98 (54.1)	0.03
Clindamycin MIC ≥1 μg/mL	47/75 (62.7)	45/98 (45.9)	0.03
Chloramphenicol MIC ≥8 μg/mL	33/75 (44.0)	11/98 (11.2)	<0.001
Tetracycline MIC ≥4 μg/mL	52/75 (69.3)	39/98 (39.8)	<0.001
Cotrimoxazole MIC ≥4/76 μg/mL†	51/75 (68.0)	47/98 (47.8)	0.008
Multidrug resistance (≥3 drugs)	55/75 (73.3)	48/98 (49.0)	<0.001

*NS, not significant; PCV7, 7-valent conjugate pneumococcal vaccine. Ciproflaxin resistance is defined by Chen et al. (*30*) as an MIC ≥4 μg/mL.
†MIC is 4 μg/mL for trimethoprim and 76 μg/mL for sulfamethoxazole.

S81F. Among 5 T1 transformants obtained with a gyrA QRDR carrying G79A and S81F, 4 carried G79A and S81F and only 1 carried S81F. Because all transformants had identical Cip MICs, results suggest that G79A is not involved in drug resistance. We could not discern the role of parC D83E and gyrA S81F in resistance, given that all R6-transformants had parC D83E and S79F and all T1 transformants had gyrA V101I and S81F. However, given the contribution to resistance of the accompanied mutations, their role in resistance is unlikely.

Table 2. Fluoroquinolone MICs of 30 low-level resistant *Streptococcus pneumoniae* isolates and 5 laboratory strains and amino acid changes in their DNA topoisomerase IV and gyrase genes, Spain, 2006*

No. isolates	Amino acid substitution *parC* S79	*parC* S80	*parC* D83	*parE* D435	*parE* E474	*gyrA* S81	*gyrA* E85	MIC, μg/mL CIP	LVX	MXF	Efflux phenotype†
1	–	–	–	–	–	–	–	4	1	0.12	None
3	–	–	–	–	–	–	–	4–8	2	0.5	CIP
1	–‡	–‡	–‡	–‡	–‡	–‡	–‡	8	4	0.5	CIP
9	F	–	–	–	–	–	–	4–8	1–2	0.25–0.50	None
3	F	–	–	–	–	–	–	4–8	2	0.12–0.25	CIP
1	F	–	–	–	–	–‡	–‡	8	1	0.12	None
1	F‡	–‡*	–‡	–‡	–‡	–‡	–‡	8	2	0.12	CIP, LVX
5	Y	–	–	–	–	–	–	4–8	2	0.12–0.25	None
1	Y	–	–	–	–	–	–	4	2	0.25	CIP
1	–	–	N	–	–	–	–	16	4	0.5	None
1	–	–	N	–	–	–	–	4	2	0.12	CIP
1	–	–	Y	–	–	–	–	4	1	0.5	None
1	–	–	Y	–	–	–	–	8	2	0.5	CIP
1	–	–	–	N	–	–	–	8	2	0.12	None
Laboratory strains§											
R6								0.5	0.25	0.12	None
$R6^{CS80P}$	–	P	–	–	–	–	–	2	1	0.25	None
T1	F	–	–	–	–	–	–	4	2	0.12	None
$T1^{AS81V}$	F	–	–	–	–	V	–	32	32	4	None
$T1^{AE85G}$	F	–	–	–	–	–	G	32	8	2	None

**par*, topoisomerase gene; *gyr*, gyrase gene; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin. Only changes involved in resistance are shown. –, no change. Additional amino acid changes not involved in resistance were parC D78N (1 isolate), parC K137 N (9), parC N91D (2 with mosaic *parC* genes), parE I460V (17), parE I476F (1), gyrA S114G (2 with mosaic *gyrA* genes), and gyrA N150H (1 with a mosaic *gyrA* gene).
†An isolate was considered to have an efflux phenotype for the indicated fluoroquinolone when a ≥2-fold decrease in its MIC in the presence of reserpine was observed.
‡Indicates that the residue is located in a recombinant gene.
§$R6^{CS80P}$, R6 derivative carrying parC S80P; $T1^{AS81V}$, T1-derivative carrying gyrA S81V; $T1^{AE85G}$, T1-derivative carrying gyrA E85K.

Table 3. Fluoroquinolone MICs of 68 high-level resistant *Streptococcus pneumoniae* isolates and amino acid changes in their DNA topoisomerase IV and gyrase genes, Spain, 2006*

No. isolates	Amino acid substitution							MIC, μg/mL			Efflux phenotype†
	parC			*parE*		*gyrA*					
	S79	S80	D83	D435	E474	S81	E85	CIP	LVX	MXF	
4	F	–	–	–	–	F	–	64	16–32	4	CIP
21	F	–	–	–	–	F	–	32–128	16–32	2–8	None
1	F	–	–	–	–	L	–	64	32	2	None
1	F	–	–	–	–	V	–	64	32	4	CIP
3	F	–	–	–	–	Y	–	64–128	16–32	4	None
1	F	–	–	–	–	–	G	32	16	4	None
2	F	–	–	–	–	–	K	32–64	16–32	2–4	None
1	Y‡	–‡	–‡	–‡	–‡	F‡	–‡	64	32	4	None
8	Y	–	–	–	–	F	–	32–64	16–32	2–4	None
1	Y	–	–	–	–	F	–	64	32	4	CIP, LVX
1	Y	–	–	–	–	Y	–	64	32	4	None
1	Y	–	–	–	–	–	K	32	16	2	None
1	–	P	–	–	–	F	–	16	4	0.5	None
1	–	–	H	–	–	F	–	32	16	2	CIP
1	–	–	Y	–	–	F	–	32	16	2	CIP
2	–	–	Y	–	–	F	–	32	8–16	2–4	None
1	–	–	N	–	–	–	K	16	8	2	None
3	–	–	–	N	–	F	–	16	8	0.5–2	None
1	–‡	–‡	–‡	N‡	–‡	F‡	–‡	16	4	0.5	CIP
1	F	–	G	–	–	F	–	64	32	4	CIP, LVX
2	F	–	G	–	–	F	–	32–64	32	4	None
1	F	–	G	–	–	L	–	64	64	16	None
1	F	–	H	–	–	F	–	64	32	4	None
2	F	–	N	–	–	F	–	32–64	16–32	4	None
2	F	–	–	N	–	F	–	64–128	32–128	4–32	None
1	F	–	–	N	–	–	K	16	32	4	None
1	F	–	–	–	K	F	–	64	32	4	None
1	F	–	–	–	–	F	A	64	16	4	None
1	F	–	–	–	–	F	K	32	32	4	None

*_par_, topoisomerase gene; _gyr_, gyrase gene; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin. Only changes involved in resistance are shown. –, no change. Additional amino acid changes not involved in resistance were parC D83E (1), parC K137 N (24), parC N91D (2 with mosaic *parC* genes), parE I460V (47), and gyrA S114G (2 with mosaic *gyrA* genes).
†An isolate was considered to have an efflux phenotype for the indicated fluoroquinolone when a ≥2-fold decrease in its MIC in the presence of reserpine was observed.
‡Indicates that the residue is located in a recombinant gene.

The contribution of classical and new mutations to Cip resistance described here enabled us to classify resistant isolates (Tables 2, 3). Five LL-CipR isolates did not show changes involved in resistance in their *parC*, *parE*, or *gyrA* QRDRs, including 1 with recombinant genes (Table 2). Four of them showed a reserpine-sensitive efflux phenotype for Cip (Table 2) as a single mechanism of resistance. Among the remaining 25 LL-CipR isolates, 24 had mutations producing changes at parC, and 1 isolate had a single change at parE. Among 68 HL-CipR isolates, 55 (80.9%) had double changes (51 in parC and gyrA and 4 in parE and gyrA), and 13 (19.1%) had triple mutations (7 had 2 changes in parC and 1 change in gyrA; 4 had 1 change in parC, 1 change in parE, and 1 change in gyrA; 2 had 1 change in parC and 2 changes in gyrA). According to Clinical and Laboratory Standards Institute guidelines (*22*), only 3 of the 30 LL-CipR isolates showed intermediate resistance to levofloxacin (MIC 4 μg/mL), and the remaining 27 isolates were susceptible to levofloxacin; all were susceptible to moxifloxacin. HL-CipR isolates showed resistance (n = 66) or intermediate resistance (n = 2) to levofloxacin. Five HL-CipR isolates were susceptible to moxifloxacin, 11 showed intermediate resistance, and 52 were resistant.

Serotype and genotype distributions of CipR isolates of 2002 (*24*) and 2006 were compared (Figure). Although isolates from 2006 belonged to 29 different serotypes, 5 serotypes (14, 9V, 8, 19A, and 6B) accounted for 44.9% of the total. The rate of PCV7 serotypes among CipR isolates decreased ($p<0.001$) in 2006 (Table 1) because of a decrease in serotypes 23F, 19F, and 6B (Figure, panel A). Forty-nine genotypes were observed among the 98 CipR isolates (Figure, panel B). Clones Spain^{9V}-ST156 (21 isolates) and Sweden^{15A}-ST63 (13 isolates) accounted for 34.7% of the CipR isolates. Capsular switch events were frequent in

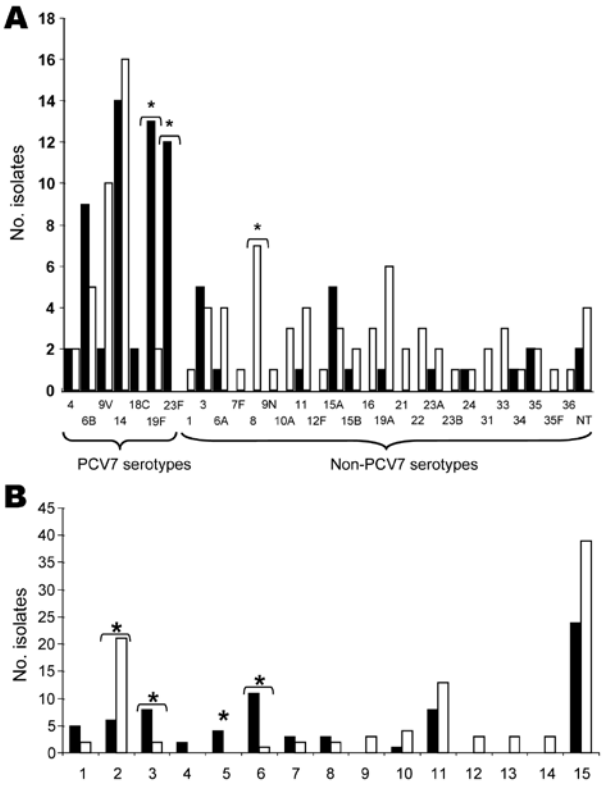

Figure. Serotype (A) and genotype (B) distributions of ciprofloxacin-resistant pneumococci isolated in Spain, 2002 and 2006. A total of 75 isolates from 2002 (black columns) and 98 from 2006 (white columns) were compared. Asterisks indicate significant differences ($p<0.05$) between the 2 years. PCV7, 7-valent conjugate pneumococcal vaccine. Baseline numbers in B indicate various genotypes. 1, Spain^{6B}-ST90; 2, Spain^{9V}-ST156; 3, Spain^{14}-ST17; 4, Netherlands^{18C}-ST113; 5, ST88^{19F}; 6, Spain^{23F}-ST81; 7, Netherlands^{3}-ST180; 8, ST260^{3}; 9, ST97^{10A}; 10, ST62^{11A}; 11, Sweden^{15A}-ST63; 12, ST570^{16}; 13, TS433^{22}; 14, ST717^{33}; 15, other.

these clones (Figure): Spain^{9V}-ST156 (12 switches) and Sweden^{15A}-ST63 (11 switches). Four new genotypes related to non-PCV7 serotypes, (ST97^{10A}, ST570^{16}, ST433^{22}, ST717^{33}, each represented by 3 isolates) emerged in 2006 (Figure, panel B).

As we observed, isolates that shared the same PFGE pattern also shared identical polymorphisms on their DNA topoisomerase QRDRs. All but 1 of the isolates belonging to the Spain^{9V}-ST156 clone had identical polymorphisms, the same found in the ATCC 700671 strain representative of this clone (*15*); the only exception was an isolate with *parC*, *parE*, and *gyrA* recombinant genes.

Discussion

We observed a stabilization during 2002–2006 in the rates of fluoroquinolone resistance in Spain. Although the rate of Cip resistance in 2002 was 2.6% (2.2% for levofloxacin), it was 2.3% (1.7% for levofloxacin) in 2006. The rates of Cip resistance were also similar for the different age groups (3.5% for adults and 0.14% for children in 2006). However, a decrease in the rate of resistance in persons >64 years of age was found in 2006. Higher levels of resistance were found in *S. pneumoniae* isolated from sputa and in isolates from people >64 years of age, who more frequently have chronic obstructive pulmonary disease and who have been treated with multiple regimens of antimicrobial drugs. In accordance, development of fluoroquinolone resistance has been reported for these patients (*31–33*). The frequency of HL-CipR resistance in adults was 2.5% (68/2,769), slightly higher than that reported for persons in other countries in Europe (*34*).

Four factors may have contributed to the observed stabilization of resistance rates. These factors are fluoroquinolone use, change in circulating clones, no recommendation of fluoroquinolones for children, and fitness cost of resistance mutations.

A direct correlation between use of fluoroquinolone and prevalence of resistance in *S. pneumoniae* has been described (*30,35*). Cip use in Spain has remained stable since 1997 at 1.1 defined daily doses (DDDs)/1,000 inhabitants-days, whereas that of levofloxacin and moxifloxacin increased during 2002–2006 (from 0.2 to 0.4 DDDs/1,000 inhabitants-days for levofloxacin and from 0.3 to 0.4 DDDs/1,000 inhabitants-days for moxifloxacin, Agencia Española de Medicamentos, Madrid, Spain; http//agemed.es). Because the borderline activity of Cip against *S. pneumoniae* favors acquisition of first-step *parC* mutations (*15,36*), we expected that the greater activity of levofloxacin and moxifloxacin would not favor the appearance of resistance, even if one considered their increased use.

Regarding circulating pneumococcal clones, the rate of PCV7 serotypes among CipR isolates decreased from 65.3% in 2002 to 35.7% in 2006 ($p<0.001$). The same finding was found among CipR isolates from adults >64 years of age (7.2% in 2002 to 4.7% in 2006; $p<0.02$) and was probably caused by decreased transmission of pneumococci from vaccinated children to adults (*37*). Consequently, we have observed a decrease in 4 multidrug-resistant clones (Spain^{23F}-ST81, Spain^{6B}-ST90, Spain^{14}-ST17, and ST88^{19F}) related to PCV7-serotypes. In addition, new clones (ST62^{11}, ST97^{10A}, ST570^{16}, ST433^{22}, and ST717^{33}) related to non-PCV7 serotypes emerged in 2006. These changes are consistent with those observed among invasive pneumococci after the introduction of PCV7 in Spain in June 2001 (*38*). At present, 2 clones, Spain^{9V}-3-ST156 and Sweden^{15A}-ST63, could be considered as the major contributors to Cip resistance in Spain, accounting for 34.7% of CipR strains.

Fluoroquinolones are not recommended for children, who are the major reservoir of pneumococci. If fluoroqui-

nolones are given to children, according to recent reports of their safety for such use (*39*), increased prevalence of resistance might occur.

Regarding fitness cost of CipR mutations in *S. pneumoniae*, CipR isolates were divided into 3 groups. The first group is composed of 5 isolates without QRDR resistance mutations. Four isolates had a reserpine efflux phenotype. The fifth isolate may have had a different efflux inhibitor or an unknown resistance mechanism. The second group is composed of 25 LL-CipR isolates with single changes at topo IV, whose distribution, 24 at parC and 1 at parE (D435N), is consistent with the low-fitness cost of parC changes (*25*) and the high-fitness cost of the parE D435N change (*40*). The third group is composed of 68 HL-CipR isolates with gyrA changes associated with topo IV changes. GyrA changes mainly occurred at S81 (62/68), whereas changes at E85 were rare (8/68) because of the high-fitness cost of E85 changes (*25*).

The frequency of CipR recombinants in 2006 remained low (5.1%, 5/98 CipR isolates), similar to that in 2002 (6.7%) and that reported previously (*3,4*). Four isolates with mosaic *parE-parC* genes and long intergenic regions (4–6 kb) containing the *ant* gene probably originated by recombination with the *S. mitis* group (*4*). One of them belongs to the Spain^{9V}-ST156 clone and was not typeable. The predominance of this clone and the fact that the recombinant *parE-ant-parC* structure did not impose a fitness cost (*25*) suggest recombinants could become more prevalent in the future.

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Bartonella quintana in Body Lice and Head Lice from Homeless Persons, San Francisco, California, USA

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Bartonella quintana is a bacterium that causes trench fever in humans. Past reports have shown *Bartonella* spp. infections in homeless populations in San Francisco, California, USA. The California Department of Public Health in collaboration with San Francisco Project Homeless Connect initiated a program in 2007 to collect lice from the homeless to test for *B. quintana* and to educate the homeless and their caregivers on prevention and control of louse-borne disease. During 2007–2008, 33.3% of body lice–infested persons and 25% of head lice–infested persons had lice pools infected with *B. quintana* strain Fuller. Further work is needed to examine how homeless persons acquire lice and determine the risk for illness to persons infested with *B. quintana*–infected lice.

The human body louse and human head louse are generally recognized as 2 subspecies of *Pediculus humanus (P. h. humanus* and *P. h. capitis*, respectively) that have distinct ecologic preferences (*1*). However, a recent genetic analysis was not able to show any differences between these 2 subspecies (*2*). The human body louse is a small, parasitic insect that lives on the body and in the clothing or bedding of its human host. Body lice feed only on human blood. In the United States, body lice infestations usually are found only on persons who do not have access to clean changes of clothes or bathing facilities (e.g., the homeless population).

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Head lice also feed only on human blood and are found on the head. Head lice infestations occur most often in school children and may also occur in the homeless population where they may be transferred by pillow cases, hats, and combs. Body and head lice are morphologically indistinguishable by the unaided human eye. Body lice are most reliably differentiated from head lice by their presence on clothing or on parts of the body other than the head or neck. These lice spend most of their time on the clothing of an infested person, visiting the body up to 5 times a day to feed. The eggs (called nits) of body lice are cemented to clothing fibers and seams or, occasionally, to body hairs (*3*,*4*).

In addition to causing discomfort and irritation, body lice can transmit disease-causing pathogens. *Bartonella quintana* is a bacterium transmitted through body lice feces that are scratched into the skin by the host. This organism can cause trench fever, endocarditis, bacillary angiomatosis, peliosis, and chronic bacteremia in infected humans (*3*). Since 1992, *B. quintana* has been recognized as a reemerging infection in homeless populations in the United States and Europe, as well as an opportunistic pathogen in patients with AIDS (*5*). Infection with *B. quintana* can cause prolonged disability in immunocompetent persons and can be life-threatening in immunodeficient patients.

Studies of homeless persons seeking medical care in clinics and hospitals in the United States and France have found that 2%–20% of persons tested had antibodies against *B. quintana* (*6–9*). In Tokyo, Japan, 57% of homeless patients had immunoglobulin (Ig) G titers ≥128 for *B. quintana* (*10*). A study in Marseille, France, found that 14% (10/71) of homeless patients who came to a hospital emergency department had blood cultures positive for *B. quintana* (*11*). In 1990, physicians in the San Francisco, California, Bay area recognized the link between *Barto-*

nella spp. infections and bacillary angiomatosis (*12*,*13*) and bacillary peliosis hepatis (*14*). A subsequent study by Koehler et al. documented the occurrence of bacillary angiomatosis in 49 patients seen over 8 years (*15*). All patients in this study were infected with either *B. quintana* or *B. henselae* (the agent of cat-scratch disease), most case-patients were immunocompromised (92% had HIV infection), and *B. quintana* infection was associated with homelessness and body lice infestation. In a subsequent study of HIV-positive patients with fever in San Francisco, Koehler and others found that 18% of 382 patients were positive for *Bartonella* spp. (*16*).

The human body louse is currently thought to play a role in the transmission of *B. quintana* among homeless persons, much as it did during the epidemics of trench fever that occurred during World Wars I and II (*3*). In the aforementioned study in Marseille, France, in 1999, body lice from 3 (20%) of 15 homeless patients were positive for *B. quintana* by PCR (*11*). In Tokyo, Sasaki et al. examined clothing from 12 homeless persons for body lice (*17*). These authors found that lice from 2 (16.7%) of 12 homeless persons were positive for *B. quintana* by PCR (*17*). Furthermore, evidence now indicates that head lice may be involved in the transmission cycle of *B. quintana* (*18*). Homeless populations in urban areas in northern California are vulnerable to body lice infestation and may be at risk for *B. quintana* infection. We studied whether body and head lice from homeless populations in a northern California city are carrying *B. quintana* or other pathogenic *Bartonella* spp.

Materials and Methods

In 2007 and 2008, staff from the Vector-borne Diseases Section of the California Department of Public Health (CDPH) participated in San Francisco's Project Homeless Connect (SFPHC). Under the auspices of SFPHC medical services, hair, body, and clothing of homeless persons were inspected for lice. Any lice found on the head with the presence of nits were considered to be head lice. Any lice on the body or clothing were considered to be body lice. Most infested persons self-referred directly to the CDPH booth at this event, with the exception of 1 physician referral. Lice were collected by using forceps, identified, sorted by subspecies, and placed in screw-top vials filled with 95% ethanol. Only a portion of the total lice infesting a person were collected for testing. The lice were shipped to the Centers for Disease Control and Prevention (Fort Collins, CO, USA) for detection and identification of *Bartonella* spp.

Lice were pooled by host and then subspecies. Samples from hosts with >20 lice were further tested individually to obtain an estimate of *Bartonella* spp. prevalence in the lice. We tested 36 pools of body lice, 7 pools of head lice, 108 individual body lice, and 4 individual head lice. Individual or pooled (2–20 lice/pool) samples were suspended in 250 μL of sterile phosphate-buffered saline and homogenized in an MM300 mixer (Retsch, Newtown, PA, USA) for 8 min. DNA was extracted from the homogenates by using a Mini Kit (QIAGEN, Valencia, CA, USA) and the Blood and Body Fluid Spin Protocol according to the manufacturer's protocol with a few minor changes. A PCR was performed in 20 μL of the mixtures containing 4–20 ng of the extracted DNA, 20 μL of 2× Ampdirect Plus, 0.5 U of Ex *Taq* Hot Start Version (Takara Bio, Otsu, Japan), and 1 pmol of each primer. *Bartonella* DNA was amplified by using *gltA* (citrate synthase gene) and *ftsZ* (cell division protein gene) primers as reported (*19*,*20*) in a thermalcycler (iCycler; BioRad, Hercules, CA, USA). A strain of *B. washoensis* was used as a positive control, and sterile deionized water was used as a negative control. Using gel electrophoresis on a 2% agarose gel, we examined the PCR products for 900-bp (*ftsZ*) and 380–400-bp (*gltA*) fragments.

The PCR amplicon of each gene was purified by using a QIA quick PCR purification kit (QIAGEN). Direct DNA sequencing of the purified PCR amplicons was conducted by using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) with the specific primers described above on a Model 3130X Genetic Analyzer (Applied Biosystems). Sequence data of each gene were aligned and compared with type strains of *Bartonella* spp. in GenBank by using MegAlign software (DNA Star Inc., Madison, WI, USA).

Results

In 2007 and 2008, 138 homeless persons had consultations at the CDPH booth at the SFPHC event. Of these persons, CDPH staff observed 33 persons with body lice infestations (23.9%) and 624 lice were collected (mean 18.9 lice/infested host). Head lice infestations were detected in 12 (8.7%) persons and 70 lice were collected (mean 5.8 lice/infested host). Six persons (4.3%) had body lice and head lice infestations.

Bartonella DNA was detected in body lice collected from 11 (33.3%) persons (Table 1) and in head lice collected from 3 (25.0%) persons (Table 2). Nine pools of body lice (n = 2–20, mean infection rate [MIR] 5%) from 9 infested persons and 2 pools of head lice (n = 7–12, MIR 8.3%) from 1 infested person showed evidence of *Bartonella* DNA. Additional lice from persons with positive pooled samples of body lice (SFB6 and SFB24) were tested individually. Sample SFB6 had 13 (87%) of 15 lice positive for *Bartonella* DNA. Sample SFB24 had 27 (64%) of 42 lice positive for *Bartonella* DNA. One of the 4 individual head louse samples (SFH2) showed amplification of *Bartonella* DNA (Table 2).

Host sample SFB16 showed no amplification of *Bartonella* DNA in its original test but when an additional 3

Table 1. *Bartonella quintana* in body lice (1–20 lice/ sample) from homeless persons, San Francisco, California, USA

Specimen ID	Date collected	No. samples tested (no. lice)	No. positive samples*	
			gltA	*ftsZ*
SFB1	2007 Feb	1 (2)	0	0
SFB2	2007 Feb	1 (1)	0	0
SFB3	2007 Feb	1 (1)	0	0
SFB4	2007 Feb	1 (4)	0	0
SFB5	2007 Feb	1 (1)	0	0
SFB6	2007 Apr	16 (35)	14	13
SFB7	2007 Apr	1 (2)	0	0
SFB8	2007 Apr	1 (4)	0	0
SFB9	2007 Aug	3 (22)	0	0
SFB10	2007 Aug	4 (23)	0	0
SFB11	2007 Aug	1 (7)	0	0
SFB12	2007 Aug	1 (14)	0	0
SFB13	2007 Aug	1 (2)	1	1
SFB14	2007 Dec	1 (2)	0	0
SFB15	2007 Dec	1 (4)	0	0
SFB16	2007 Dec	5 (91)	1	0
SFB17	2007 Dec	2 (40)	1	1
SFB18	2007 Dec	1 (6)	1	0
SFB19	2007 Dec	1 (1)	1	0
SFB20	2007 Dec	1 (5)	0	0
SFB21	2008 Jan	1 (16)	1	0
SFB22	2008 Jan	2 (21)	1	0
SFB23	2008 Jan	1 (2)	0	0
SFB24	2008 Jan	43 (62)	26	24
SFB25	2008 Jan	6 (25)	0	0
SFB26	2008 Jan	1 (3)	1	0
SFB27	2008 Jan	8 (27)	1	0
SFB72	2008 Jun	6 (25)	0	0
SFB73	2008 Jun	7 (7)	0	0
SFB74	2008 Jun	2 (2)	0	0
SFB76	2008 Jun	9 (85)	0	0
SFB77	2008 Jun	10 (10)	0	0
SFB78	2008 Jun	12 (12)	0	0

**gltA*, citrate synthase gene; *ftsZ*, cell division protein gene.

pools of 20 lice and 11 individual lice were tested, 1 pool of 20 lice was positive. This host had a massive body louse infestation; 91 lice were collected from his clothing. Host sample SFB27 was also negative in its first test of a pool of 20 lice; 7 additional lice tested afterwards produced a single detection of *B. quintana* DNA in a body louse (14%).

Samples from 1 person who was co-infested with body lice and head lice were positive for *Bartonella* DNA by the *gltA* gene PCR (SFB17, 1 pool of 20 lice) in body lice, but not in the head lice pool (SFH7, n = 4). Samples from another co-infested person were negative for *Bartonella* DNA in 1 pool of 5 body lice (SFB10). *Bartonella* DNA was detected in a pool of 12 head lice (SFH3, MIR 8.3%) (Tables 1, 2).

Thirteen (86.7%) and 12 (80.0%) body lice from host SFB6 had *Bartonella* DNA amplification by *gltA* and *ftsZ*, respectively. Twenty-five (59.5%) and 23 (54.8%) of individual body lice from host SFB24 had *Bartonella* DNA amplification by *gltA* and *ftsZ*, respectively. Two samples from hosts SFB6 and SFB24 were sequenced and found to be identical with *B. quintana* type strain Fuller for both genes. Host sample SFB13 had *Bartonella* DNA amplification for both genes, and showed a sequence identical to *B. quintana* type strain Fuller for the *gltA* product and 99.9% homology to the same type strain for the *ftsZ* product. One of the individual head lice samples, SFH2, showed positive amplification of the *ftsZ* and *gltA* genes. Sequencing showed that this sample was *B. quintana* type strain Fuller for the *gltA* product.

Discussion

Our study has shown that homeless persons in the San Francisco Bay area have body and head lice that harbor *B. quintana* type strain Fuller. Prevalence of *B. quintana* in body lice from homeless persons (33.3%) in our study was slightly higher than the prevalence reported by Sasaki et al. in Tokyo, where body lice in 2 (16.7%) of 12 homeless persons were infected with *B. quintana* (*17*). Furthermore, similar prevalence of *B. quintana* infection in body lice has been reported from Russia (12.3%) (*21*) and Marseille, France (20%) (*11*).

Although Sasaki et al. detected *B. quintana* DNA in head lice by using molecular detection methods, their samples came from children in Nepal who also had body lice (*18*). However, there is no strong evidence that head lice are vectors of this organism between human hosts. Moreover, Fournier and others tested 143 head lice from schoolchildren from 8 countries and found no *B. quintana* (*22*) We have detected *B. quintana* in head lice from persons without a known concurrent body louse infestation. Further work is needed to examine how homeless persons acquire lice and which groups may be predisposed to louse infestation and *B. quintana* infection.

Table 2. *Bartonella quintana* in head lice (1–20 lice/sample) from homeless persons, San Francisco, California, USA

Specimen ID	Date collected	No samples tested (no. lice)	No. positive samples*	
			gltA	*ftsZ*
SFH1	2007 Feb	1 (7)	1	0
SFH2	2007 Apr	1 (1)	1	1
SFH3	2007 Aug	2 (32)	1	0
SFH4	2007 Dec	1 (15)	0	0
SFH5	2007 Dec	1 (2)	0	0
SFH6	2007 Dec	1 (2)	0	0
SFH7	2007 Dec	1 (4)	0	0
SFH8	2008 Jan	1 (1)	0	0
SFH75	2008 Jun	2 (2)	0	0
SFH79	2008 Jun	1 (1)	0	0
SFH80	2008 Jun	2 (2)	0	0
SFH81	2008 Jun	1 (1)	0	0

**gltA*, citrate synthase gene; *ftsZ*, cell division protein gene.

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Ms. Bonilla is an associate public health biologist at the California Department of Public Health. Her research interests are surveillance, prevention, and control of vector-borne diseases; control of vectors such as lice, bed bugs, and scabies in urban settings; and the ecology of tick-borne disease.

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Drought, Smallpox, and Emergence of *Leishmania braziliensis* in Northeastern Brazil

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Cutaneous leishmaniasis caused by *Leishmania (Vianna) braziliensis* is a major health problem in the state of Ceará in northeastern Brazil. We propose that the disease emerged as a consequence of the displacement of persons from Ceará to the Amazon region following the Great Drought and smallpox epidemic of 1877–1879. As the economic and social situation in Ceará deteriorated, ≈55,000 residents migrated to the Amazon region to find work, many on rubber plantations. Those that returned likely introduced *L. (V.) brazilensis* into Ceará, where the first cases of cutaneous leishmaniasis were reported early in the 20th century. The absence of an animal reservoir in Ceará, apart from dogs, supports the hypothesis. The spread of HIV/AIDS into the region and the possibility of concurrent cutaneous leishmaniasis raise the possibility of future problems.

The emergence of cutaneous leishmaniasis in northeastern Brazil in the state of Ceará illustrates how environmental and human factors combine to influence human health. Cutaneous leishmaniasis is an important health problem for residents of Ceará. In the 20 years from 1986 to 2005, >49,000 new cases were reported (Figure 1) (*1*). Given the difficulties in reporting in rural areas, the true number is likely substantially higher. Today, the disease is distributed across several areas in the state, but this has not always been the case. In fact, historical data suggest that cutaneous leishmaniasis is a relatively recent arrival and may have come from the Amazon region as a consequence of drought, smallpox, and social and economic conditions that led to human migration.

The first well-documented cases of cutaneous leishmaniasis in Ceará date back to 1925 when photographs of patients with classic skin ulcers and advanced mucosal leishmaniasis (Figures 2, 3) were published in a report on endemic diseases in the state (*2*). The author, A. Gaviao-Gonzaga, hypothesized that the disease may have been introduced from the Amazon. Sales (*3*), the first to map the geographic distribution of the disease in the state, also suggested this possibility, but neither report provided specific evidence to support the hypothesis.

We investigated the possible origin of cutaneous leishmaniasis in Ceará and postulate that the disease emerged as a consequence of the displacement of >55,000 persons from Ceará to the Amazon region at the end of the 1800s (*4*) after the Great Drought of the 1870s (*5,6*) and an unprecedented epidemic of smallpox that left a death toll of >100,000 persons (*7–9*). We propose that migrants became infected in the Amazon and carried the disease with them when they returned to Ceará and elsewhere in eastern Brazil. As a result, the disease was introduced into northeastern Brazil and subsequently emerged as an important health problem.

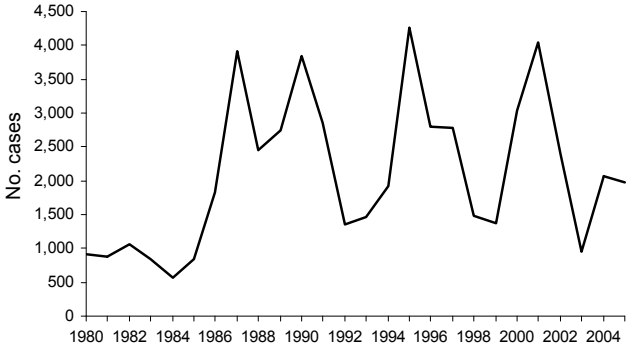

Figure 1. Cases of cutaneous leishmaniasis in the state of Ceará, Brazil, 1980–2005. Source: Ministry of Health, Brazil.

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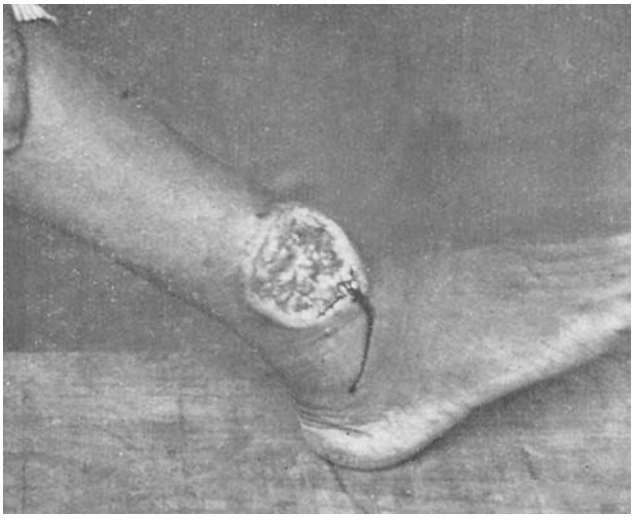

Figure 2. Weeping ulcer (pizza-like lesion) in a patient with typical cutaneous leishmaniasis, Ceará, Brazil. Source: (*2*).

The Great Drought

Drought is a natural phenomenon that has afflicted the lives of millions of persons over the centuries and remains an important cause of human illness in many regions of the globe. Its greatest effects frequently occur in developing areas with agrarian societies and few natural resources. Depending on the region, drought can cause serious problems in humans through famine and later malnutrition, in addition to the death of livestock, alteration of the area's wildlife, and acceleration of deforestation. Drought frequently stimulates migration and displacement of large groups of people. These events are well documented in the history of northeastern Brazil.

The state of Ceará in northeastern Brazil is one of the poorest in the country in respect to natural resources. Its population is >8 million inhabitants, representing 4.2% of the country's population. Ceará is known as the "land of light." Many attribute the saying to its hot, sunny, and dry weather that occurs almost year round; the real reason is that it was the first state in Brazil to abolish slavery. Unfortunately, droughts have occurred periodically in the region for as long as records have been kept. A list of the droughts since the beginning of the 17th century is available, but it is no doubt incomplete because less severe droughts were not registered (*9*). Studart, in discussing the history of drought in Ceará, wrote the following: "If Ceará was a land of regular rains and well distributed, in Brazil no state would compete with it; however, the drought that persecutes it, impairs its flight to incomparable destinies" (*9*).

The first severe drought in Ceará in the 19th century occurred in 1824. A second drought followed in 1844. Because 2 decades had elapsed between those droughts, it was thought that there was a 20-year drought cycle. When no drought occurred in 1864–65, many thought Ceará was free of them (*4,9*). Rapid development followed. Cotton exports to the United States were increased because of the US Civil War. These exports to the United States decreased after the war, and exports also decreased to England after incentives were provided for cotton plantations in some of England's colonies (*4*). However, the agricultural economy remained strong because ample rainfall from 1870 to 1876 enhanced the production of other crops. By 1872, Ceará had a population of 721,686 persons, and Fortaleza, its capital city, had grown to 23,500 (*6*).

The Great Drought, the most severe ever recorded in Brazil, began in Ceará in 1877 and lasted 3 years. Drought conditions also occurred in several other northeastern states (*5*), but Ceará was by far the most severely affected. The delayed and inadequate response of the central Brazilian government exacerbated the problems (*8*).

As the drought continued, the number of persons needing assistance grew quickly and by 1878 had reached 480,000. Those persons in rural areas in the central part of the state migrated to the capital and other cities in mountainous areas and along the coast where the effects of the drought were less severe. Fortaleza was in a state of calamity. As persons arrived in the city, they lived on the outskirts of the city in inhumane conditions with very little food and or sanitation. The situation was ripe for disaster, and it happened.

The Smallpox Epidemic

Smallpox was beyond a doubt one of the greatest scourges of humanity. It was responsible for much human

Figure 3. Advanced mucosal leishmaniasis in a patient who had cutaneous leishmaniasis, Ceará, Brazil. Source: (*2*).

illness, millions of deaths over centuries, and the elimination of entire societies, for example, the Mississippian chiefdoms in North America between 1491 and the late 1600s (*10,11*).

In September 1878, smallpox arrived in Ceará, brought by a sailor from a ship that had anchored at a coastal city near Fortaleza (*8*). The disease quickly reached Fortaleza and spread to other cities. An estimated 95% of the population in Fortaleza and ≈100% of those living in the other cities had not been vaccinated and were susceptible to infection (*8*). Theophilo (*8*) wrote that the disease spread like a fire in dried leaves fanned by a strong wind.

In Fortaleza, 62 people died of smallpox in September 1878. In October, the number had risen to 592 and in November to 9,844. In December, 15,435 persons died, >500 deaths per day. December 10, 1878, was the saddest day of all; 1,004 persons died of smallpox in Fortaleza alone. Many were left unburied because of the lack of healthy persons to bury them. This tragedy was brought home in a poignant way in 1994 when excavation for a new sewage system unearthed skeletons in shallow graves (Figure 4) in Fortaleza believed to have been buried on the "day of one thousand deaths" (*12*).

Thirty years later, Studart (*9*), who had only recently graduated from medical school when the drought began, recalled the horror:

> "The year of 1878 started, and with it grew to the infinite the anguish of the people from Ceará. People died of hunger, purely of hunger on the streets and on the roads, after they had eaten roots of many different plants. The starving people ate the most repugnant meat; reptiles, dogs, vultures and crows. Although rare, cases of anthropophagy occurred; individuals were seen eating human flesh. Bodies were found with part of the limbs partially eaten due to the extreme despair of human famine. … December 10 [1878], I remember that horrific day; 1,004 people died of smallpox in Fortaleza. They were brought to the cemetery and many were not buried due to the tiredness of the buriers. It is registered that an average of 500 individuals died a day; however, the numbers must have been much higher, because around the city, into the bushes and into closed houses where whole families had died, bodies were found in a state of putrefaction."

Rodolpho Theophilo (*12*), a local pharmacist, began producing the smallpox vaccine in a laboratory he had built with his own money because the vaccine from Rio de Janeiro was not apparently protective. He personally vaccinated thousands of persons over a 4-year period (Figure 5) and created a chain of volunteers in other cities in the state to whom he sent the vaccine with a packet insert containing instructions. He recalls the situation (*8*):

> "The excessive heat of 33 degrees centigrade in the shade in that fatal December added to the epidemic. There was total disorientation. In the tenth of the month the cemetery received 1,004 dead bodies, that horrifying obituary of just one day, let all those who received the news in panic. … At the end of the day 230 dead bodies were left unburied. … In the next morning when the buriers returned to continue their work, they found dogs and vultures feeding on human bodies."

News of the terrible calamity reached the New York Herald, the Medical Times and Gazette of London, and the New York Times (*6*). Articles in the New York Times appeared on November 17, 1877, and on February 24, 1879. The latter article, entitled "Pestilence and famine in Brazil," described the disaster (*7*): "During the 3 years (1877–1879) of drought, 150,000 persons died in Ceará state, most of them in 1878 (118,927 deaths). In Fortaleza, 67,267 deaths occurred in the 3-year-period, 57,780 of those in 1878."

As the economic and social situation in Ceará worsened in 1878, a call went out for workers in the Amazon region where rubber production was rapidly developing. In

Figure 4. Discovery of human skeletons during excavation in 1994 of new sewage system in Ceará, Brazil, of persons who died of smallpox during the epidemic of 1877–1879. Photo courtesy of Jornal O Povo.

Figure 5. Rodolpho Theophilo (second from right) during a smallpox vaccination campaign he led during 1901. Photo courtesy of Nirez Archives.

that year, 54,875 (*4*) persons migrated to the Amazon. The migration to the Amazon occurred in the period known as the rubber boom (*13*), several decades after the discovery of vulcanization by Charles Goodyear in 1839. In the following years, many more people went to the Amazon in pursuit of better living conditions. Many returned to their families in Ceará; others died of malaria, a frequent cause of death in the Amazon in those days (*14*).

The Emergence of Cutaneous Leishmaniasis in Ceará

Archeologic evidence suggests that *Leishmania* species that cause cutaneous leishmaniasis were present in South America long before the arrival of Europeans. Human disease has been recognized in Peru since Inca and pre-Inca times. Facial mutilations consistent with mucosal leishmaniasis have been observed in Peruvian pottery images (*15*). Written description of the Peruvian form of leishmaniasis called *uta* dates back to 1764, when the disease was already endemic in many areas of the country (*16*).

Although human leishmaniasis was known in Peru in antiquity, it has been recognized in Brazil for little more than a century. The first clear clinical description of cutaneous leishmaniasis in Brazil was made in 1895 in Bahia (*17*); however, Rabello (*18*) cites a report of a missionary trip in the Amazon region in a publication dated 1827, which noted that it was common to see persons with ulcers in their arms and legs as well as destructive lesions around the mouth and nose and that those were caused by mosquito bites. The descriptions are consistent with leishmaniasis (*18*).

We have been unable to find any reference to cutaneous or mucosal disease consistent with *Leishmania braziliensis* infection in Ceará in a careful review of government documents, books, and newspapers from 1830 until Studart's 1909 report (*9*) of a skin condition that might have been leishmaniasis. The first official reference to cutaneous leishmaniasis appeared in a 1917 government report (Public Library, Ceará 1917). In 1912, Gaspar Vianna (*19*), who discovered trivalent antimony treatment for cutaneous leishmaniasis, reported treating patients from many states in Brazil, including Ceara (*20*). Accounts of the first well-documented autochthonous cases in Ceará were published in 1925 with photographs of persons with cutaneous and mucosal lesions (*2*). An alternative hypothesis proposed that *L. braziliensis* was present in animals before the Great Drought and smallpox epidemic of the 1870s, but the lack of early reports suggests that this was not the case, and even today no animal reservoir other than dogs has been identified in Ceará.

Cutaneous leishmaniasis is currently endemic in a number of areas of Ceará. Most are located in mountainous regions and in areas adjacent to the coast where people immigrated during the Great Drought. Although the disease may have previously existed there, and healthcare workers failed to observe or report the chronic skin and mucosal lesions, we believe that it is more likely that persons who had immigrated to the rubber plantations in the Amazon after the Great Drought and smallpox epidemic brought *L. braziliensis* infection to Ceará, either through human or animal infection. Several observations support this finding.

Considering that leishmaniasis was known to exist in Peru for centuries, why it did not emerge in Brazil earlier? The reason is not totally clear. The Incas did not settle in the Amazon Basin, presumably for economic reasons and due to their preference for vertical landscapes (*21*). The disease may well have been present for many years among Indian tribes in the Amazon region, but they had little communication and interaction with the rest of the country until the start of the rubber industry, which intensified after vulcanization was discovered.

The parasite was first identified in 1909. Lindenberg (*22*) and Carini and Paranhos (*23*), working independently, identified the protozoan during an epidemic of "ulcera de Bauru" or Bauru sore that accompanied the construction of a railroad between the cities of Bauru in São Paulo and Corumba in Mato Grosso states. The name *L. braziliensis* was given by Vianna in 1911 (*24*). He observed the parasite in smears from a person with disseminated cutaneous leishmaniasis, an uncommon manifestation of infection (*25*). Vianna, in examining smears from the lesions, described the morphologic features of the parasite, including the kinetoplast and a thin linear structure that is not seen with currently used Wright and Wright-Giemsa stains. Vianna concluded that he was dealing with a new species of *Leishmania.* It is likely that the linear structure was the inner lying flagellum, which is easily seen by electron microscopy (*26*). d'Utra e Silva (*20*), who worked with Vianna, explained the staining methods.

L. (*Viannia*) *braziliensis* has the widest geographic distribution of the *Leishmania* species endemic in Latin America. It has been documented to cause human disease in 14 countries (*27*). In Brazil, it is responsible for most cases of

leishmaniasis (*28*), and in Ceará, 272 isolates from patients with cutaneous leishmaniasis, representing all disease-endemic areas, were characterized as *L. braziliensis* (*29*).

The spectrum of disease caused by *L. braziliensis* is broad. It includes an early lymphadenophathic form, the classic weeping cutaneous ulcers, disseminated cutaneous leishmaniasis, and mucosal disease (*25*,*29*,*30*). Mucosal involvement may occur simultaneously with the cutaneous lesion(s), but most cases are diagnosed months to years after the cutaneous ulcer has healed (*31*).

Leishmaniasis in the 21st Century

Much has transpired in northeastern Brazil since the Great Drought and smallpox epidemic of the 1870s. Cutaneous leishmaniasis has grown into a major health problem in the region and across Brazil. The total number of cases reported in the country from 1980 to 2005, was 613,644 (Figure 6). At least 9 Brazilian states now have >1,000 cases each year. Ceará is among the top 5 states, and in some years, it ranks first in the total number of cases (*1*).

Although smallpox has been eradicated, HIV infection has emerged. There is great concern that concurrent infection with HIV and *Leishmania* may pose major problems in the future (*32*). It appears that each infection can worsen the course of the other, acting in synergistic ways to shorten the incubation periods and increase progression of both (*33*). The incidence of HIV/AIDS is increasing in northeastern Brazil, and HIV is extending into rural areas where the prevalence of cutaneous leishmaniasis is high (*34*).

It is important to note that persons who are infected with *Leishmania* species, with or without symptoms, appear to remain infected. Reactivation can occur if cellular immunity diminishes as a result of HIV. Identifying co-infected persons will be crucial so that appropriate highly active antiretroviral therapy and antileishmanial therapy can be initiated. The skin load of *Leishmania* species is much higher in HIV-infected persons, and although not proven, these patients may be a source of infection for the sand flies in disease-endemic areas.

For decades, the treatment of *L. braziliensis* infection has been based on parenteral administration of the pentavalent antimony. The drug is toxic and variably effective. A more effective, safer, cheaper, and, preferably, oral alterative is badly needed. Some progress has been made in recent years in new drug development (*35*,*36*). Prospects for an effective vaccine in the near future seem limited. Finally, a sylvatic reservoir has not been identified for *L. braziliensis* in Ceará and other areas. Dogs appear to be the most important reservoir in domestic and peridomestic transmission (*37*). Preliminary data on the use of deltamethrin-impregnated dog collars appear promising (*38*), but additional studies and government sponsorship are needed if they are to be widely implemented. Advances in one or more of these areas are essential to reverse the effects of *L. braziliensis* in the region and elsewhere.

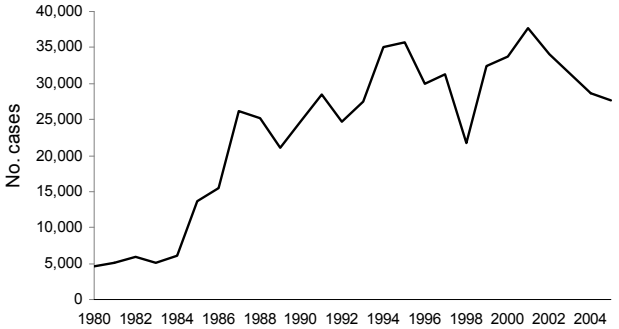

Figure 6. Cases of cutaneous leishmaniasis, Brazil, 1980–2005. Source: Ministry of Health, Brazil.

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Tropheryma whipplei in Fecal Samples from Children, Senegal

Florence Fenollar, Jean-François Trape, Hubert Bassene, Cheikh Sokhna, and Didier Raoult

We tested fecal samples from 150 healthy children 2–10 years of age who lived in rural Senegal and found the prevalence of *Tropheryma whipplei* was 44%. Unique genotypes were associated with this bacterium. Our findings suggest that *T. whipplei* is emerging as a highly prevalent pathogen in sub-Saharan Africa.

Tropheryma whipplei is known mainly as the bacterial pathogen responsible for Whipple disease (*1*). Until recently, it was thought to be a rare bacterium typically causing disease in white men (*1*). However, recent studies have shown 1%–11% prevalence of the bacterium in fecal samples from the healthy general adult population in Europe (*2*,*3*). *T. whipplei* also was viable in a fecal sample from a patient with Whipple disease (*4*). In addition, *T. whipplei* DNA has been detected in sewage and is more prevalent in fecal samples of sewer workers (12%–26%) than in the general population, supporting this environment as a likely ecologic niche (*2*,*3*). *T. whipplei* was highly prevalent in fecal samples of children 2–4 years of age in France who have gastroenteritis but was not detected in a control group of children of the same age who did not have diarrhea (*5*).

For these reasons, we speculated that if *T. whipplei* is transmitted through the fecal–oral route, it might be more prevalent in countries with poor sanitation, such as developing countries. Because no information is available about *T. whipplei* and Whipple disease in Africa, we conducted a study to assess the prevalence of *T. whipplei* in fecal samples from healthy children in Senegal, specifically in the villages of Dielmo and Ndiop (Figure 1).

The Study

In early April 2008, we sampled fecal specimens from 150 healthy children (79 girls) 2 months–10 years of age (mean 3.5 years ± 2.5 years) living in 2 villages in Senegal (Ndiop, 77 children; Dielmo, 73 children) (*6*). These villages are included in the Dielmo project, initiated in 1990 for long-term investigations of host–parasite associations in the entire village population, which was enrolled in a longitudinal prospective study (*6,7*). At the beginning of the current study, parents or legal guardians of all children gave individual informed consent. The national ethics committee of Senegal approved the project (*6*). Eight wells in the 2 villages (5 from Dielmo, 3 from Ndiop), which are the only sources of drinking water for the communities, also were sampled.

After collection, each fecal specimen was mixed with 2.5 mL of absolute ethanol for storage and transportation to our laboratory at room temperature. On arrival, DNA was extracted by using the BioRobot MDx workstation (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's recommendations and protocols. *T. whipplei* quantitative PCR assays were performed as previously described (*8*). A case was defined as 2 positive quantitative PCR results in assays targeting 2 different *T. whipplei* DNA sequences. *T. whipplei* genotyping using 4 variable sequences was performed by using samples from children with high bacterial loads, as previously reported (*9*).

Among the 150 healthy children, the prevalence of *T. whipplei* was 11% (2/18) in children <8 months of age, 37% (10/27) in children 8–24 months of age, and 44% (46/105) in children 2–10 years of age (Figure 1). None of the 8 water wells sampled were positive for *T. whipplei*.

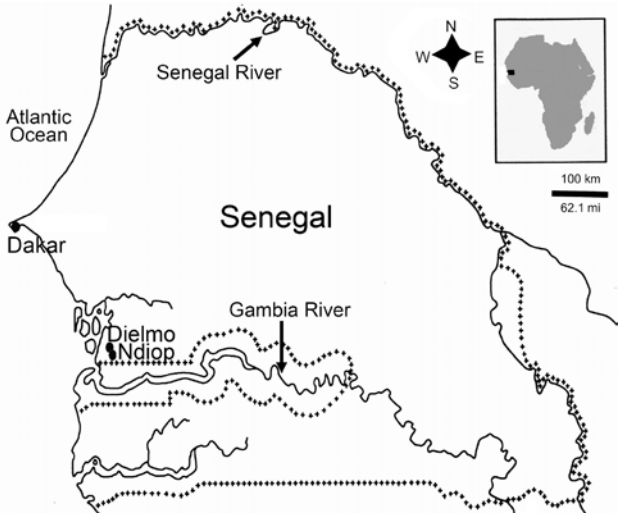

Figure 1. Location of Dielmo and Ndiop in Senegal, Africa. Plus-symbol lines define the Senegal frontiers. The number of fecal samples positive for *Tropheryma whipplei* and number tested for children in each age group was as follows: Dielmo, 1/13 from children <8 months of age, 5/9 from children 8–24 months of age, and 19/54 from children 2–10 years; Ndiop, 1/5 for children <8 months of age, 5/18 from children 8–24 months of age, and 27/51 from children 2–10 years of age.

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A complete genotype was obtained from 18 healthy children (Figure 2). We found 10 new genotypes in 3 independent clusters, all of which were unique to Senegal. Four genotypes were detected only in Ndiop and 5 only in Dielmo. One genotype common to both villages was classified as epidemic to Ndiop.

Conclusions

In our study, 44% of children >2 years of age who live in rural Senegal carried *T. whipplei*. Our requirement for 2 positive test results ruled out sample contamination, and the 4 different PCR amplifications and sequencing reactions used for genotyping resulted in the discovery of novel sequences. This pathogen in Europe, Asia, and America has been reported at much lower prevalences than we found in Senegal (*1*–*3*,*5*). The unique genotypes we discovered in Senegal have not been demonstrated elsewhere through global DNA-based comparisons. This specific type of geographic distribution of genotypes also has been reported for *Mycobacterium tuberculosis,* for which researchers named genotypes using the same genotyping method (*10*). Clonal diffusion of a specific genotype within a single area favors a human-to-human transmission hypothesis, which the circulating clones exemplified in Senegal.

In Senegal, children are contaminated with *T. whipplei* at an early age, and the high carriage rate we observed indicates a potential public health problem. *T. whipplei* may be responsible for numerous undiagnosed infections, including gastroenteritis, in Africa. The classic form of Whipple disease, characterized by histologic periodic acid-Schiff–stained bacilli in infected small-bowel macrophages, may represent only 1 rare clinical variant of infection that *T. whipplei* can cause. The higher number of Whipple disease cases in white men may be related to a genetic factor or might reflect a large number of unrecognized cases in developing countries. However, *T. whipplei* also can cause localized infections, such as endocarditis, spondylodiscitis, meningoencephalitis, and uveitis (*1*). The bacterium also was detected in a child with pneumonia who resided in the United States (*11*).

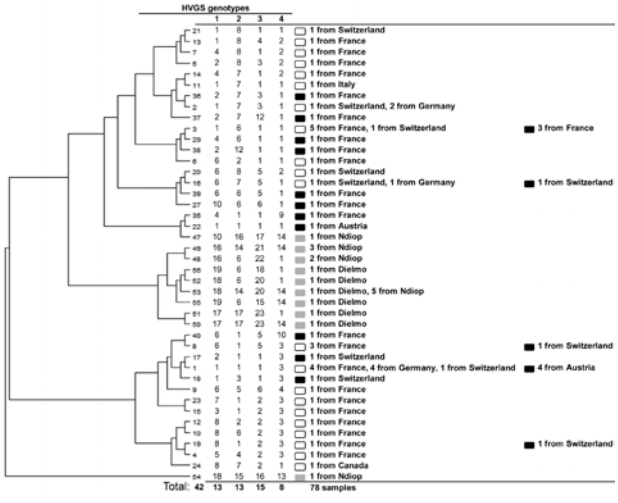

Figure 2. Dendrogram constructed using the unweighted pair group method with arithmetic mean showing the phylogenetic diversity of 42 genotypes from the 78 *Tropheryma whipplei* strains detected in 18 healthy children (gray squares) in Senegal, 39 adults in whom Whipple disease was diagnosed (white squares), and 21 symptomatic carrier adults (including 11 sewer workers; black squares) from Europe. Phylogeny assembly was based on the sequences of 4 variable sequences, which were concatenated to construct the dendrogram.

Our study provides evidence that *T. whipplei* is common in fecal samples from children in Senegal and that local strains circulate in the 2 villages investigated. We suspect *T. whipplei* infection results directly from human-to-human transmission because water from all 8 village wells tested negative by PCR. *T. whipplei* is present throughout the world, and specific genotypes often are linked to geographic sources. We speculate that *T. whipplei* infection might be a major public health concern in sub-Saharan Africa. Additional studies are needed to investigate the role of this extremely common emerging pathogen in developing countries.

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Phocine Distemper Virus in Northern Sea Otters in the Pacific Ocean, Alaska, USA

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Phocine distemper virus (PDV) has caused 2 epidemics in harbor seals in the Atlantic Ocean but had never been identified in any Pacific Ocean species. We found that northern sea otters in Alaska are infected with PDV, which has created a disease threat to several sympatric and decreasing Pacific marine mammals.

In northern Europe, phocine distemper virus (PDV) caused 2 epidemics that resulted in 23,000 harbor seal deaths in 1988 and >30,000 deaths in 2002 (*1*). PDV has also been associated with seal deaths on the eastern coast of the United States and Canada, which shows the persistent threat of this virus to Atlantic marine mammal populations (*2*). Serologic surveys before 2000 indicated that Pacific marine mammals had not been exposed to PDV (*3*,*4*), and this virus had never been identified as the cause of illness or death in the North Pacific Ocean. In this region, specifically in Alaska, northern sea otters (*Enhydra lutris kenyoni*) are one of many species that have had population decreases since the 1980s. Steller sea lion (*Eumetopias jubatus*), northern fur seal (*Callorhinus ursinus*), and most recently, harbor seal (*Phoca vitulina*) populations have all decreased (*4*–*6*).

The Study

In 2004 and 2005, strong serologic evidence of exposure to a PDV-like morbillivirus was obtained by serum neutralization for ≈40% (30/77) of live captured sea otters sampled in the eastern Aleutian Islands (Fox Island, South Alaska Peninsula) and Kodiak Archipelago (T. Goldstein et al., unpub. data) (Figure 1, panel A, southwest stock). These captures were part of an investigation into potential causes of a precipitous decrease in the population that resulted in a US Endangered Species Act listing. Although northern sea otters are found along the Pacific coast of Alaska, Canada, and Washington and in the Aleutian Islands, only the southwest stock in Alaska has been decreasing (*9*) (Figure 1, panel A). As little as 50% of the southwest stock remains since the 1980s, and the Aleutian Archipelago population decreased from ≈74,000 to 8,742 sea otters by 2000.

In 2006, the US Working Group on Marine Mammal Unusual Mortality Events declared an unusual mortality event for northern sea otters; large numbers of deaths were documented in southcentral Alaska adjacent to the threatened southwest stock (V. Gill, unpub. data) (Figure 1, panel A). Necropsies showed a high prevalence of valvular endocarditis (43%) and septicemia in mature adults associated with various strains of *Streptococcus infantarius* subsp. *coli* (*S. bovis/equinus* complex) and inconsistent intracytoplasmic inclusions were present. However, a primary site of bacterial infection could not be identified in most infected animals, despite this high prevalence of lesions. In humans, *S. bovis* is a major cause of valvular endocarditis and is often associated with preexisting pathologic changes of the colon, underlying disease, and immunosuppression (*10*). This disease is often sporadic and secondary to chronic recurrent bacterial seeding from a primary site of infection or secondary to heart valve abnormalities. The lack of underlying bacterial infection or heart valve defects indicated the presence of a primary immunosuppressive viral infection.

To further investigate serologic evidence and necropsy findings, we looked for morbilliviral nucleic acid in nasal swabs archived from live otters and in tissue (brain, lung, lymph node) from 9 stranded carcasses from Kachemak Bay (southcentral stock, Figure 1, panel A) examined during 2005–2008. Total RNA was extracted by using Tri Reagent (Sigma, St. Louis, MO, USA) and complimentary DNA was transcribed by using Superscript III (Invitrogen, Carlsbad, CA, USA) with random nonamers. A heminested PCR was performed with universal morbillivirus primers and a PDV-specific primer for the phosphoprotein gene (*11*). Products of the expected size were sequenced.

Morbilliviral nucleic acid was amplified from 8 nasal swabs from live otters (10%, 8/77) and from lung, lymph node, or brain from 3 dead otters. Sequence analysis identified a PDV fragment identical to that of the isolate from the 2002 outbreak in northern Europe. This PDV fragment differed from the 1988 isolate at 2 nucleotide positions (online Technical Appendix, available from www.cdc.gov/EID/content/15/6/925-Techapp.pdf; Figure 2). The PDV-positive nasal swabs were from 5 juveniles and 3 adults, 7

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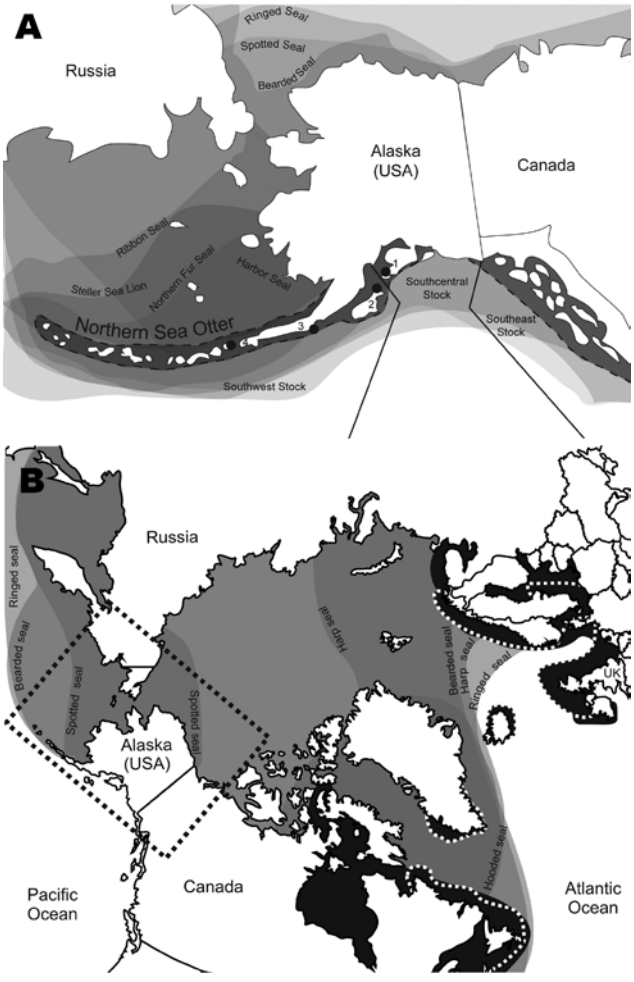

Figure 1. Distribution of Arctic and sub-Arctic pinnipeds in relation to Arctic ice coverage representing a unique area where distribution ranges of multiple seal species overlap (*7*,*8*). A) North Pacific Ocean region showing the range of the northern sea otter (*Enhydra lutris kenyoni*) in Alaska, its population stock delineations, and sample collection locations for the study. 1, Kachemak Bay; 2, Kodiak Archipelago; 3, South Alaska Peninsula; 4, Fox Island; seal species ranges overlap. This overlap indicates potential for phocine distemper virus disease transmission among Arctic and sub-Arctic pinniped species in this highly productive region. B) Circumpolar Arctic region showing species overlap among Arctic pinnipeds and the potential for disease transmission from the Atlantic Ocean through the Arctic Ocean to Alaska (outlined) by migrating seal species. The black areas indicate ranges of Atlantic harbor and gray seals; the areas exclusive to gray seal are bordered with a broken line. The boxed region corresponds to the Arctic region containing sea otter populations shown in panel A.

from the Kodiak Archipelago and 1 from the Eastern Aleutians in 2004 and 2005. Seven of these 8 otters were also positive for antibodies to PDV by serum neutralization. The dead PDV-positive otters were 2 adults and 1 juvenile from Kachemak Bay sampled during 2005–2007. The cause of death in these animals included meningoencephalitis and/or sepsis with or without valvular endocarditis. This finding mirrors the secondary bacterial infections characteristic of infected and immunosuppressed European harbor seals during PDV epidemics (*1*).

Conclusions

These results demonstrate that PDV has been introduced to the North Pacific Ocean since 2000. All Pacific marine mammal species are now at risk for phocine distemper–induced population decreases. Although additional work is needed to determine if PDV has played a role in the decrease in the sea otter population, its association with lesions in carcasses, especially in animals that have died of bacterial infections, suggests it may contribute to ongoing deaths. Viral nucleic acid in nasal swabs from free-ranging, live-captured otters confirms viral shedding. Therefore, otters are capable of transmitting PDV to conspecifics and other species.

Because the PDV fragment isolated from Alaskan otters is identical to that of the 2002 Atlantic isolate, this virus was likely transmitted to the North Pacific Ocean after the 2002 European epidemic, although it is remotely possible that it may have originated in the North Pacific Ocean during 2000–2002. Several ranges of seal species overlap across the Atlantic and Arctic Oceans (Figure 1, panel B). Arctic and sub-Arctic migrating seals have also been suggested to be carriers of PDV (*1*). In the Atlantic Ocean, gray seals (*Halichoerus grypus*) are vectors of PDV that enable spread of disease to harbor seal populations and provide contact between North Sea and Arctic Ocean species (*12*) (Figure 1, panel B). Although PDV vector species are largely unknown, the close phylogenetic relationship and geographic range of susceptible seals with other seal species makes this intraspecies contact the likely method of transmission through the Arctic to the Pacific Ocean. Now that PDV is in the Pacific Ocean, the diversity and abundance of seal and sea lion species creates the potential for viral transmission (Figure 1).

Serologic evidence indicates that the 1988 Atlantic PDV virus did not reach the Arctic or Pacific regions of Alaska. The decrease in sea ice during the 14 years between these epidemics may have affected movement of Arctic seal populations (online Technical Appendix Figure 2). This reduction was even more pronounced in 2004 and 2005, years in which PDV was confirmed to have infected sea otters (online Technical Appendix). Ice coverage is at its lowest level during August and September (*14*). In 1988 and 2002, the PDV epidemic had reached gray and harbor seal populations in the North Sea and Norwegian Sea by August. This sea ice reduction may have altered seal haulout and migration patterns, resulting in contact between Atlantic, Arctic, and Pacific Ocean species that was not possible in 1988 and the few years afterwards.

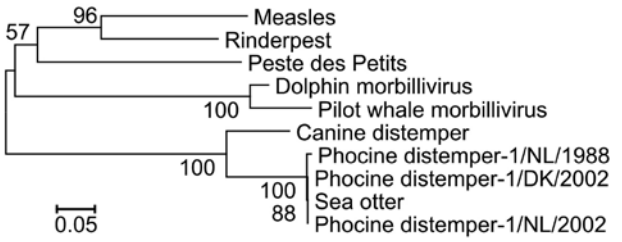

Figure 2. Neighbor-joining bootstrap tree (1,000 replicates, pairwise deletion comparisons, Tamura-Nei model) shows that morbillivirus fragments isolated from northern sea otters are identical to those of the 2002 PDV isolates. All known corresponding phosphoprotein gene fragments from morbilliviruses (online Technical Appendix, available from www.cdc.gov/EID/content/15/6/925-Techapp.pdf) were compared by using Molecular Evolutionary Genetics Analysis software version 3.1 (www.megasoftware.net/mega.html). Scale bar indicates number of nucleotide substitutions per site.

Now that PDV has been found in the Pacific Ocean, its role in population decreases and future deaths among currently uninfected species of marine mammals in Alaska must be assessed. A subspecies of the susceptible Atlantic harbor seal, the Pacific harbor seal is potentially vulnerable to PDV, and with a range from Alaska and along the West coast of the United States, they have enormous potential to spread the virus. Additionally, because terrestrial and marine Arctic species from Canada have previously been exposed to PDV, the risk for predatory and scavenging North Pacific Ocean carnivore species must not be overlooked (*15*). All seal species in the Arctic and Pacific Oceans are threatened, especially those with limited numbers, and epidemic management strategies must be in place to protect critically small populations.

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Diversity of *Anaplasma phagocytophilum* Strains, USA

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We analyzed the structure of the expression site encoding the immunoprotective protein MSP2/P44 from multiple *Anaplasma phagocytophilum* strains in the United States. The sequence of *p44ESup1* had diverged in *Ap-variant 1* strains infecting ruminants. In contrast, no differences were detected between *A. phagocytophilum* strains infecting humans and domestic dogs.

Anaplasma phagocytophilum (order Rickettsiales) has a broad host range and infects humans as well as numerous other animal species (*1*). It has been known as a ruminant pathogen in Europe since at least 1932 but in recent years has emerged as a cause of disease in humans in the United States and Europe. The number of cases reported to the Centers for Disease Control and Prevention has increased from 537 in 2004 to 834 in 2007. Similarly, the number of dogs with clinical anaplasmosis has apparently increased. Strains clearly differ; not all appear to be capable of infecting humans or mice (*2*) or to cause persistent infections. These differences have stimulated the search for molecular markers of strain phenotypes and host tropisms. Although much strain variation has been identified, these markers have not been clearly linked to host tropisms except for 16S rRNA and the US *Ap-variant 1* (*Ap-V1*). *Ap-V1* differs from a human strain (*Ap-ha*) by a 2-bp substitution in the 16S rRNA sequence (*3*) and appears to be restricted to ruminant species (*2*,*4*). Because a 2-bp difference in 16S rRNA is minimally informative, we selected the *msp2/p44* expression site to investigate other potential differences between *Ap-V1* and other strains. In the related organism, *A. marginale*, the genomic repertoire of *msp2* pseudogenes has been associated with the ability, or lack of ability, of strains to superinfect and cause epidemic spread of the organism (*5*).

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The Study

Different isolates of the *Ap-V1* strain were obtained from Rhode Island and Minnesota. Genomic DNA was extracted from infected goat blood, infected *Ixodes scapularis* ticks, and cell culture–grown isolates (ISE6) as described by Massung et al. (*6*). Other genomic DNA was isolated as described previously (*7*) from whole infected blood or HL-60 cultures. Dog blood was obtained from naturally infected animals identified by private practitioners in New York and Minnesota. Genomic DNA from the horse MRK strain of *A. phagocytophilum* (*8*) was isolated from infected equine neutrophils.

PCR amplification, sequencing, and analysis of the *msp2/p44* expression site were performed by methods described previously (*7*). We used oligonucleotide primers AB1207 (5′-GGGAGTGCTCTGGTTAGATTTAGG-3′) and AB1221 (5′-ATAGAACAAGAGCAGGGAGAAGAC-3′) or AB1207 and AB1058 (5′-GAACCATCCCCTTA AAATACTTTC-3′) to amplify the *msp2/p44* gene, the upstream gene *p44ESup1,* and the intergenic region between them. To amplify just the *msp2/p44* gene in the expression site and to determine hypervariable region sequences, we used AB1221 and AB1266 (5′-GAAGAAGAGATTGGACTTTTGATCTGTC-3′) or AB1221 and AB1267 (5′-GAGGAAGAGATTGGA CTTTTGAGCTGTC-3′). The sequences determined here have been assigned GenBank accession nos. FJ467331–FJ467340.

The expression site encoding MSP2/P44 is composed of the MSP2/P44 coding region itself, including a central hypervariable region, an intergenic region containing binding sites for a regulatory transcription factor ApxR, and an upstream gene known either as *p44ESup1* or *omp-1n* (*9*,*10*). In our study, the *p44ESup1* gene appeared to be experiencing purifying or stabilizing selection. Evolutionary analysis using MEGA4 (*11*; Nei-Gojobori method with the Jukes-Cantor correction) showed a low ratio of nonsynonymous-to-synonymous substitutions (mean dN 0.053, dS 0.296; dN/dS ratio 0.179). The *Ap-V1* strains from Rhode Island and Minnesota shared many substitutions with a sheep strain from Norway that were not present in the other *A. phagocytophilum* strains (Figure 1). The *p44ESup1*gene in strains isolated from 5 persons from Wisconsin, Minnesota, and New York most closely resembled *p44ESup1* in strains from clinical infections identified in 3 dogs from New York and Minnesota.

When we performed a concatenated analysis of the *p44ESup1* and intergenic region *p44ESup1* to *msp2(p44)* using maximum-likelihood methods, we found strong support for 3 clades: a clade of strains from eastern North America; a clade of strains from western North America; and a clade comprising a sheep strain from Norway, a dog strain from Sweden, and the 4 *Ap-V1* strains from North America

(Figure 2). Except for *Ap-V1*, the strains from eastern North America appeared to be closely related among themselves;

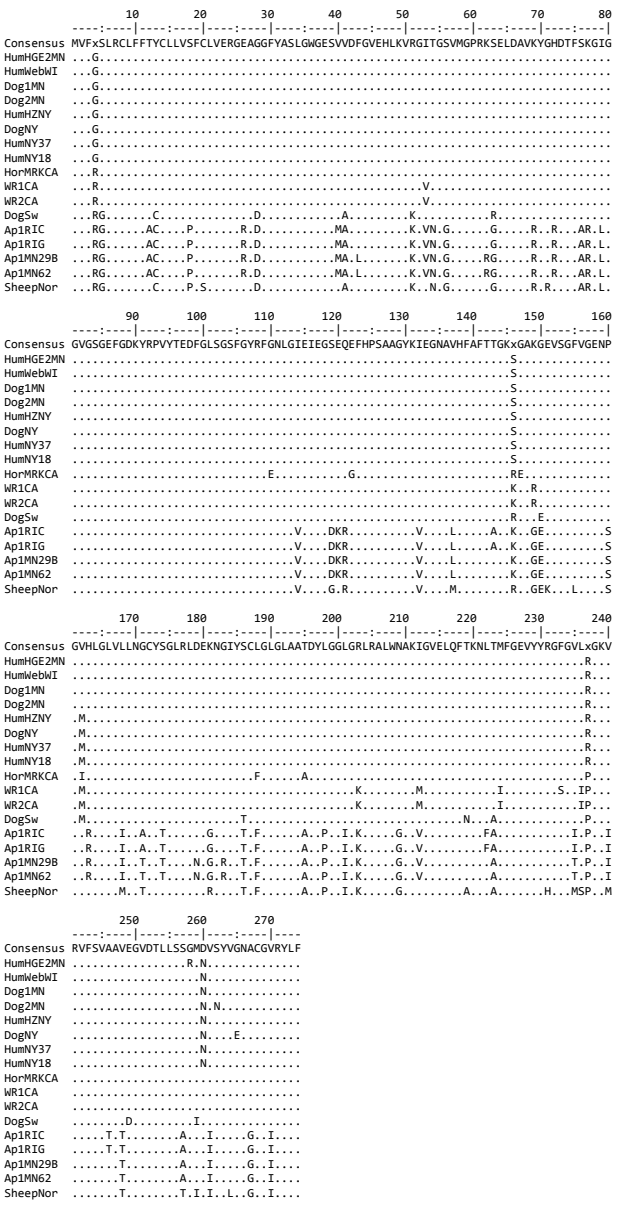

Figure 1. Diversity in the amino acid sequences encoded by *p44ESup1/omp-1n* in US and European strains of *Anaplasma phagocytophilum*. All strains are from the United States (the state is indicated in the strain designation) except for the strain from the sheep from Norway (SheepNor) and the dog from Sweden (DogSw). Human-origin strains are HZNY, NY18, NY37, WebWI, and HGE2MN; dog strains are Dog1MN, Dog2MN, and DogNY; wood rat (*Neotoma fuscipes*) strains are WR1CA and WR2CA; the horse strain is HorMRKCA; *Ap-V1* strains are Ap1RIC (culture derived), Ap1RIG (isolated from an infected goat), Ap1MN29B, and Ap1MN62 (both Ap1MN strains were derived from naturally infected *Ixodes scapularis* ticks). Sequences were from either this study or GenBank: accession nos. DQ519565 (SheepNor), DQ519566 (DogSw), CP000235 (HZ), AY164490 (NY18), AY137510 (NY37), AY164491 (Webster), and AY164492 (HGE2).

the dog and human strains of *A. phagocytophilum* were indistinguishable from each other. Of note, a strain isolated from a dog in Sweden with clinical disease is on a separate branch from all US strains, including those from dogs in the United States.

The central hypervariable regions of *msp2/p44* and the flanking conserved sequences from 34 *Ap-V1* sequences were also aligned. The alignments showed the typical structure, including flanking LAKT residues and conserved framework residues such as C and WP described previously (*9*,*12*). Also, multiple hypervariable region variants were identified in each population of *A. phagocytophilum* (organisms characterized at a single time point from a single host). Some of the same variants were identified in different Rhode Island populations. No shared expression site variants were found between the Rhode Island and Minnesota *Ap-V1* strain sequences.

When comparing the *Ap-V1* expression site variants to genomic copies of the sequenced US human HZ strain, we found sequence identities >90% between 20/34 *Ap-V1* variants, including 100% identities of 5/34 *Ap-V1* variants. This level is comparable to that seen in most other US *A. phagocytophilum* strains. When compared with variants (non-HZ) identified directly from human infections, 10/34 *Ap-V1* variants were >90% identical. In contrast, none of the *Ap-V1* variants matched, with at least 70% identity, any previously identified MSP2/P44 expression site variants from strains from sheep in Norway. In general, little similarity was found between the *msp2/p44* hypervariable regions of US and European strain variants.

Conclusions

Despite finding clear differences in the MSP2/P44 hypervariable region repertoire between US and European strains, we did not discover distinct repertoires in any US strains, including in *Ap-V1*. These findings agree with previous data that showed few differences by pulsed-field gel electrophoresis of 7 US strains (*13*) or by comparative microarray hybridization of 3 US strains (*14*). Our analysis focused on those hypervariable regions found frequently in the expression site. Because the genome repertoire contains ≈100 functional pseudogenes in each strain, complete genome sequencing may show differences in this repertoire not detected here.

The *p44ESup1*gene, upstream from *msp2/p44* on the same polycistronic mRNA transcript, gave the most phylogenetically useful information. This gene clearly distinguished *Ap-V1*from other US strains. Moreover, the resemblance of the *p44ESup1* gene in *Ap-V1* and in a strain from a sheep in Norway suggests that it may be a marker for a ruminant tropism of *A. phagocytophilum*. Also, phylogenetic trees based on the *p44ESup1* gene grouped *A. phagocytophilum* strains that cause clinical infections in US dogs

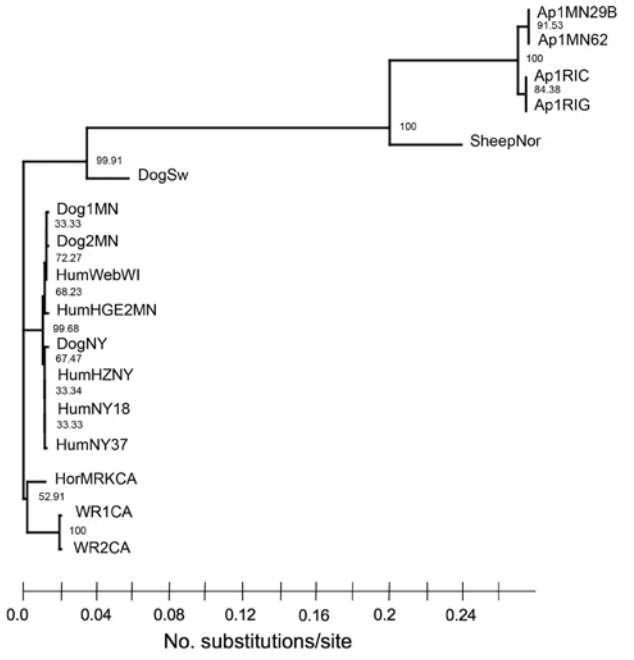

Figure 2. Maximum-likelihood phylogram of different variants of *Anaplasma phagocytophilum* based on the *p44ESup1/omp-1n* and intergenic region gene sequences created by using TREEFINDER (www.treefinder.de) with default values. The number of substitutions per site over 1,092 total sites is shown under the tree, and bootstrap support for each split (percentage of times recovered) is shown next to each branch of the tree.

or humans on the same branch. In fact, the genes from the 2 sources are indistinguishable, which may suggest a recent and common evolutionary origin of the US dog and human strains. Because these US data were obtained from a relatively small sampling of *A. phagocytophilum* infections (although from at least 2 states for the human, dog, and *Ap-V1* strains), these findings should be verified in a larger dataset.

The sequence divergence between strains in *p44E-Sup1* is similar to that in the downstream intergenic region. This intergenic region includes 2 divergent (54% and 58% identity in *Ap-V1*) binding sites for the transcription factor ApxR, which has been postulated to upregulate *msp2/p44* transcription in mammalian cells (*15*). Either the ApxR transcription factor has low specificity for sequence compared with secondary structure or it does not have the same biological mode of action in *Ap-V1* as in some other strains.

In summary, the *Ap-V1* expression site encoding *msp2/p44* was most similar to a strain from sheep in Norway. Strains causing clinical disease in humans and domestic dogs in the United States were indistinguishable.

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Increasing Incidence of Zoonotic Visceral Leishmaniasis on Crete, Greece

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To determine whether the incidence of canine leishmaniasis has increased on Crete, Greece, we fitted infection models to serodiagnostic records of 8,848 dog samples for 1990–2006. Models predicted that seroprevalence has increased 2.4% (95% confidence interval 1.61%–3.51%) per year and that incidence has increased 2.2- to 3.8-fold over this 17-year period.

Zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum* is a disease of humans and domestic dogs (the reservoir) transmitted by phlebotomine sandflies. According to the World Health Organization (*1*), ZVL was first recorded on Crete in 1907, after which it featured prominently in medical literature as a serious public health problem. In Chania, Crete, the annual incidence in the 1930s was 50 cases/30,000 population (*2*), and 21% of 1,115 dogs were positive for ZVL by the formol-gel serologic test; 30% of those were symptomatic (*1*).

After World War II, use of DDT against malaria vectors and focal destruction of *Leishmania* spp.–infected dogs is thought to have reduced ZVL on Crete (*3*). Sandflies were not found in villages systematically sprayed during 1946–1949 compared with unsprayed villages (*4*). During 1951–1975, only 33 alleged human ZVL cases were recorded on Crete (*5*), and reports of canine ZVL were scanty. In 1983 a serosurvey of 72 stray dogs in Chania identified only 1 infected dog (*1*).

By the late 1980s, *Phlebotomus neglectus*, the putative vector of *L. infantum* on Crete (*6*), was abundant in stone walls inside and outside villages around Heraklion (*7*). During 1999–2004, *P. neglectus* was found in abundance in human dwellings and rural locations (hollows in olive trees and near rodent burrows) (*8*). Since 1991, 38 persons who came to hospitals in Heraklion were confirmed as having cases of ZVL. Today, canine infection is confirmed throughout the island (M. Antoniou, unpub. data), and seroprevalences (30%–40%) are some of the highest reported in Europe. These accounts anecdotally suggest that the incidence of ZVL has increased on Crete during the past decade, which, if so, would be relevant to the public and veterinary health sectors.

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The Study

We statistically examined diagnostic records of 8,848 dogs sampled in the eastern Crete districts of Lasithi and Heraklion during 1990–2006. Data were supplied by the Faculty of Medicine, University of Crete (n = 1,205 dogs from 97 villages tested by using an indirect immunofluorescent antibody test [IFAT], cutoff titer 160, during 1999–2006, accompanied by demographic and geographic records); and by the Ministry of Agriculture Serology Laboratory, Heraklion (n = 7,643 dogs tested by using an IFAT, cutoff titer 200, during 1999–2005, but without accompanying records). Samples were collected by veterinarians in private practice from any dog initially brought to their clinic for any reason, or by government veterinarians for any dog encountered, irrespective of clinical condition, during prearranged visits to villages. Numbers of villages and dogs sampled in any year depended on available resources at the time.

To reduce potential sampling bias, we first tested serologic data from 6 villages (located 9–45 km apart) consistently sampled annually during 1999–2006 (n = 744 dogs). The age-adjusted annual prevalence increased significantly with sample year (odds ratio [OR] 1.18, 95% confidence interval [CI] 1.089–1.271, $p<0.001$) and showed no significant differences in slope or intercept (by using robust standard errors) compared with data from 91 less consistently sampled villages with demographic records (n = 461 dogs; slope × village group interaction OR 0.91, 95% CI 0.800–1.023, p not significant; intercepts OR 5.35, 95% CI 0.902–3.688, p not significant).

These combined datasets were then compared with crude prevalence data for 1990–2005 (n = 7,643 dogs) calculated from the ministry records; no difference was detected in prevalence slopes (slope × data source interaction OR 1.06, 95% CI 0.995–1.30, p not significant; Figure). In univariable or multivariable logistic regression that controlled for dog age and clustering on villages (1999–2006, n = 1,205 dogs), no statistical confounding of the probability of a dog being seropositive was attributed to dog use (companion, guard, or hunting dog), sex, crude habitat type, or village altitude ($p\geq0.215$; see online Technical Appendix Table, available from www.cdc.gov/EID/content/15/6/932-

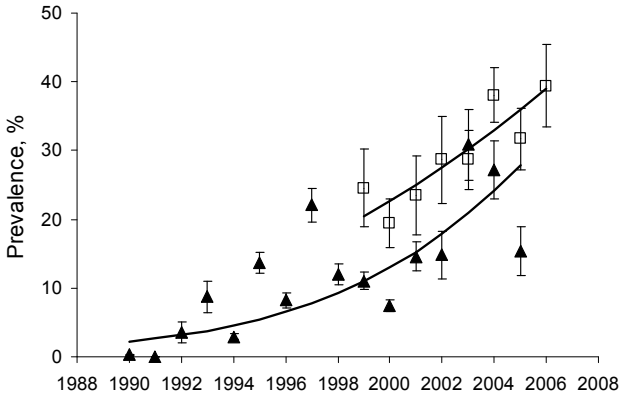

Figure. Annual seroprevalence of zoonotic visceral leishmaniasis in dogs on Crete, Greece, 1990–2006. Shown are logistic fits of age-adjusted prevalences (line and squares) for dogs from 97 villages (indirect immunofluoresecent antibody test [IFAT] cutoff titer 160) and crude seroprevalences (line and triangles) calculated from records of the Veterinary Laboratory of Heraklion, Crete, Hellenic Ministry of Rural Development and Food (www.minagric.gr) (IFAT cutoff titer 200). Binomial standard error bars are shown.

Techapp.pdf). The final fit of seroprevalence against time was significant (OR 1.24, 95% CI 1.221–1.264, p<0.001), and the linearized difference in model prevalence (and binomial confidence limits) over the 17-year study equated to a mean prevalence increase of –ln(1 – 0.321)/16 = 2.4% (95% CI 1.61%–3.51%) per year.

To assess the change in infection incidence, our principal aim, we used 3 standard epidemiologic models (*9–11*; online Technical Appendix) to calculate infection rates accounting for time, dog age, and potential loss of infection. The first method (model 1) used the cross-sectional age-prevalence data (IFAT cutoff titer 160), in which the proportion of seropositive dogs in each age class is fitted by varying the rates of infection and recovery. A second method (model 2) used these same data to describe the infection rate as it varied with both time and age until reaching the best fit. The third method (model 3) estimated the infection rate from longitudinal data of previously unexposed dogs ≤12 months of age that were followed up during 1 transmission season.

Results from the 3 models were consistent (Tables 1, 2) and showed a relative increase in the mean infection rate estimated to be 2.20–3.78-fold higher during 2005–2006 than during 1999–2000. The models differed in approach and age of dogs considered by necessity of the model, number of estimated parameters, or model reduction. Inclusion of a parameter describing loss of infection (Table 1, model 1) did not significantly lower the infection rate estimates as might be expected compared with a single parameter (Table 1, model 2) or longitudinal (Table 2, model 3) model, both of which identified younger (≤2 years of age) dogs to be at substantially greater risk for infection (p<0.0001).

Conclusions

The potential contribution of any improvements in diagnostic test sensitivity or vigilance to the increasing incidence of ZVL infection is unclear. The difference in cutoff titers between data sources minimally shifted the absolute prevalence values, but not relative prevalence slopes, with time (Figure). Any loss of infection with age (Table 1, model 1) could result from nonmutually exclusive biologic processes including recovery from infection, death, or reduced past exposure (*9,11*). The latter possibility is unlikely on Crete because of the higher risk identified in young dogs in all biannual periods. Disproportionate numbers of deaths of seropositive dogs is not suggested by a decline in ZVL clinical signs in older dogs in this study (data not shown) or elsewhere (*11,12*). Loss of detectable *Leishmania*-specific antibody is the more likely explanation because the observed rates of serorecovery are not dissimilar to those (e.g., 0.062/month) estimated by cohort studies elsewhere (*12*).

Actual infection rates are likely to be higher than those shown here because IFAT sensitivity is <100%. Similarly, absolute prevalences, particularly low values for 1990–1991, should be treated with caution because the official leishmaniasis control program on Crete (1984–1995) began before this period when infection was presumably sufficient on the island to warrant intervention. The intervention comprised elimination of IFAT-seropositive dogs (cutoff titer 400) but did not include insecticide spraying (DDT spraying ceased in 1950; V. Chatzistefanou, pers. comm.).

The continual increase in canine seroprevalence during the latter part of the intervention (Figure) suggests that the culling policy was unsuccessful in reducing transmission;

Table 1. Variation in incidence over time estimated from cross-sectional data for 1,205 dogs with accompanying demographic records, Crete, Greece, 1990–2006*

Period	Model 1				Model 2		No. dogs
	Incidence/mo	95% CI	Loss of infection/mo	95% CI	Incidence/mo	95% CI	
1999–2000	0.016	0.0107–0.0206	0.045	0.0260–0.0645	0.015	0.0093–0.0213	237
2001–2002	0.029	0.0114–0.0455	0.071	0.0201–0.1211	0.023	0.0166–0.0298	219
2003–2004	0.030	0.0216–0.0381	0.049	0.0320–0.0667	0.029	0.0221–0.0367	401
2005–2006	0.059	0.0233–0.0946	0.106	0.0383–0.17430	0.032	0.0205–0.0477	348

*Only model 1 is designed to estimate loss of infection (serorecovery). CI, confidence interval. A full description of these models is available in the online Technical Appendix (available from www.cdc.gov/EID/content/15/6/932-Techapp.pdf).

Table 2. Variation in incidence over time estimated from longitudinal data for 179 dogs with accompanying demographic records, Crete, Greece, 1990–2006*

Period	Model 3		
	No positive/ no. tested	Incidence/ mo	95% CI
1999–2000	6/56	0.014	0.0061–0.0280
2001–2002	7/30	0.033	0.0153–0.0612
2003–2004	12/56	0.030	0.0172–0.0490
2005–2006	10/37	0.039	0.0210–0.0670

*CI, confidence interval. A full description of this model is available in the online Technical Appendix (available from ww.cdc.gov/EID/content/15/6/932-Techapp.pdf).

likely reasons for the low efficacy of dog culling in other leishmaniasis-endemic regions have been described (*13*). Officially, destruction of seropositive dogs is still required today unless the owner agrees to veterinary treatment of the dog or to keep the dog under sandfly-proof netting. However, there is no current policy on Crete to combat vectors. We conclude that the results of our study are consistent with a postwar reemergence and current increasing incidence of ZVL infection on Crete.

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Avian Influenza in Wild Birds, Central Coast of Peru

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To determine genotypes of avian influenza virus circulating among wild birds in South America, we collected and tested environmental fecal samples from birds along the coast of Peru, June 2006–December 2007. The 9 isolates recovered represented 4 low-pathogenicity avian influenza strains: subtypes H3N8, H4N5, H10N9, and H13N2.

Limited data are available on genotypes of avian influenza virus (AIV) circulating among wild birds in South America (*1*–*6*). To determine whether AIVs are present and circulating in wild birds in South America, we collected and examined bird environmental fecal samples.

The Study

From June 2006 through December 2007, environmental fecal samples were collected from resident and migratory wild birds from 4 wetland areas along the central coast of Peru (Figure) and tested for evidence of avian influenza. Each wetland was visited 1×/week for 6 weeks; after a sampling period was completed, we would sample another wetland. Each wetland was sampled at least 2 times, with the exception of Villa, which was sampled only 1 time.

Bird colonies were identified from 7 AM to noon and observed for 15–20 min by persons experienced in bird identification. Size and accessibility to the bird colonies affected which species were sampled. We focused on collecting samples from single-species flocks; however, mixed flocks were also sampled and identified to the family level or as a mix of species. Immediately after the colony left the resting area, fresh fecal samples were collected. Each sample was collected with a sterile-tipped applicator, placed in a cryovial containing transport media (RPMI 1640 with 5% bovine serum albumin; Sigma-Aldrich, St. Louis, MO, USA) in a Styrofoam box with ice packs at ≈4°C, and transported to the laboratory to be processed within 24 h of collection.

Samples were processed in the Avian Pathology Laboratory at the Veterinary School, San Marcos University. Virus isolation was conducted by standard methods (*7*). Samples were pooled according to species, date, and wetland; each pool consisted of 8–12 samples. Pools were centrifuged and filtered before inoculation into the allantoic cavity of 5 specific-pathogen-free 9-day-old chicken embryos. Eggs were incubated for 6 d; survival was checked daily. Allantoic fluid of each egg was tested for hemagglutinating agents by a direct hemagglutination assay (*7*). First-passage negative pools were passaged a second time to confirm absence of avian influenza. All hemagglutination-positive allantoic fluids were tested by antigen capture tests for influenza virus A and sent to the US Naval Medical Research Center Detachment, Peru, for further molecular characterization. Allantoic fluids were also sent to the US Centers for Disease Control and Prevention (Atlanta, GA, USA) for virus typing and sequencing. RNA extracts were prepared from 100 μL of allantoic fluids with the MagNA

Figure. Locations of 4 sites (large circles) along coast of Peru where wild bird fecal samples were collected, June 2006–December 2007.

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Pure Compact automated RNA extraction system (Roche Applied Science, Indianapolis, IN, USA). One-Step RT-PCR (Invitrogen, Carlsbad, CA, USA) was used to amplify hemagglutinin (HA) and neuraminidase (NA) genes with universal HA and NA oligonucleotide primers (*8*). Amplicons were purified by agarose gel electrophoresis followed by purification using the MiniElute Gel Extraction kit (QIAGEN, Valencia, CA, USA), then sequenced on an automated Applied Biosystems 3730 system (Foster City, CA, USA) using cycle sequencing dye terminator chemistry. Each gene segment sequence was analyzed by BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) analysis against the Influenza Sequence Database of Los Alamos National Laboratories (Los Alamos, NM, USA) for identification of avian influenza genotype.

A total of 2,405 samples, representing 27 species, were processed and analyzed (Table 1). Nine AIV isolates, from 7 species, representing 5 avian families, were recovered from 3 of the 4 wetlands sampled. Hemagglutinin subtypes H3, H4, H10, and H13 and neuraminidase subtypes N2, N5, N8, and N9 were identified (Table 2).

For the samples from Medio Mundo, sequences of the HA gene were 100% identical; however, the NA genes differed by 1 nt. Similarly, for the samples from Paraiso, the HA genes from both bird species were 100% identical, but the NA genes differed by 4 nt. From the Puerto Viejo samples, 2 different influenza virus A subtypes were recovered: H3N8 in ducks and H10N9 in 2 ruddy turnstones and an American oystercatcher. The HA and NA genes of the H10N9 were 100% identical in 3 isolates from 2 species. All samples from Villa were negative.

Conclusions

We isolated no highly pathogenic AIVs; however, several low-pathogenicity avian influenza (LPAI) strains were identified. AIVs were recovered from both migratory and resident (nonmigratory) birds. Each strain was first isolated from a migratory bird and, in most instances, at least 1 week thereafter from resident species. Although this finding seems to support unidirectional transmission of the virus from migratory to resident birds, further research should be conducted to test this hypothesis. Furthermore, although subtype H13 is considered restricted to gulls (*9*), we isolated this subtype from a flock purely of whimbrels; this finding may have resulted from a spillover event, or perhaps gulls are not the sole reservoir species for this strain. Orders Charadriiforme and Anseriforme have a greater isolation index than other orders (*9*), and subtype

Table 1. Bird species sampled along central coast of Peru, June 2006–December 2007

Family	Species	Wetland, no. sampled				Total*
		Medio Mundo	Puerto Viejo	Paraiso	Villa	
Anatidae	*Anas bahamensis*		44		10	54
	Anas cyanoptera		37		20	57
	Oxyura ferruginea	10				10
	Mix†	46	38	115	30	229
Ardeidae	*Ardea alba*	10	14			24
	Egretta thula	8	37			45
	Egretta caerulea		10			10
	Nycticorax nycticorax		4			4
	Mix†	43	10	134	10	197
Laridae	*Larus pipixcan*	38	24	20	10	92
	Larus modestus	67	24		10	101
	Larus dominicanus	10				10
	Larus belcheri				20	20
	Mix†	53	16	65		134
Rinchopidae	*Rynchops niger*	79	19	10		108
Scolopacidae	*Calidris mauri*			10	21	31
	Calidris alba	105	23	41	50	219
	Calidris pusilla	31				31
	Arenaria interpres		61		20	81
	Numenius phaeopus		35	10	40	85
Charadridae	*Charadrius vociferus*		6	10		16
	Charadrius semipalmatus			10	10	20
	Pluvialis squatarola	9				9
Recurvirostridae	*Himantopus mexicanus*		3		10	13
Threskiornithidae	*Plegadis ridgwayi*			39		39
Rallidae	*Gallinula chloropus*		30			30
	Fulica ardesiaca	21	39	10		70
Pelecanidae	*Pelecanus occidentalis thagus*	86		56		142
Phalacrocoracidae	*Phalacrocorax brasilianus*	174	99	70	39	382
Haematopodidae	*Haematopus paliatus*	108	34			142
Total		898	607	600	300	2,405

*Does not represent the exact number because some were included in the mixed samples.
†≥2 species from same family.

Table 2. Birds with positive avian influenza virus test results, central coast of Peru, June 2006–December 2007

Date	Wetland	Common name (taxonomic name)	Hemagglutinin	Neuraminidase
2006 Oct 17	Puerto Viejo	Ruddy turnstone (*Arenaria interpres*)	H10	N9
2006 Oct 24	Puerto Viejo	Ruddy turnstone (*Arenaria interpres*)	H10	N9
2006 Nov 6	Puerto Viejo	White-cheeked pintail (*Anas bahamensis*) and cinnamon teal (*Anas cyanoptera*)	H3	N8
2006 Nov 7	Puerto Viejo	American oystercatcher (*Haematopus palliates*)	H10	N9
2007 Feb 6	Medio Mundo	White-cheeked pintail (*Anas bahamensis*) and cinnamon teal (*Anas cyanoptera)*	H4	N5
2007 Feb 13	Medio Mundo	White-cheeked pintail (*Anas bahamensis*) and cinnamon teal (*Anas cyanoptera)*	H4	N5
2007 Feb 13	Medio Mundo	Peruvian pelican (*Pelecanus occidentalis thagus*)	H4	N5
2007 Nov 20	Paraiso	Whimbrel (*Numenius phaeopus*)	H13	N2
2007 Nov 20	Paraiso	Dominican gulls (*Larus dominicanus*)	H13	N2

patterns observed in this study appear to be the same as those observed in North America.

Migrating birds make frequent stopovers when moving between breeding and nonbreeding areas (*10*). The strains identified in our study may have been carried from Alaska and Canada through the continental United States, perhaps disseminating these viruses on their stopover sites before arriving in Peru. Thus, in the future, these strains may arise from within other countries along the north-to-south flyway.

We identified positive samples at the beginning and end of the migratory season, as has been found in previous studies (*9–11*). Our inability to detect viruses throughout the year, over multiple seasons, may be partially explained by insufficient sample size. The sample size was based on the assumption that the prevalence rate of circulating virus should be at least 1%; however, the rate may be lower.

The proximity of the Peru wetlands to human habitation, swine farms, and chicken farms could represent a risk for transmission of influenza viruses from wild birds to poultry, humans, and pigs. Wild birds were suspected to be the source of the avian influenza outbreak in Chile in 2002. This hypothesis was later supported by Spackman et al. (*6*), who identified an LPAI virus from a cinnamon teal; this virus was almost an exact match to the LPAI strain circulating among poultry during the Chile outbreak. Although the strains identified in our study were LPAI, a genetic shift, which likely occurred during the Chile outbreak, is possible.

Another study limitation is that field identification of bird species is sometimes difficult and can result in misidentification. Although most birds in a flock are generally of the same species, it is not uncommon to find additional species, albeit few, intermingled within the flock (e.g., calidrids and gulls). Therefore, bird sources of the fecal samples collected may have been misidentified. Although the probability that a sample may be recovered from 1 rogue species within a group of a different species is low, to minimize this uncertainty we assigned a few samples to the family level or as a mixture of 2–3 species. We were also unable to determine the prevalence of avian influenza in the wetlands sampled because environmental sampling limited our ability to determine whether multiple samples were from the same bird.

Our systematic evaluation of avian influenza strains among migratory and resident aquatic bird populations in South America used a cost-effective and efficient surveillance method to monitor AIV in bird populations at a specific location (*12*). Although all isolates were LPAI strains, our data support the hypothesis that migratory birds can serve as vectors for the spread of AIV among nonmigratory species. More data are needed to determine the role that migratory birds can play in spreading AIVs throughout the region.

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Japanese Encephalitis Viruses from Bats in Yunnan, China

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Genome sequencing and virulence studies of 2 Japanese encephalitis viruses (JEVs) from bats in Yunnan, China, showed a close relationship with JEVs isolated from mosquitoes and humans in the same region over 2 decades. These results indicate that bats may play a role in human Japanese encephalitis outbreaks in this region.

Bats have been increasingly recognized as an important source of zoonotic viruses responsible for some of the recent major disease outbreaks, including Hendra, Nipah, severe acute respiratory syndrome–associated, and Ebola viruses (*1*). *Japanese encephalitis virus* (JEV) is a member of the family *Flaviviridae* and the genus *Flavivirus* (*2*) and is the etiologic agent of severe encephalitic diseases in humans. In addition to humans, JEV has been isolated from various hosts, e.g., mosquitoes, birds, pigs, and horses (*3,4*). The role of bats in JEV epidemiology has been poorly defined, although the virus has been isolated from bats of various species since 1963 in multiple locations (*5,6*). To date, no nucleotide sequence information has been available for bat JEV isolates.

Yunnan Province, in southern China, has a tropical to subtropical climate and a diverse biota. The annual case rate of Japanese encephalitis in Yunnan is >2× the average case rate of the whole country (*7*). We report the molecular and virulence characterization of 2 bat JEV isolates from Yunnan: B58, obtained from a Leschenault's rousette (*Rousettus leschenaultia*), a fruit bat, in 1989; and GB30, obtained from a little tube-nosed bat (*Murina aurata*), an insectivore, in 1997.

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The Study

The viruses used in this study were isolated from homogenates of the brains of bats by direct intracranial (i.c.) inoculation of 3-day-old suckling mice. Although isolate B58 was obtained in 1989, no further identification or characterization was conducted until this study. GB30 was identified as JEV by serologic analysis (*8*). Inoculation in suckling mice was conducted following procedures approved by the Animal Ethics Committee of the Institute for Viral Disease Control and Prevention, China. Mice were observed 2× per day after inoculation, and every 2 hours after the onset of clinical signs. After euthanasia, supernatant from brain homogenate was used to inoculate C6/36 cells. After the appearance of cytopathic effect (CPE), supernatant was harvested and passaged 3 more times. Virus stock was prepared from the previous passage and stored at –85°C.

Neurovirulence of the 2 bat JEV strains and of a mosquito-derived JEV strain, M10 (*9*), were determined. All viruses were consecutively passed 3 times in mice, and virus suspension (defined as the 10^{-1} stock) was prepared from the third passage. For determination of a 50% lethal dose (LD_{50}), suckling mice (5 per group) were inoculated i.c. with dilutions from 10^{-1} to 10^{-9}. Animals were monitored daily for survival, and the LD_{50} values were calculated by using a standard method (*10*).

Viral RNA was isolated by using the Viral RNA Mini Kit (QIAGEN, Hilden, Germany). First strand cDNA was synthesized using the Ready-To-Go kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Flavivirus-specific primers (*11*) and primers designed from the sequence of JEV Beijing-1 (L48916) were used for PCR and sequencing. Sequence assembly was conducted by using the ATGC software package, version 4.0 (GENETYX Corp., Tokyo, Japan). Homology and alignment analysis were conducted by using ClustalX version 1.8 (www.clustal.org/download/1.X/ftp-igbmc.u-strasbg.fr/pub/ClustalX) and MegAlign (DNASTAR, Madison, WI, USA). MEGA 3.1 (*12*) was used for phylogenetic analysis.

For initial studies, suckling mice were inoculated i.c. with the supernatant of clarified brain homogenate. The 2 groups (n = 8 for each) inoculated with B58 and GB30, respectively, displayed clinical signs after 42 h postinoculation (hpi), whereas the control group with buffer only (n = 8) displayed no clinical signs. Clinical signs included refusing sucking, no interest in grouping, neck rigidity, tremors and muscular spasms, ataxia, and hind-limb paralysis. All mice had to be euthanized from 70 to 78 hpi. The supernatant of brain homogenate was used to inoculate C6/36 cells, and CPE was visible at ≈96 hpi for the first passage and at 72 hpi for second and third passages.

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The identity of B58 as JEV was confirmed by PCR sequencing. The complete genome sequence of both isolates was then determined. The 2 genomes have identical size at 10,977 nt with a 95-nt 5′ nontranslated region (NTR) and a 583-nt 3′ NTR. The single open reading frame codes for a polyprotein of 3,432 aa. The genomes have similar guanine-cytosine content (51.44% for B58 and 51.39% for GB30).

The 2 bat JEV isolates have an overall sequence identity of 99.9% both at nt and aa levels. When compared to 55 known JEV isolates of known complete genome sequences, the nt sequence identity varies from 88.6% to 99.3%, and aa sequence identity from 97.0% to 99.3%. Analysis of the NTR sequences showed that the bat JEV isolates have the same 5′ NTR as do the others, but the 3′ NTRs of the bat JEVs have a G insertion at nt 307, the same as that observed in the Nakayama strain (GenBank accession no. EF571853).

Phylogenetic trees derived from nucleotide sequences of the complete genome or the most variable envelope protein gene of selected JEV strains (Table 1) indicated that both JEV isolates from bats are members of genotype III as defined by Solomon et al. (*3*). A more detailed analysis indicated that the bat JEVs are most closely related to human isolate LiYujie and mosquito isolate BN19 within cluster 6 (Figure). Similar phylogenetic trees were obtained based on other gene sequences, such as PrM (data not shown).

Neurovirulence of the 2 bat JEV isolates was determined as described above, and the LD_{50} for suckling mice was 8.0 $\log_{10}$/0.02 mL, compared with 3.5 $\log_{10}$/0.02 mL for the mosquito strain M10. Neurovirulence of these 2 bat isolates also was predicted from aa sequence comparison to those known to have high neurovirulence (*13,14*). As shown in Table 2, all residues important for virulence and neurotropism were conserved between the bat JEV isolates and the Nakayama strain.

Conclusions

In this study, we analyzed the complete genome sequences of 2 bat JEV isolates. Although previous serologic studies (*15*) have indicated the occurrence of JEV in a Leschenault's rousette, we demonstrate JEV infection of *Marina aurata* bats, confirming that the same JEV genotype can infect bats of many species. Both bats are commonly present

Table 1. Background information of selected strains of Japanese encephalitis virus used in this study*

Strain	Source	Year	Location	GenBank accession no.	Genotype
B58	Bat	1986	China	FJ185036	III
GB30	Bat	1997	China	FJ185037	III
Beijing-1	Human	1949	China	L48916	III
p3	Human	1949	China	U47032	III
Nakayama	Human	1935	Japan	EF571853	III
JaOH0566	Human	1966	Japan	AY508813	III
Ling	Human	1965	Taiwan	L78128	III
ML17-_live	Human	1981	Taiwan	AY508812	III
Vellore P20778	Human	1958	India	AF080251	III
GP78	Human	1978	India	AF0723	III
FU	Human	1995	Austria	AF217620	II
WHe	Pig	NA	China	EF107523	III
SA14-14-2	SA-14 derivative	1954	China	AF315119	III
SA-14	Mosquito	1954	China	U14163	III
SH0601	Mosquito	2006	China	EF543861	III
JaGAr01	Mosquito	1959	Japan	AF069076	III
Ishikawa	Mosquito	1998	Japan	AB051292	I
JaOArS982	Mosquito	1982	Japan	M18370	III
K87P39	Mosquito	1987	South Korea	AY585242	III
K94P05	Mosquito	1994	South Korea	AF045551	I
RP-9	Mosquito	1985	Taiwan	AF014161	III
CH2195LA	NA	1994	Taiwan	AF221499	III
BN19	Mosquito	1982	China	FJ185038	III
Liyujie	Human	1979	China	FJ185039	III
YN86-86266	Mosquito	1986	China	DQ404134	I
WTP-70-22	Mosquito	1970	Malaysia	D00998	II
47	Human	1950s	China	AY243810	III
Tla	Human	1971	China	AY243808	III
NACH-13	Human	NA	China	AY243813	III
YNJH04-18	Mosquito	2004	China	DQ404078	III
Chiang Mai	Human	1964	Thailand	U70393	III
G8924	Mosquito	1956	India	EF688636	III
733913	Human	1973	India	AB379813	III
782219	Human	1978	India	EF688655	III
VN118	Mosquito	1979	Vietnam	D00975	III
JKT7003	Mosquito	1981	Indonesia	AY184215	IV

*__Boldface__ indicates strains with complete genome sequences in GenBank. NA, information not available.

in Yunnan Province and other parts of China. The bats tend to roost in trees, caves, and roofs of residential properties in close proximity to rice paddocks and pig pens, providing ample opportunity for cross-species transmission among bats and between bats and other animals. Notably, the 2 JEV strains most closely related to the bat viruses were all isolated from Yunnan Province, LiYujie from a human in 1979 and BN19 from mosquitoes in 1982 (Figure).

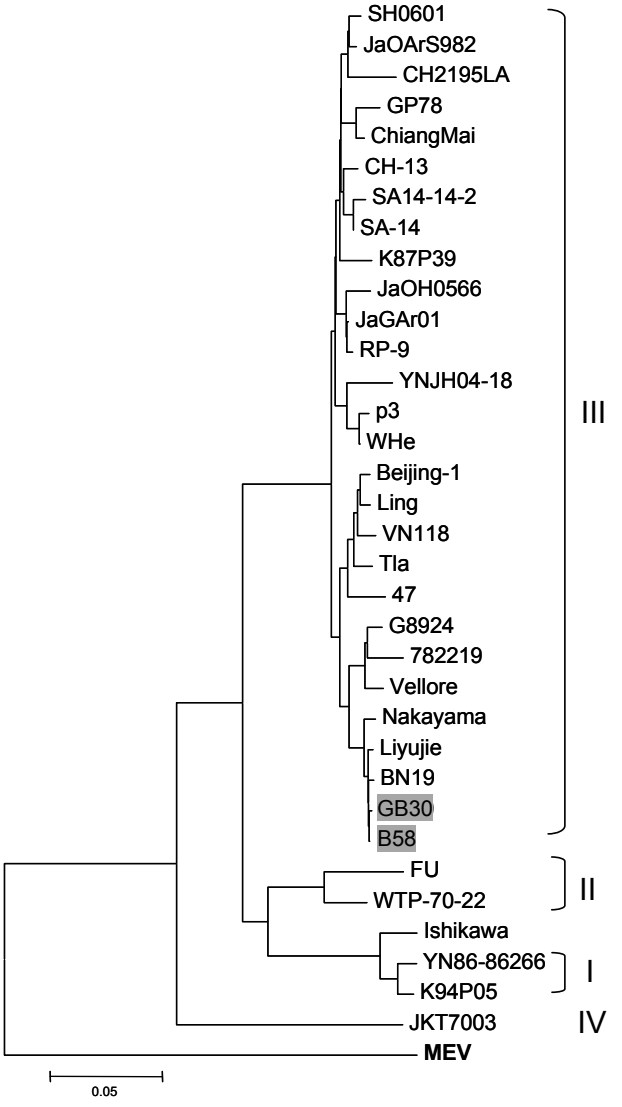

Figure. Phylogenetic tree based on the envelope (E) protein gene of selected Japanese encephalitis virus strains. Murray Valley encephalitis virus (MEV) E gene (in **boldface**) was used as an outgroup. Genotypes are indicated on the right. The 2 bat virus isolates used in this study are indicated by shading. Scale bar indicates number of nucleotide substitutions per site. See Table 1 for more details of the strains used in this analysis and their GenBank accession numbers.

Our study indicates that the same virus is circulating in hosts of at least 4 different species (human, mosquito, and 2 different bat species), and likely in birds as well, highlighting the broad host range of JEVs in this area. We emphasize that the 4 closely related strains (B58, GB30, LiYujie, and BN19) were isolated over 2 decades, which suggests that the virus was stably maintained in the region, perhaps by circulating in disparate hosts. The findings from our current study highlight the potential importance of bats in human JE outbreaks in the region. Needed additional studies of JEV in bats should include the determination of viremia in bats of different species and potential seasonal variation of viral loads among different bats at different geographic locations. This data would provide a better assessment of risks posed by JEV in bats.

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Table 2. Comparison of key amino acid residues of the E protein of Japanese encephalitis virus important for neurovirulence*

Strain	E107	E138	E176	E177	E264	E279	E315	E439
SA14-14-2	Phe	Lys	Val	Ala	His	Met	Val	Arg
B58	Leu	Glu	Ile	Thr	Gln	Lys	Ile	Lys
GB30	Leu	Glu	Ile	Thr	Gln	Lys	Ile	Lys
Nakayama	Leu	Glu	Ile	Thr	Gln	Lys	Ile	Lys

*These 8 aa residues of the envelope protein are shown to play a key role in neurovirulence. They vary substantially between the attenuated vaccine strain (SA14-14-2) and the virulent strain (Nakayama).

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Vancomycin-Resistant *Staphylococcus aureus*, Michigan, USA, 2007

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Vancomycin-resistant *Staphylococcus aureus* (VRSA) infections, which are always methicillin-resistant, are a rare but serious public health concern. We examined 2 cases in Michigan in 2007. Both patients had underlying illnesses. Isolates were *vanA*-positive. VRSA was neither transmitted to or from another known VRSA patient nor transmitted from patients to identified contacts.

Vancomycin continues to be used as a first-line antimicrobial agent for the treatment of infection with methicillin-resistant *Staphylococcus aureus* (MRSA). Because alternative treatments are limited, development of resistance to vancomycin can make treatment of MRSA infections increasingly difficult. Fortunately, only 7 cases of vancomycin-resistant *S. aureus* (VRSA) infection, which is always methicillin-resistant, have been reported in the United States (Table) (*1*); 5 of these cases occurred in Michigan. We report 2 additional cases of VRSA that occurred in Michigan in 2007. The Michigan Department of Community Health (MDCH) examined the patients' records, compared genetic characteristics of isolates, assessed possible transmission to contacts, and assessed infection control practices at facilities providing patient care.

The Cases

From each patient's medical records, we collected information about demographics and concurrent illness, antimicrobial drug history, history of prior MRSA and vancomycin-resistant *Enterococcus* spp. (VRE) infections, and VRSA site co-infections. Initial isolate identification and antimicrobial drug susceptibility testing were conducted by 2 independent Michigan hospitals. Confirmatory organism identification by conventional biochemical methods and antimicrobial drug susceptibility testing were performed by MDCH's Bureau of Laboratories (*2*,*3*). Vancomycin resistance is defined as MIC ≥16 μg/mL (*4*). Isolates were submitted to the Centers for Disease Control and Prevention (CDC) for PCR testing for *van* genes, which encode vancomycin resistance, and for genetic analysis by pulsed-field gel electrophoresis (PFGE) and plasmid restriction digest to compare with other VRSA isolates (*5*–*7*).

By following the CDC guide for investigating and controlling VRSA (*8*), we defined periods of potential transmissibility. The length of this period is flexible: start date depends on recent culture results, patient care settings, and clinical assessment; end date is determined by 2 negative cultures, which are submitted weekly posttherapy. To develop a list of potential patient contacts, we assessed healthcare visits, community activities, and personal acquaintances from this period. Contacts were then screened for VRSA, starting with persons who had had the most extensive contact (*8*). Swabs of bilateral anterior nares and open wounds were collected from each contact and spread onto blood agar (TSA with sheep blood) and mannitol salt agar (both from Remel, Lenexa, KS, USA). Plates were incubated for 72 h at 35°C and then for 72 h at room temperature; results were reported as negative when no growth occurred after incubation at these conditions. Serial swabs were collected from contacts who had ongoing exposure. Infection control practices were assessed at all facilities that had provided care to each patient.

On October 12, 2007, VRSA and MRSA were cultured from a right plantar foot wound of a 48-year-old patient (patient 1) who had a history of insulin-dependent diabetes, chronic foot ulcers, and prior concurrent MRSA and VRE infections. The patient had recently received vancomycin and ceftriaxone for 7 months to treat osteomyelitis of the right metatarsals. The patient's VRSA infection was treated with linezolid and meropenem for 15 weeks. Final VRSA-negative posttreatment swabs were collected on February 26, 2008. The investigation was closed 134 days

Table. Vancomycin-resistant *Staphylococcus aureus* isolates detected in the United States, 2002–2006

Isolate no.	State	Date isolated
1	Michigan	2002 Jun
2	Pennsylvania	2002 Sep
3	New York	2004 Mar
4	Michigan	2005 Feb
5	Michigan	2005 Oct
6	Michigan	2005 Dec
7	Michigan	2006 Oct

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after initial VRSA-positive culture (Figure 1).

On December 13, 2007, VRSA, VRE, and *Citrobacter youngae* were cultured from a left plantar foot wound of a 54-year-old patient (patient 2) who had inadequately controlled insulin-dependent diabetes. This patient had no documented history of MRSA infection and had recently received vancomycin and levofloxacin for 4 weeks to treat osteomyelitis of the left metatarsals. The patient's VRSA infection was treated with daptomycin for 6 weeks. Final VRSA-negative posttreatment swabs were collected on March 4, 2008. The investigation was closed 81 days after initial VRSA-positive culture (Figure 1).

The VRSA isolates from each patient were highly resistant to vancomycin (each MIC 1,024 μg/mL) but susceptible to daptomycin, linezolid, quinupristin/dalfopristin, rifampin, tetracycline, and tigecycline. The isolate from patient 1 was additionally susceptible to chloramphenicol. Isolates from both patients were resistant to trimethoprim/sulfamethoxazole, whereas the 7 VRSA isolates tested previously had been susceptible. VRSA isolates from the 2 patients were PCR positive for the *vanA* gene, 1 of the 7 *van* genes that encode vancomycin resistance. PFGE results for both isolates differed from all other US VRSA isolates (data not shown). VRSA isolates from the 2 patients reported here had distinct plasmids (Figure 2), and plasmid restriction patterns of these isolates differed from the other 7 US VRSA isolates (*7*).

The period of potential transmissibility for patient 1 began October 5, a total of 7 days before the date of positive culture, because of possible exposures during a recent hospitalization; the period ended February 26. The period of potential transmissibility for patient 2 began December 13, the date of positive culture, and ended March 4 (Figure 1). Contacts for patient 1 were evaluated at 7 locations and for patient 2 at 5 locations. For patient 1, a total of 111 swabs were collected from 75 (99%) of 76 identified contacts; 19 (25%) contacts were positive for *S. aureus*; 5 (7%) were positive for MRSA**.** For patient 2, a total of 140 swabs were collected from 126 (98%) of 128 identified contacts; 40 (32%) contacts were positive for *S. aureus,* 13 (10%) were positive for MRSA. No contacts of either patient were positive for VRSA. No infection control breaches were identified.

Conclusions

These 2 recent cases are consistent with cases reported in the review by Sievert et al. (*1*): each patient had substantial underlying concurrent conditions that contributed to the illnesses, genetic analysis of these isolates indicates that VRSA was not transmitted to or from another known VRSA patient, and no identified transmission occurred from patients to contacts. Also consistent with most previous cases, each patient reported here had a history of VRE and of vancomycin use ≤3 months before VRSA infection. However, patient 2 did not have a documented history of MRSA infection or colonization. Given the patient's history of diabetes and chronic foot wounds, MRSA might have been present but undiagnosed.

Data from the other 7 US cases support the hypothesis that patients at risk for VRSA are co-infected or co-colonized with VRE and MRSA, which enables transfer of the *vanA* gene from VRE to MRSA in a biofilm environment, resulting in a VRSA strain. Despite attempts, only 1 laboratory has reported in vitro transfer of vancomycin resistance from VRE to *S. aureus*, demonstrating that interspecies transfer is not frequent (*9*). However, in vitro transfer of vancomycin resistance from VRSA to *S. aureus* has been demonstrated, reinforcing concerns about potential intraspecies transfer of vancomycin resistance among staphylococci (*10*).

Although VRSA infection continues to be rare and no transmission has been identified, it remains a serious public health concern, especially in Michigan where 7 of the 9 US cases have occurred. MDCH continues to educate healthcare providers about correct infection control strategies (*11*) and prudent antimicrobial drug use. MDCH's Bureau of Laboratories provides guidance to hospitals on methods of VRSA detection. MDCH field staff educate patients and their household contacts about wound care, hand and personal hygiene, and the importance of regular monitoring and control of diabetes, a common underlying condition with VRSA infection. Despite these efforts, questions

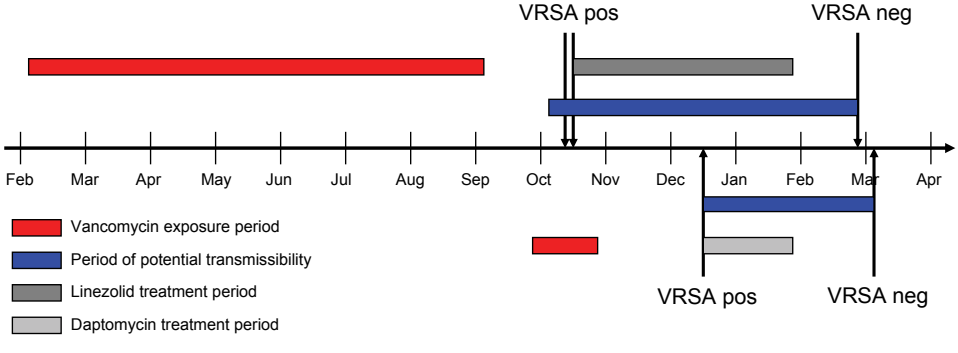

Figure 1. Vancomycin-resistant *Staphylococcus aureus* (VRSA) culture, treatment, and period of potential transmissibility timelines, 2 patients, February 2007–April 2008. Top, patient 1; bottom, patient 2; pos, positive; neg, negative.

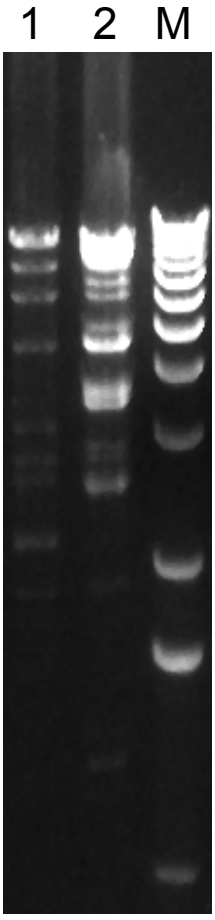

Figure 2. Restriction enzyme (*Hind*III) digest of plasmids prepared from vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates from 2 patients in Michigan, USA, 2007. Each lane is labeled with the VRSA isolate number; lane M, 1-kb molecular marker.

remain unanswered, including why 7 of the 9 US VRSA cases occurred in Michigan. Before targeted prevention strategies can be developed, more research is needed to improve understanding of the microbiologic, clinical, and epidemiologic risk factors for VRSA.

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Rabies in Ferret Badgers, Southeastern China

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Ferret badger–associated human rabies cases emerged in China in 1994. We used a retrospective epidemiologic survey, virus isolation, laboratory diagnosis, and nucleotide sequencing to document its reemergence in 2002–2008. Whether the cause is spillover from infected dogs or recent host shift and new reservoir establishment requires further investigation.

Rabies is an acute encephalomyelitis caused by rabies or rabies-related viruses. Although dogs are the main reservoir worldwide, all mammals are believed to be susceptible. When rabies is widely distributed, affected wildlife may constitute a public health threat to local residents. For example, the Chinese ferret badger (*Melogale moschata*) has been associated with human rabies for several years, although diagnoses have not been confirmed (*1*–*4*). Rabies has also been reported in other subspecies, such as honey badgers (*Mellivora capensis*) and European badgers (*Meles meles*) in Africa and Europe. Transmission was presumed to occur independently among the population or as spillover from other reservoirs, such as jackals, dogs, or foxes (*5*,*6*). However, none of these animals have been reported to be associated with human deaths. The Chinese ferret badger, which dwells mainly in southeastern China, is a different subspecies than the badgers in Africa and Europe. These mustelids have several names in southern China—crab-eating mongoose, rice field dog, viviparid-eating dog, loach-eating dog, and white face weasel—mainly because of their omnivorous behavior and external appearance. Recently, human rabies associated with Chinese ferret badgers has seemed to reemerge.

Because the People's Republic of China has no governmental surveillance network, few data exist on wildlife rabies in China, and therefore the natural behavior and habitats of Chinese ferret badgers are not clear (*7*). Most background information about this animal species in this report was obtained from local hunters. Chinese ferret badgers are solitary and nocturnal. Those observed during daylight are usually sick. The animals are distributed widely in China but are concentrated mainly in Anhui, Zhejiang, and Jiangxi provinces (Figure 1). However, the detailed population density of the badgers is largely unknown.

Ferret badger–associated human rabies cases in China were first reported in 1997 but had actually emerged in 1994 (*1*). During that year, 6 patients with clinical signs of rabies received a preliminary diagnosis at Huzhou Second Hospital, Huzhou District, Zhejiang. In 1995, a similar case was reported in the same hospital. Among the 7 case-patients, 6 were reported to have been bitten on the hands by ferret badgers. This could be the first alleged epizootic of ferret badger–associated human rabies. From 1999 through 2003, 4 ferret badger–associated human rabies cases were reported in Huzhou, and 14 cases were reported in Hangzhou (*8*,*9*), the capital district of Zhejiang. In 2004, 1 human case in Huzhou and 3 human cases in Hangzhou were recorded (*10*,*11*). From 1994 through 2004, 12 (60%) of 20 human rabies cases in Huzhou, and 17 (77%) of 22 human rabies cases in Hangzhou were associated with ferret badger exposure. Ferret badger–associated human rabies in the western counties of Hangzhou were frequently reported in local news (http://zjajcdcsy.zjwst.gov.cn/col71/info.htm1?infoid=605, http://news.sina.com.cn/c/2003-07-08/09391300011.shtml, www.zj.xinhua.org/old/200212/4/100021681.htm, and www.jksoso.com/html/0F1A6B60.htm).

In Jing County, which is located in eastern Anhui and is adjacent to the western border of Zhejiang, 3 human rabies cases associated with ferret badger bites were reported

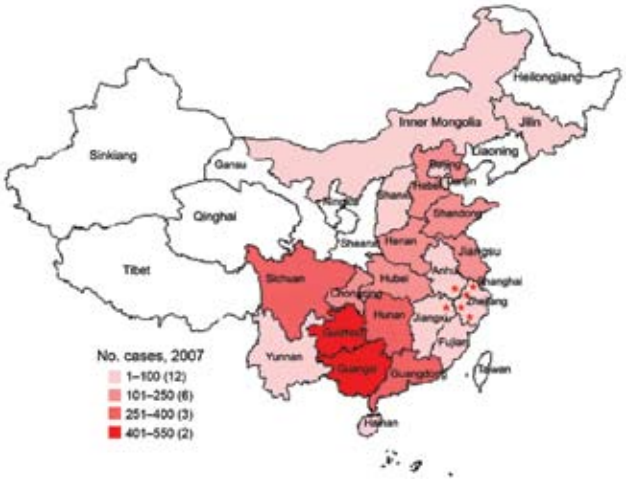

Figure 1. Distribution of human rabies cases in mainland China, 2007. Red stars indicate ferret badger–associated human rabies cases. Numbers in parentheses in key indicate number of affected provinces.

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successively in 1999, 2000, and 2001 (*4*,*12*). An incorrect photograph of the ferret badger was cited in a previous brief report (*3*).

To determine whether ferret badger–associated rabies is reemerging in China, we conducted a retrospective epidemiologic survey in the affected regions from 2002 through 2008. To document ferret badger–associated rabies, we used virus isolation, laboratory diagnosis, and nucleotide sequencing.

The Study

During 2002–2004, many sick badgers were seen at the bases of mountains, on village roads, and within residential houses. At the same time, rabies in livestock was reported in the nearby villages. Concomitantly, the highest number of human rabies cases was recorded during that period. Local residents stated that dead animals were seen everywhere; however, accurate numbers and distribution of affected animals in these areas were difficult to estimate.

Table 1. Rabies fluorescent antibody virus neutralization assay results of ferret badger serum samples, China

Date	No. samples (no. positive)	IU/mL*
2005–2007	63 (0)	0
Apr–Jul 2008	30 (5)	0.20, 0.33, 0.45, 0.5, 0.8

*Arbitrary cut-off value for seroconversion is 0 IU/mL.

During 2005–2007, ferret badger hunters were recruited to help capture the animals for further investigation; 1–2 badgers were captured each week. The badgers were no longer commonly seen in the fields, probably the result of depopulation by the disease. Among the 58 specimens collected in Lin'an, Chun'an, and Jiande counties of Hang-

Table 2. Rabies virus isolates or strains used to construct phylogenetic tree (Figure 2)*

Isolate or strain	GenBank accession no.		Region of origin	Host	Year isolated
	Nucleoprotein	Glycoprotein			
BD06	EU549783	EU549783	Hebei	Dog	2006
CTN-33	DQ787145	DQ767896	Shangdong	Dog	1957
GC07	EU828655	EU828656	Hebei	Dog	2007
GN07	EU828653	EU828654	Guangdong	Dog	2007
Guangxi_YL66	DQ666287	EU267744	Guangxi	Dog	2006
Guizhou_A10	DQ666288	EU267745	Guizhou	Human	2004
Guizhou_A103	DQ666290	EU267747	Guizhou	Dog	2004
Guizhou_Qx5	DQ666296	EU267751	Guizhou	Dog	2004
GX01	DQ866105	NA	Guangxi	Dog	2006
GXWXp	DQ866121	NA	Guangxi	Dog	2006
Hebei0(H)	EU267777	EU267752	Hebei	Human	2007
Henan_Hb10	DQ666297	EU267753	Henan	Dog	2004
Henan_Sq59	DQ666306	EU267759	Henan	Dog	2004
Hubei070308	EF611081	EF643518	Hubei	Buffalo	2007
Hunan_DK13	DQ666307	EU267762	Hunan	Dog	2004
Hunan_Wg12	DQ666308	EU267763	Hunan	Dog	2004
Hunan_Xx33	DQ666317	EU267769	Hunan	Dog	2004
Jiangsu_Wx1-06	DQ666321	EU267773	Jiangsu	Dog	2004
Jiangsu_Wx0(H)	DQ666320	EU267772	Jiangsu	Human	2004
MRV	DQ875050	DQ875050	Henan	Mouse	1987
WJ07-1	EU828657	EU828658	Hebei	Dog	2007
Yunnan_Md06	EU095330	EU253477	Yunnan	Dog	2006
Yunnan_Qj07	EU275245	EU275240	Yunnan	Dog	2007
Yunnan_Tc06	EU275243	EU275242	Yunnan	Dog	2006
Zhejiang Wz0(H)	EF556197	EF556198	Zhejiang	Human	2007
ZJ-LA	**FJ598135**	**FJ719756**	**Zhejiang**	**Ferret badger**	**2008**
ABLV	NC003243	AF006497	Australia	Bat	1996
ERA	AF406695	EF206707	France	Vaccine strain	2003
HEP-Flury	AB085828	AB085828	Japan	Vaccine strain	2003
Mokola	NC006429	NC006429	France/USA	Bat	1997
Nishigahara	AB010494	AB044824	Japan	Vaccine strain	1998
Ni-CE	AB128149	AB128149	Japan	Vaccine strain	2007
PV	M13215	M13215	France	Vaccine strain	1993
RC-HL	D16331	D16330	Japan	Vaccine strain	1994
SAD-B19	M31046	M31046	USA	Vaccine strain	1990
SHBRV-18	AY705373	AY705373	USA	Bat	1996
SRV9	AF499686	AF499686	Clone of SAD-B19	Vaccine strain	2006

*NA, not available; **boldface** indicates the isolate reported in this article.

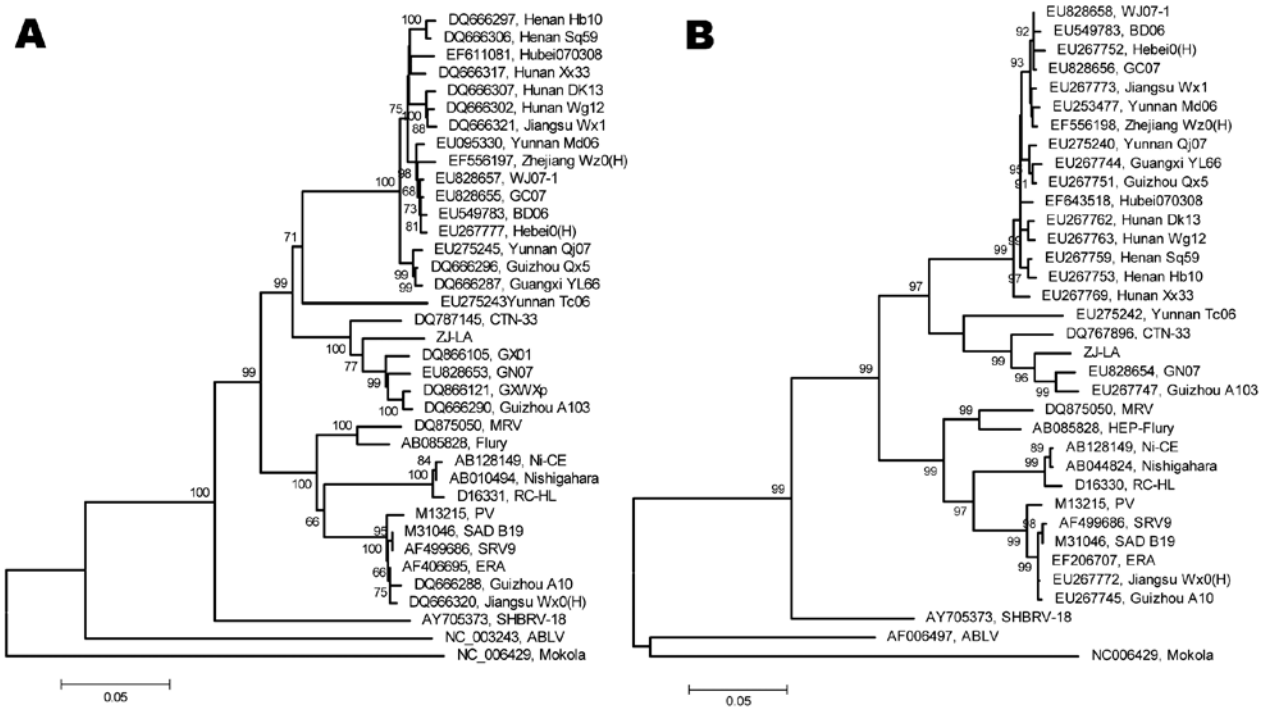

Figure 2. Position of a ferret badger–associated rabies virus isolate (ZJ-LA) in a phylogenetic tree constructed based on the nucleoprotein genes from representative dog rabies virus isolates and common vaccine strains in China (A) and the glycoprotein genes from representative dog rabies virus isolates and common vaccine strains in China (B). This figure was drawn by MEGA 4 (www.megasoftware.net) with maximum composite likelihood model. Bootstrap values are calculated from 1,000 repetitions. Scale bars represent phylogenetic distance between isolates.

zhou, none of the brain tissue samples were positive for rabies by standard direct fluorescent assay. Serum samples from the 63 animals captured in the 3 counties mentioned above did not have detectable rabies virus–neutralizing antibodies according to the fluorescent antibody virus neutralization test (Table 1).

During 2007–2008, the population of the ferret badgers in the same regions seemed to recover, and rabies infection in badgers began to increase. Since the summer of 2008, sick and dead badgers have been seen by local residents inside houses, in the fields close to the residential areas, and on the roads in Hangzhou District. Of the 71 brain samples collected in 2008, 4 had positive direct fluorescent assay results. Of 30 serum samples, 5 had positive results for rabies virus–neutralizing antibody (Table 1). In addition, a human rabies case was recorded in April 2008 in Lishui County, Zhejiang. Our most recent retrospective epidemiologic investigation of human rabies cases from the end of 2007 through 2008 showed that in Wuyuan County, Jiangxi, adjacent to Hangzhou, Zhejiang, 4 of 5 recorded human rabies cases were caused by badger bites.

Phylogenetic analysis using the nucleoprotein and the glycoprotein genes (Table 2) demonstrated that the ferret badger rabies virus isolate (ZJ-LA, isolated from a badger in Lin'an County of Hangzhou, Zhejiang) had 89.0% homology with a local dog rabies virus isolate (Zhejiang Wz0) and overall 86.5%–95.9% homology with other isolates from China (Figure 2). The ZJ-LA strain had the highest homology with a dog rabies virus isolate (GN 07, from Guangning County, Guangdong Province) and a vaccine strain CTN-33 (originally from a person who died of rabies in Ji'nan, Shandong Province, in 1957). Because dog-associated human rabies has been reported only sporadically in Zhejiang Province, whether the ferret badger–associated rabies is a spillover event from dogs, or the animals now serve as a natural reservoir in the rabies-endemic area, needs further investigation.

Conclusions

Rabies in ferret badgers occurred during 2 alleged epizootics (1994–1995 and 2002–2004) in southeastern China (Figure 1) (*13*). Our preliminary data suggest another probable epizootic of rabies in ferret badgers during 2007–2008. Rabies in ferret badgers is becoming a greater public health threat to humans in eastern Anhui, middle to western Zhejiang, and northern Jiangxi provinces in China.

Because no practical rabies vaccine has been developed for wildlife in China, a rabies epidemic in ferret badgers is almost inevitable without intervention, and the threat to public health is immediate. Lack of communication and

cooperation among the Chinese Center for Disease Control and Prevention, Ministry of Agriculture, and wildlife services from the Bureau of Forestry makes the situation more complicated than canine rabies control. Whether rabies in ferret badgers is a spillover event from rabid dogs or whether ferret badgers serve as a natural reservoir remains to be addressed. In addition to more detailed epidemiologic investigations, control and elimination of rabies in dogs is a primary suggestion to test the latter hypothesis.

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Nipah Virus Infection in Dogs, Malaysia, 1999

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The 1999 outbreak of Nipah virus encephalitis in humans and pigs in Peninsular Malaysia ended with the evacuation of humans and culling of pigs in the epidemic area. Serologic screening showed that, in the absence of infected pigs, dogs were not a secondary reservoir for Nipah virus.

During September 1998–April 1999, a viral disease associated with pigs resulted in at least 265 human cases of febrile encephalitis in Peninsular Malaysia; case-fatality ratio was 38% (*1*). The etiologic agent, Nipah virus (NiV; family *Paramyxoviridae,* genus *Henipavirus*), is believed to have entered pig populations in Perak state, central Malaysia, from a fruit-bat reservoir (*2*) before spreading by transport of pigs among farms. Bukit Pelanduk, Negeri Sembilan state, and adjoining Sepang, Selangor state, had the largest number of cases. The epidemic in that region was controlled by cessation of animal movement, destruction of pigs on affected farms, public education, use of personal protective equipment, and evacuation of humans from and quarantine of farms and villages within the epidemic area (*3*).

Although humans were most frequently infected after contact with live pigs (*4–6*), 8% of patients reported having had no direct contact with pigs, which suggests other sources of transmission to humans. One study reported an association with sick or dying dogs; case-patients were more likely than controls to report an increase in the number of sick or dying animals, including dogs (*5*).

During the outbreak, evidence of NiV infection was found in domestic animals such as goats and cats, but especially dogs (*2*). NiV infection was confirmed by immunohistochemical examination of 1 dead and 1 dying dog from the epidemic area. Both showed histologic evidence of severe disease (*7*). After pig populations were destroyed but before residents were allowed to return to their homes in the epidemic area, studies were undertaken to determine whether domestic animal populations maintained active infection in the absence of infected pigs. Dogs were especially suspected because they live commensally with both pigs and humans. Many dogs sampled from the Bukit Pelanduk and Sepang epidemic area around the time of the pig culling had antibodies to NiV or a Nipah-like virus (*2*). To test the hypothesis that NiV was being transmitted from dog to dog, we looked for evidence of the spread of infection among dogs outside the immediate disease-endemic area.

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The Study

The disease-epidemic zone in southwestern Malaysia was an area of small pig farms associated with a cluster of small towns in the states of Negeri Sembilan (Bukit Pelanduk, Sungai Nipah, Kampong Sawah) and adjacent Selangor (Sepang) (Figure). For 3 days (May 11–13, 1999), stray and pet dogs were sampled along 2 transects following major paved roads, through rural areas, leading from the periphery of the recognized disease-epidemic area (Figure). On days 1–3, samples were collected within 15–20, 8–15, and 0–8 km, respectively, of each transect.

Sampling followed 2 methods. For household pets, blood samples were collected from the dogs, geographic coordinates of the house were measured by using a geographic positioning system, and owners were asked about the animal's potential exposure history (e.g., where it was kept, whether it was allowed to roam, health, diet, any presence on a pig farm, and if it had been sick during the previous 12 months). Free-roaming stray dogs without collars were killed by animal control personnel following routine protocols for rabies control and returned to a field laboratory in Bukit Pelanduk for sampling. Geographic coordinates and form-directed data (sex, age, apparent health) were recorded for each stray. A veterinary team collected blood and tissue samples (spleen, kidney, liver, lung) from each animal.

For comparison, blood samples from 109 dogs from the Kuala Lumpur area (29 from veterinary clinics, 19 from pounds, and 61 stray dogs) were collected. Hendra virus (HeV) is highly cross-reactive with NiV and was successfully used for initial screening in humans and animals during the outbreak (*8*). Samples were tested by using an indirect immunoglobulin (Ig) G ELISA with HeV antigen as described (*9*).

During the 3 days, 249 dogs were sampled; 161 blood samples were from pets, and 88 blood and tissue samples were from stray dogs. The daily number of samples increased along both transects as the study progressed, reflecting the improved efficiency of sampling teams (Figure).

Of the 249 blood samples, 4 (1.6%; 2 from each transect) had detectable antibodies reactive with HeV. The 56 blood samples from animals 15–20 km from the epidemic area had no detectable antibodies. There was 1 antibody-positive animal in the 8–15 km zone and 3 in the 0–8-km zone (Figure). Of the 4 antibody-positive dogs, 3 were pets from the 0–8-km zone. The antibody-positive stray was from the 8–15-km zone. The 109 blood samples from pet and stray dogs in Kuala Lumpur were HeV antibody negative.

Conclusions

The finding that all 109 dogs from Kuala Lumpur (including at least 29 vaccinated pets) were antibody negative indicated that the immunoassay did not detect antibody elicited by common canine vaccines (canine distemper, hepatitis [adenovirus type II], parainfluenza, leptospira, and canine parvovirus) or other common paramyxovirus infections. The absence of NiV antibody in dogs >15 km from the epidemic area and the low prevalence in populations nearer the epidemic area provides evidence that the virus was not spreading by dog-to-dog transmission and that the dog population was not acting as an amplifying reservoir for NiV in the absence of infected pigs. In addition, there were no reports of unusual numbers of dead or sick dogs outside the immediate disease-endemic area.

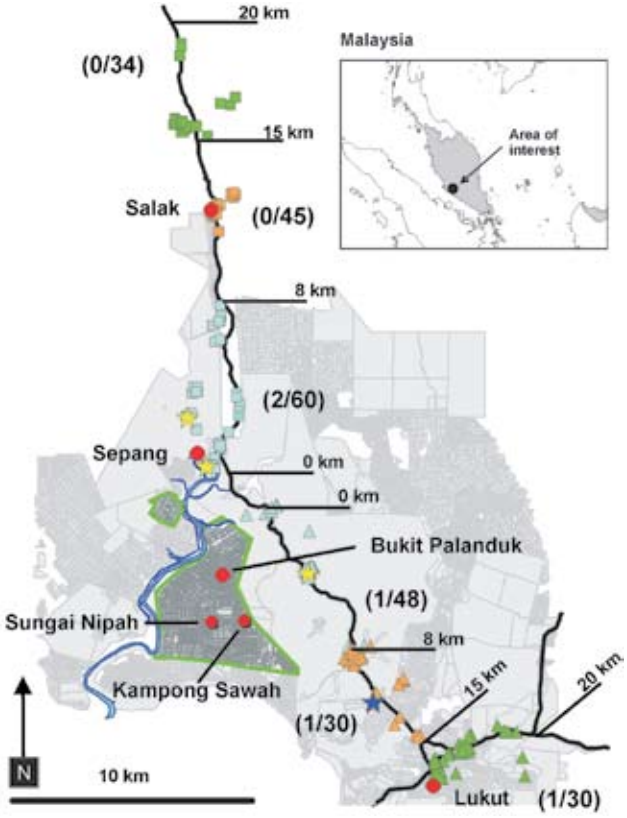

Figure. Sampling locations for 161 pet and 88 stray dogs along 2 transects that followed major roads leading north (squares) and southeast (triangles) from the Nipah virus encephalitis disease-epidemic areas in Bukit Pelanduk and Sepang, Malaysia. Color-coded squares and triangles refer to the transect interval where they were taken (light blue, 0–8 km; orange, 8–15 km; green, 15–20 km). The recognized disease-epidemic areas in pigs and humans are outlined by thick green lines. Sampling sites of Nipah virus antibody–positive dogs are indicated by stars (yellow, pet; blue, stray). Numbers in parentheses beside each transect indicate number antibody positive/number tested from each transect. Distances are in road kilometers; however, all antibody-positive animals were sampled within 5-km linear distances from the epidemic area.

Infected pigs in the area of the epidemic were destroyed March 1–April 16, 1999. Prevalence of NiV antibody in 63 dogs from within this area April 3–14, 1999, was 57%. Prevalence in 19 dogs from the same area April 23–May 4, 1999, was 26% (unpub. data). Although this reduction in prevalence is not significant ($p = 0.23$, 2-tailed Fisher exact test), it suggests that the virus was not being rapidly transmitted among dog populations after destruction of the pigs. Some of the animals that had been exposed to infected pigs died or were killed and were probably replaced by uninfected immigrant dogs. Alternatively, infection in the dog population may have been local and patchy, and the apparent temporal differences in prevalence may reflect geographic sampling bias. Dogs sampled during the earliest study period were taken by patrolling animal control personnel. The exact locations of sampling are unknown, but they were areas abandoned by humans.

The greater number of infected pets than strays reflects their greater representation in the sampled population. Antibody prevalences (3/161 [1.9%] for pets vs. 1/88 [1.1%] for strays) did not differ significantly ($p = 1.00$, 2-tailed Fisher exact test).

Of the 3 antibody-positive pet dogs, none was reported to have been sick within the past year, 2 were "always" allowed to roam free, and the third was "rarely" allowed to roam free. However, the owners of the third dog reported feeding it "pig bones." We believe that these animals could have become infected through direct contact with infected pigs or by eating uncooked pork products. Some animals classified as strays may have been pets that were allowed to roam free without collars.

We cannot exclude the possibility that dogs may have remained infectious for some period after infection or that other dogs or even humans may have become infected through contact with infected dogs. However, our results indicate that such infection was rare and was insufficient to maintain and spread NiV in dog populations in the absence of infected pigs.

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Methicillin-Resistant *Staphylococcus aureus* USA300 Clone in Long-Term Care Facility

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We performed a longitudinal analysis of 661 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates obtained from patients in a long-term care facility. USA300 clone increased from 11.3% of all MRSA isolates in 2002 to 64.0% in 2006 (p<0.0001) and was mostly recovered from skin or skin structures (64.3% vs. 27.0% for non-USA300 MRSA; p<0.0001).

Since 2001, a dramatic increase in methicillin-resistant *Staphylococcus aureus* (MRSA) infections has been observed in the United States, mostly related to emergence of the USA300 clone in the community (*1*) and subsequently in hospitals (*2*,*3*). Residents of long-term care facilities (LTCFs) are at risk for colonization with antimicrobial drug–resistant bacteria, including MRSA. After they have been colonized, these residents are at increased risk for infections (*4*). Although it is assumed that transfer of patients between acute-care hospitals and LTCFs provides an ongoing cycle for the introduction of MRSA between these facilities (*5*), few studies have described the molecular epidemiology of MRSA in LTCFs. We report the prevalence and distribution of MRSA genotypes among clinical isolates obtained over a 10-year period (1997–2006) at the main LTCF in San Francisco, California, USA.

The Study

The San Francisco Laguna Honda Hospital is a 1,000-bed LTCF. We conducted a retrospective review of electronic records for all cultures positive for *S. aureus* that originated from hospital residents during 1997–2006. Colonization screening was not performed during the study period. Data collection was approved by the Committee on Human Research, Office of Research Administration, at the University of California, San Francisco.

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Isolates were tested for oxacillin resistance by the salt agar method, and the presence of the *mec*A gene was confirmed by PCR. Susceptibility to other antimicrobial drugs was determined by using microbroth dilution with the MicroScan WalkAway 96 instrument (Dade Behring, Deerfield, IL, USA). Inducible clindamycin-resistance testing (D-zone test) was performed by using the agar disk-diffusion method for isolates obtained during 2005–2006. Results were interpreted in accordance with guidelines (M7-A5) of the Clinical and Laboratory Standards Institute (Wayne, PA, USA; www.clsi.org).

Nonduplicated MRSA isolates were genotyped by pulsed-field gel electrophoresis (PFGE) after digestion of chromosomal DNA with *Sma*I (*6*), *spa* typing (*7*), and multilocus sequence typing (MLST) (*8*). USA300 was defined by the presence of Panton-Valentine leukocidin (PVL) genes (*luk*F-PV and *luk*S-PV) and the arginine catabolic mobile element (ACME), detected by PCR. Staphylococcal cassette chromosome *mec* (SCC*mec*) type was identified by using a PCR-based protocol (*9*). All strains underwent *spa* typing and were tested for PVL and ACME genes. MLST was conducted for 12 strains that could not be characterized otherwise.

Chi-square tests were used for bivariate analysis, and χ^2 tests for trend were used to evaluate secular trends. All statistical analysis was conducted by using Stata version 9.1 (Stata Corp., College Station, TX, USA).

S. aureus was isolated from 1,284 patients. Of these isolates, 744 (57.9%) were MRSA. The proportion of MRSA among *S. aureus* isolates increased from 38.1% (56/147) in 1997 to 72.3% (99/137) in 2006 (p<0.0001; Figure 1). Median age (interquartile range [IQR]) was 67 years (54–78 years) for patients with MRSA and 64 years (49–78 years) for those with methicillin-susceptible *S. aureus* (MSSA) (p = 0.22). Male:female ratio was 1:2.2 for MRSA and 1:1.7 for MSSA (p = 0.12). For MRSA specimen sources, we observed a decrease in the proportion of urinary or respiratory specimens from 69.7% (131/188) during 1997–2000 to 49.5% (236/477) during 2001–2006, and a concomitant increase in the proportion of skin or skin structure specimens from 22.3% (42/188) to 42.8% (204/477) (p<0.0001; Figure 2). No change in specimen sources was observed for MSSA.

Three PFGE clonal groups (USA100 [ST5, SCC*mec* type II], USA300 [ST8, SCC*mec* type IV], and USA500 [ST8, SCC*mec* type IV]) accounted for 85.2% (563/661) of genotyped MRSA isolates. USA300 was first isolated in 2001 and accounted for 11.3% of all MRSA (7/62) in 2002, 30.1% (25/83) in 2003, 47.9% (45/94) in 2004, 49.5% (49/99) in 2005, and 64.0% (55/86) in 2006 (p<0.0001;

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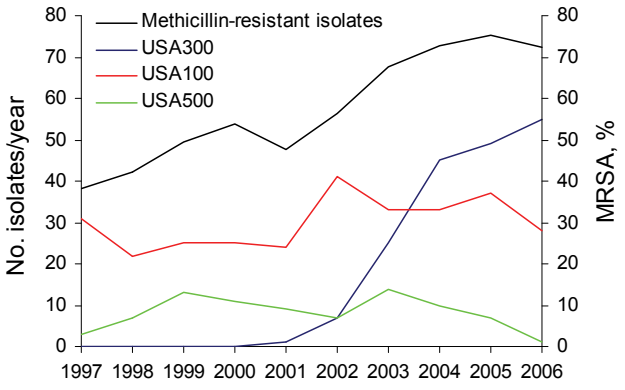

Figure 1. Longitudinal dynamics of methicillin resistance and methicillin-resistant *Staphylococcus aureus* (MRSA) clones at a long-term care facility, San Francisco, California, USA, 1997–2006.

Figure 1). Compared with non-USA300 MRSA, USA300 was most frequently isolated from skin or skin structures (64.3% vs. 27.0%, risk ratio 2.38, 95% confidence interval 1.98–2.87, p<0.0001) and was recovered from younger patients with a median age (IQR) of 62 years (51–75 years) vs. 68 years (54–79 years; p = 0.001).

Among all MRSA isolates, gentamicin resistance decreased from 46.9% in 1997 to 4.7% in 2006, and clindamycin resistance decreased from 71.4% to 47.6% for the same years (p<0.0001). Overall, 285/744 (38.3%) MRSA isolates were multidrug resistant (resistant to ≥3 non-β-lactam antimicrobial drugs), including 177/299 (59.2%) USA100, 39/182 (21.4%) USA300, and 32/82 (39.0%) USA500. Inducible clindamycin resistance was detected in 15/281 (5.3%) MRSA isolates during 2005–2006. The rate of multidrug resistance for USA300 was lower than for non-USA300 (p<0.0001) but increased from 0% in 2001 to 14.3% (1/7) in 2002, 12.0% (3/25) in 2003, 13.3% (6/45) in 2004, 24.5% (12/49) in 2005, and 30.9% (17/55) in 2006 (p = 0.015). USA300 was less frequently resistant than non-USA300 to clindamycin (p<0.0001), gentamicin (p = 0.0004), and trimethoprim/sulfamethoxazole (p = 0.0001) and more frequently resistant to tetracycline (p = 0.0002) (Table). Resistance to vancomycin, linezolid, dalbavancin, and daptomycin was not detected.

Conclusions

Increasing incidence of MRSA infections in this LTCF during 1997–2006 is attributable to 2 clonal groups; USA100 predominated until 2003, and USA300 predominated during 2004–2006. Emergence of a new MRSA clone in healthcare facilities may be followed by a decrease in incidence of other MRSA clones (*10*). Unfortunately, this decrease was not observed in this LTCF, where the incidence of the previously predominant clone USA100 remained unabated. As a consequence, emergence of USA300 led to a 2-fold increase in MRSA incidence and an increase to 73% in the rate of methicillin resistance among *S. aureus*. Moreover, because USA300 has a tropism for skin and skin structure infections (*3*), its emergence caused a shift in MRSA specimen sources.

Although primarily encountered in urinary and respiratory specimens until 2001 in this study, as in previous studies performed in LTCFs, MRSA isolates have mostly originated from skin or skin structure since 2002. This finding has important implications for MRSA transmission and should be taken into account when designing infection control policies in LTCFs. For example, nasal decolonization as a means to prevent MRSA infection implies that MRSA reservoirs reside in endogenous sources. However, skin–skin and skin–fomite contact may represent common alternative routes of transmission for USA300 (*11*).

In contrast to reports characterizing USA300 strains as typically not multidrug resistant (*1*), we found that up to 30.9% of USA300 isolates were multidrug resistant. This finding suggests that USA300 isolates were acquired under antimicrobial drug pressure in the LTCF or during a stay in another hospital rather than while in contact with the community. Results from this study and others illustrate the fitness trait of USA300 clonal lineage (*12*). To prevent further spread of multidrug-resistant USA300 in LTCFs would require enhanced infection control policies, which may include isolation or cohorting of infected patients, antimicrobial drug stewardship, and systematic use of alcohol-based handwashing products. However, given that these policies have proven difficult to implement in tertiary care hospitals, it may be even more challenging in LTCFs, which have limited staff and infection control resources (*5*,*13*–*15*).

This study had some limitations. Increased incidence of MRSA-positive cultures could be related to changes in sampling policies in this institution (e.g., more frequent sampling, surveillance cultures). However, the increased incidence rate for MRSA is not likely related to these changes because the annual number of cultures positive for any pathogen gradually decreased over the study period,

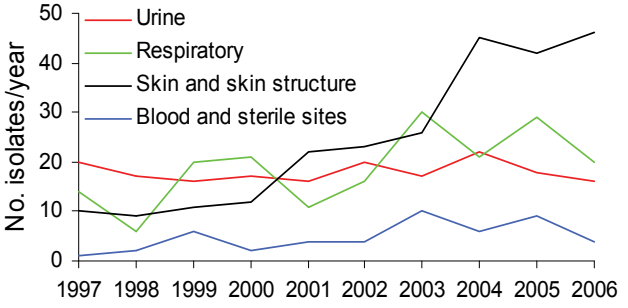

Figure 2. Evolution of methicillin-resistant *Staphylococcus aureus* isolate specimen sources at a long-term care facility, San Francisco, California, USA, 1997–2006.

Table. Analysis of MRSA clones obtained in a long-term care facility, San Francisco, California, USA, 1997–2006*

	Antimicrobial drug resistance, %						Specimen source, %			
Clonal group	Ery	Cli	Cip	Sxt	Gen	Tet	SST	Respiratory tract	Urine	Blood†
USA100 (n = 299)	96.2	84.9	95.9	3.3	16.9	2.6	22.7	36.4	35.1	5.8
USA300 (n = 182)	87.8	15.0	79.4	0.6	6.1	13.2	64.3	18.1	10.4	7.1
USA500 (n = 82)	84.7	27.8	71.7	28.2	20.8	6.0	30.5	28	31.7	9.8
LFT (n = 98)	87.3	65.6	62.8	10.9	13.4	7.8	37.2	26.6	26.6	9.6
All MRSA (n = 744)	91.8	64.6	87.0	7.1	21.6	5.1	32.7	25.1	23.1	6.3

*MRSA, methicillin-resistant *Staphylococcus aureus*; Ery, erythromycin; Cli, clindamycin; Cip, ciprofloxacin; Sxt, trimethoprim-sulfamethozazole; Gen, gentamicin; Tet, tetracycline; SST, skin and skin structure; LFT, low frequency types.
†Blood and other typically sterile sites.

from 1,433 in 1997 to 862 in 2006, as did the incidence of MSSA-positive cultures, from 91 in 1997 to 38 in 2006.

Although our study was limited by its reliance on retrospective data collection, it illustrates the need for further investigations in LTCFs regarding risk factors and appropriate interventions to minimize further transmission of MRSA. LTCFs, long thought to be reservoirs of nosocomial MRSA clones, now emerge as an important reservoir for USA300 and could play a role in the emergence of multidrug-resistant USA300.

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Leishmaniasis, Autoimmune Rheumatic Disease, and Anti–Tumor Necrosis Factor Therapy, Europe

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We report 2 cases of leishmaniasis in patients with autoimmune rheumatic diseases in Greece. To assess trends in leishmaniasis reporting in this patient population, we searched the literature for similar reports from Europe. Reports increased during 2004–2008, especially for patients treated with anti–tumor necrosis factor agents.

We report 2 new cases of leishmaniasis involving patients with autoimmune rheumatic diseases who received anti–tumor necrosis factor (anti-TNF) agents. We also reviewed all similar cases from Europe reported in the literature, and we discuss the implications of leishmaniasis in the setting of anti-TNF therapy, which is associated with increased risk for opportunistic infections (*1*).

The Study

Patient 1, a 55-year-old man who had received a diagnosis of ankylosing spondylitis 7 years previously, was admitted to Laikon Hosptal, Athens, Greece, in May 2005 for evaluation of encrusted vesicular lesions on the face. The lesions were painless but mildly pruritic. The patient had been receiving nonsteroidal antiinflammatory agents until 12 months before admission, when his medications were changed to infliximab (3 mg/kg) plus methotrexate (10 mg weekly) because of his deteriorating clinical condition. He was living in a leishmaniasis-endemic area in Athens, had no pets in his house, and had no history of recent travel abroad. The central scale was removed from one of the lesions, and scrapings from the base of the lesion were stained with Giemsa stain, which showed intracellular amastigotes with peripheral nuclei and rod-shaped kinetoplasts. Results of indirect immunoflorescent antibody (IFA) testing were positive for *Leishmania* parasites (titer 6,400). Infliximab and methotrexate therapy was discontinued, and treatment with liposomal amphotericin B was started at a dose of 3 mg/kg, for days 1 to 5, and 2 additional doses (3 mg/kg) on days 14 and 21. Eighteen months later, treatment with etanercept was begun due to the patient's severe spondyloarthritis; 2 years after the new anti-TNF treatment, he is well, with no signs or symptoms of leishmaniasis.

Patient 2, a 71-year-old woman who had giant cell arteritis, was admitted to the Euroclinic Hospital, Athens, in May 2005 with a high fever and fatigue. The patient had been treated with infliximab (0.25 mg/kg) and variable doses of methylprednisolone for the previous 2 years. Methotrexate (10 mg/week) was added 1 year before admission. She was also living in an Athens suburb, which is leishmaniasis-endemic, and had 4 dogs. Laboratory tests showed a high level of C-reactive protein (163 mg/L, reference range 0–6 mg/L), high erythrocyte sedimentation rate (77 mm/h), pancytopenia (hemoglobin level 12.5 g/dL, leukocyte count 3,300/mm^3, platelet count 122,000/ mm^3), and diffuse hyperglobulinemia. The examination of Giemsa-stained smears from bone marrow aspirate demonstrated abundant *Leishmania* parasites, and IFA was marginally positive for *Leishmania* antibodies (titer 400). PCR was positive for the detection of the *Leishmania* genome in peripheral blood. Infliximab and methotrexate treatment was discontinued, and treatment with intravenous liposomal amphotericin B was started at a dose of 3 mg/kg for 5 days. Two days later, the fever subsided, and within the next few days, the patient recovered from pancytopenia, while the inflammatory markers showed a gradual decrease. She received 2 additional doses of liposomal amphotericin B (3 mg/kg) on days 7 and 14, and by that time, she exhibited no signs or symptoms of visceral leishmaniasis.

We then searched Medline, EMBASE, and Current Contents databases for all reports on leishmaniasis in Europe and the Mediterranean area among patients with autoimmune rheumatic diseases, which are often treated with anti-TNF agents. In our search strategy, we used medical subject heading terms and text words, including rheumatoid arthritis, juvenile rheumatoid arthritis, Still's disease, seronegative arthritis, psoriatic arthritis, Behçet's disease, ankylosing spondylitis, reactive arthritis, vasculitis, giant cell arteritis, Wegener's granulomatosis (ANCA [antineutrophil cytoplasmic antibody]–associated vasculitis), panarteritis nodosa, leishmaniasis, *Leishmania*, and anti-TNF. We searched the reference list of each resulting report for additional publications. We used no language or time restrictions.

All retrieved articles were case reports. We found 13 additional cases of leishmaniasis in patients with autoimmune rheumatic diseases (*2–14*), all published after the introduction of anti-TNF agents in 1998 (Table). All 15 patients (including our 2 patients) were treated in the past or at the time of the diagnosis of leishmaniasis with ≥1

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Table. Detailed characteristics of 15 patients with autoimmune rheumatic disorders in whom leishmaniasis developed, Europe*

Patient no.	Country	Age, y/sex	Disease	Anti-TNF treatment		Other immunosuppressive treatments		Form of *Leishmania* infection	Ref.
				Agent	Duration, mo	Agent(s)	Duration, mo		
1	France	66/M	ANCA-associated vasculitis	NA	NA	Cyclophosphamide, methotrexate, corticosteroids	120	Visceral	(*7*)
2	Israel	56/M	Rheumatoid arthritis	NA	NA	Methotrexate, corticosteroids	120	Cutaneous	(*8*)
3	Italy	35/M	Behçet disease	NA	NA	Chlorambucil, corticosteroids	36	Visceral	(*9*)
4	Spain	50/M	Rheumatoid arthritis	NA	NA	Methotrexate, corticosteroids	120	Visceral	(10)
5	Italy	60/M	Polyarteritis nodosa	NA	NA	Cyclophosphamide, corticosteroids	2	Visceral	(*11*)
6	Spain	55/M	Psoriatic arthritis	Infliximab	9	No details given	300	Visceral	(*2*)
7	Italy	76/M	ANCA-associated vasculitis	NA	NA	Cyclophosphamide, corticosteroids	36	Visceral	(*12*)
8	France	53/F	Rheumatoid arthritis	Infliximab	12	Azathioprine, corticosteroids	12	Visceral	(*3*)
9	Italy	69/F	Rheumatoid arthritis	Adalimumab	25	Methotrexate, corticosteroids	360	Visceral	(*4*)
10	Greece	60/F	Rheumatoid arthritis	Etanercept	18†	Cyclosporine, corticosteroids, anakinra	96	Visceral	(*5*)
11	France	9/F	Juvenile rheumatoid arthritis	NA	NA	Cyclosporine, methotrexate, corticosteroids, anakinra	60	Visceral	(*13*)
12	Greece	45/M	Psoriatic arthritis	Infliximab	60	Methotrexate, corticosteroids	60	Visceral	(*6*)
13	Greece	65/F	Rheumatoid arthritis	NA	NA	Methotrexate	96	Visceral	(*14*)
14	Greece	71/F	Giant cell arteritis	Infliximab	24	Methotrexate, corticosteroids	24	Visceral	This study
15	Greece	55/M	Ankylosing spondylitis	Infliximab	12	Methotrexate	12	Cutaneous	This study

*TNF, tumor necrosis factor; Ref., reference; ANCA, anti-neutrophil cytoplasmic antibody; NA, not applicable.
†All biologic treatments had been terminated 6 mo before leishmaniasis occurred.

standard immunosuppressive agents, including corticosteroids (11/14 [78.5%]) patients for whom treatment details were reported), methotrexate (9/14 [64.3%]), cyclosporine (3/14 [21.4%]), cyclophosphamide (3/14 [21.4%]), azathioprine (2/14 [14.3]), and chlorambucil (1/14 [7.1%]). Seven (46.6%) patients received an anti-TNF agent along with standard immunosuppressive agents. Two of the 15 reported patients had been treated with a recombinant interleukin–1 receptor antagonist (anakinra; Amgen Inc., Thousand Oaks, CA, USA) (*5*,*13*). In most of the patients, visceral leishmaniasis developed (13 patients, 86.6%), while cutaneous leishmaniasis developed in 2 patients (1 was receiving an anti-TNF agent). All patients were living in leishmaniasis-endemic areas of Europe (Figure).

The anti-TNF agents were introduced into clinical practice in 1998, and the first case of leishmaniasis associated with anti-TNF blockade occurred in 2001 (*4*). During the 6-year period (1998–2003), a total of 6 reports were made of leishmaniasis in patients with rheumatic diseases; 1 (16.6%) occurred in a patient treated with an anti-TNF agent. During the ensuing 5 years (2004–2008), 9 cases of leishmaniasis were reported, 6 (66.6%) in patients receiving anti-TNF agents.

The median duration of previous immunosuppressive therapy, before the diagnosis of leishmaniasis, was 60 months (range 2–360 months) for all 15 patients (Table). For the 7 patients who received anti-TNF agents, the median duration of anti-TNF treatment was 18 months (range 9–60 months). Six of these 7 patients were receiving anti-TNF agents when symptoms and signs of leishmaniasis occurred. In 1 patient (*5*), biologic treatments had been discontinued 6 months before the diagnosis of leishmaniasis (Table). Only 1 patient had been tested for antibodies against *Leishmania* spp. before immunosuppressive therapy was begun, and the results were negative (*6*). Therefore, this is the only case with compelling evidence that leishmaniasis was a primary infection and not reactivation of a latent infection.

Figure. Reported cases of leishmaniasis in patients with autoimmune rheumatic diseases in Europe, indicated by stars (1 case from Israel not shown). Dark gray shading, distribution of leishmaniasis; light gray shading, distribution of leishmaniasis vector sandfly. Source: World Health Organization, 2004 (www.who.int/tdr/svc/publications/tdr-research-publications/swg-report-leishmaniasis).

Conclusions

Our data suggest that the introduction of TNF blockade into the clinical practice is associated with increasing reports of leishmaniasis in patients with autoimmune rheumatic diseases who live in leishmaniasis-endemic areas of Europe. Notably, in most reported cases, patients had not received anti-TNF agents but other immunosuppressants. However, all cases of leishmaniasis in patients with autoimmune rheumatic diseases were reported after 1998, the year of introduction of anti-TNF agents, and most (9/15) of the reported leishmaniasis cases occurred during the past 5 years (2004–2008), mainly among patients receiving anti-TNF agents (6 of the 9 patients with leishmaniasis; 66.6%). This increase coincides with the increasing use of anti-TNF agents during the same period, as prescription practice started changing toward treating patients with lower disease activity (*15*). Another indirect piece of evidence that TNF blockade may increase the risk for leishmaniasis is that the median duration of previous anti-TNF treatment before the diagnosis of leishmaniasis was significantly shorter than the median duration of immunosuppressive therapy for all 15 patients (18 vs. 60 months).

Our report has limitations. It is unclear for all cases (with 1 exception) presented in this article whether leishmaniasis was primary infection or reactivation of latent disease. We cannot also exclude the possibility that the concomitant, long-term use of other immunosuppressants, and not the anti-TNF agents per se, played a crucial role in the development of leishmaniasis. Different prescribing patterns of anti-TNF agents might influence the number of cases reported from each disease-endemic European country. However, the small number of reported cases and the lack of data on differences in the anti-TNF prescribing policies do not allow any conclusions to be reached. Finally, due to underreporting, the reported cases may underestimate the real incidence of leishmaniasis among patients with autoimmune rheumatic diseases.

Prospective studies to estimate the incidence of the disease, the impact of risk factors and the need for serologic screening for leishmaniasis before initiation of anti-TNF agents or any other immunosuppressive treatment are clearly needed. This is particularly important since currently only a few patients with autoimmune rheumatic diseases receive anti-TNF agents (*15*). Therefore, the use of anti-TNF treatment is likely going to increase, possibly causing a parallel increase in opportunistic infections such as leishmaniasis.

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Merkel Cell Polyomavirus Strains in Patients with Merkel Cell Carcinoma

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We investigated whether Merkel cell carcinoma (MCC) patients in France carry Merkel cell polyomavirus (MCPyV) and then identified strain variations. All frozen MCC specimens and 45% of formalin-fixed and paraffin-embedded specimens, but none of the non-MCC neuroendocrine carcinomas specimens, had MCPyV. Strains from France and the United States were similar.

Although infectious agents have been recognized as etiologic agents in ≈20% of cancers (*1*), the list of oncogenic infectious agents is limited. A new virus, Merkel cell polyomavirus (MCPyV), recently was discovered in humans with Merkel cell carcinoma (MCC), a relatively rare, aggressive primary cutaneous neuroendocrine carcinoma. Feng et al. (*2*) reported PCR detection of MCPyV in most MCC specimens (*2*), and clonal integration of the viral genome has been identified.

Polyomaviruses are small nonenveloped DNA viruses, with a double-stranded circular DNA genome of ≈5 kb packaged within a capsid 45–50 nm in diameter and composed of 3 proteins: VP1, VP2, and VP3 (*3*). Twenty members of the polyomavirus family have been identified in mammals and birds (*4*). Four viruses, including the ubiquitous BK and JC viruses, which cause persistent or latent infections, infect humans. Although BK virus, JC virus, and simian virus 40 are tumorigenic in experimental animals and can transform mammalian cells in vitro, no convincing epidemiologic evidence exists for their role in human cancers. We investigated whether patients in France who had MCC carry MCPyV and aimed to identify the strain variations.

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The Study

We conducted our study in 2008 on samples collected during 1991–2008. The study comprised 39 patients with MCC (50–93 years of age, mean 76.9 years; sex ratio 0.95 [19 men, 20 women]). Formalin-fixed and paraffin-embedded (FFPE) tissue specimens from 27 patients and frozen resection specimens from 12 other patients were investigated for MCPyV. In addition, frozen tissue from 8 patients with non-MCC high-grade neuroendocrine carcinomas (5 small-cell lung carcinomas and 3 well-differentiated intestinal carcinomas) and an FFPE tissue specimen from a patient with high-grade neuroendocrine carcinoma of the cervix (human papillomavirus 16 DNA positive) were investigated for MCPyV (43–79 years of age, mean 54.0 years; sex ratio 0.80). All tissue samples were collected for diagnostic purposes, and participants gave written consent in accordance with French ethics regulations.

For DNA preparation from FFPE tissues, 8–10 consecutive sections were subjected to deparaffinization, tissues were then lysed by proteinase K, and DNA was purified by phenol-chloroform extraction. For DNA preparation from frozen specimens, tissue was directly treated with proteinase K and processed as above.

MCPyV was detected by nested PCR by using a first PCR amplification with the LT1 and VP1 primer sets published by Feng et al. (*2*). PCR was performed with 31 cycles for each primer set. A second run of amplification was performed with 2 nested pairs of primers (LT1n, forward 5′-GGCATGCCTGTGAATTAGGA-3′ and reverse 5′-TGTAAGGGGGCTTGCATAAA-3′; and VP1n, forward 5′-TGCAAATCCAGAGGTTCTCC-3′ and reverse 5′-GCAGATGTGGGAGGCAATA-3′) with PCR products from the first round of amplification. Amplification products were subjected to electrophoresis, stained with ethidium bromide, and examined under UV light. To avoid false-negative results from unsuitable DNA quality, a seminested PCR with β-globin primers was run. The first PCR was performed with primers PC04/GH20, and the second PCR used primers PC04/PC03 (*5*). Water was used as PCR-negative controls, and a DNA extract from frozen tissue from an MCC patient was used as a positive control in all experiments. β-Globin amplicons were observed for all frozen MCC tissues investigated (sex ratio 1.0; mean age 71.3 years), whereas only 20 (74%) of 27 FFPE MCC tissues were positive for β-globin by PCR. We found no statistically significant differences in sex ratio and mean age between patients with samples that were FFPE β-globin positive (sex ratio 0.73, mean age 78.5 years) and those

that were β-globin negative (sex ratio 0.75; mean age 82.1 years). β-Globin amplicons were detected in all patients with non-MCC neuroendocrine carcinoma (sex ratio 0.86; mean age 60.4 years).

Samples from 21 (66%) of the 32 β-globin–positive MCC patients were PCR positive for MCPyV (Table). All 12 frozen samples of MCC were MCPyV DNA positive, in contrast to FFPE MCC samples in which MCPyV was detected in only 9 (45%) of the 20 investigated. This low level of detection is similar to the 43% reported by Garneski et al. (*6*) and the 54% reported by Ridd et al. (*7*), but lower than the 85% reported by Becker et al. (*8*) in which a smaller DNA segment (80 bp) was amplified by using quantitative PCR. Identity of the PCR products was verified by sequencing or Southern blotting (data not shown). For this purpose, 1 LT1- and 1 VP1-nested PCR products were cloned, sequenced, and used to prepare digoxygenin-labeled probes. VP1 amplicons of ≈350 bp were observed after the first PCR amplification in 9 of the frozen samples from 12 MCC patients. Amplicons of smaller size (≈250 bp) corresponding to a 90-bp deletion in the VP1 open reading frame, as observed by Kassem et al. (*9*) in 1 of 14 patients, were not detected. In contrast, MCPyV DNA was not detected for any of the 9 patients with non-MCC neuroendocrine carcinomas (Table).

In addition, we investigated the possibility of amplifying the entire VP1 open reading frame by encoding the major capsid protein of MCPyV, using VP1F/VP1R primer sets (5′-CCTGAATTACAAGTAATTGAAGATGGCACC-3′ and 5′-CTGAATAGGAATGCATGAAATAATTCTCAT-3′, respectively). The VP1 gene was amplified from 7 of the frozen samples (online Technical Appendix, available from www.cdc.gov/EID/content/15/6/960-Techapp.pdf), and 6 of these VP1 amplicons of ≈1,300 bp were cloned and then sequenced. We compared the sequences obtained with the MCPyV sequences from isolates from the United States, Sweden, and Japan (MCC339, EU375804.1; MCC350, EU375803.1; MKL-1, FJ173815; and TKS, FJ464337). The results confirmed the MCPyV VP1 sequence, and only point mutations were observed in the VP1 sequences from the isolates from France compared with the VP1 sequences published (online Technical Appendix). The VP1 amino acid sequence from 4 French isolates of MCPyV was identical to that of the Swedish MKL-1 isolate (*10*), and 1 (MKT-23) was identical to that of the MCC339 strain (*2*). MKT-26 showed 2 point mutations that were not reported in any of the other isolates. No French isolate was similar to the U.S. strain MCC350 (*2*), nor to the recently described Japanese isolate (FJ464337). Moreover, 3 silent nucleotide changes were observed in all French isolates, compared with the MCC339 strain, and 1–4 different silent point mutations were observed in isolates MKT-21, MKT-23, MKT-26, MKT-31, and MKT-33.

Because deletions in the viral genome have been reported in the VP2 sequence and the regulatory region of hamster polyomavirus (*11*), a virus that causes lymphomas, the sequence encompassing part of the VP2 protein and the regulatory region (4,876–238) of 7 MCPyV isolates were PCR amplified with the primer set RegF/RegR (5′-TGTTCAGCTGTGAACCCAAG-3′ and 5′-GAGC CTCTCTTTCTTTCCTATTT-3′, respectively), cloned, and sequenced. The N-terminal part of the VP2 of the French isolates was similar to those of the MCC339 U.S. strain and the MKL-1 Swedish strain and differed by 1 amino acid (E41D) from that of the MCC350 U.S. strain. Only minor nucleotide changes were observed within the regulatory region in comparison with the MCC339 strain, except for a deletion of 5 bp (5022–5026) in 5 of the 7 French isolates (online Technical Appendix). This deletion has been reported in the MCC350 U.S. strain and the MKL-1 strain. The MKT-23 isolate was similar in the VP1, VP2, and regulatory region to the MCC350 strain.

Conclusions

Our study confirms the association of MCPyV with MCC (*2*,*6*,*8*,*9*,*12*). However, the primer sets used were not effective for detecting MCPyV DNA in FFPE tissues. In contrast, frozen tissues from MCC patients were all PCR positive, and the entire VP1 gene was easily amplified in 7 of 12 MCC tissues. Our findings demonstrate that strains circulating in Europe are highly conserved and relatively similar to the MCC339 strain in the United States and the MKL-1 isolate from Sweden, suggesting this virus is genetically stable. However, the VP1 sequence of these isolates is relatively different from the VP1 sequence of the

Table. Detection of Merkel cell polyomavirus by PCR in patients with Merkel cell carcinoma using primers sets within LT and VP gene sequences, France, 2008*

Sample	No. patients	LT1, no. (%)	VP1, no. (%)	Total, no. (%)
Merkel cell carcinoma				
Paraffin-embedded	20	6 (30)	6 (30)	9 (45)
Frozen tissue	12	10 (83)	12 (100)	12 (100)
Other neuroendocrine carcinomas				
Paraffin-embedded	1	0	0	0
Frozen tissue	8	0	0	0

*Non–Merkel cell carcinoma high-grade neuroendocrine carcinomas were 5 small-cell lung carcinomas, 3 well-differentiated intestinal carcinomas, and 1 high-grade neuroendocrine carcinoma of the cervix.

MCC350 strain identified in the United States. The MKT-26 VP1 sequence, isolated from an 80-year-old MCC patient, differed from all other isolates.

MCC represents a promising direction for future studies. The MCPyV life cycle needs to be characterized and a greater understanding reached of the natural history of MCPyV infection in humans, including determination of whether MCPyV is associated with other human diseases or malignancies.

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Diagnosis of Queensland Tick Typhus and African Tick Bite Fever by PCR of Lesion Swabs

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We report 3 cases of Queensland tick typhus (QTT) and 1 case of African tick bite fever in which the causative rickettsiae were detected by PCR of eschar and skin lesions in all cases. An oral mucosal lesion in 1 QTT case was also positive.

Queensland tick typhus (QTT) is endemic to Sydney, New South Wales, Australia (*1–6*). A prospective study of 80 serologically confirmed QTT cases (63 acquired in Sydney) yielded 68 cases with rash, of which 62% and 27%, respectively, were vesicular and pustular (*6*). This study yielded an isolate of *Rickettsia australis* from a patient who acquired QTT in Sydney (*1*). Eschar biopsies and removed eschars have been used for PCR confirmation of rickettsial and scrub typhus infections (*7*). We report 3 cases of QTT and 1 case of African tick bite fever (ATBF) in which the causative rickettsiae were detected by PCR of eschar and skin lesions in all cases and by an oral mucosal lesion in one of the QTT cases. Clinical details of the cases are summarized below.

The Cases

Case 1

A 45-year-old woman sought treatment at the emergency department in one of our district hospitals after being bitten by a tick in suburban Sydney. Clinical signs were inguinal eschar, tender local lymphadenopathy, fever to 39.8°C, severe headache, myalgia, arthralgia, and generalized sparse rash characterized by maculopapular and vesiculopustular lesions.

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Case 2

A 32-year-old woman, a resident of suburban Sydney, sought treatment for an acute febrile illness (fever to 41°C), severe headache, myalgia, arthralgia, and rash. She did not recall a tick bite. Clinical examination showed an eschar on the torso, generalized sparse rash characterized by maculopapular and vesiculopustular lesions, plus oral mucosal lesions and a palatal lesion.

Case 3

A 79-year-old woman sought treatment for gradual onset of fever (to 38.8°C) after being bitten by a tick at her home in suburban Sydney. Examination showed an eschar on the scalp, tender local lymphadenopathy, and generalized rash with maculopapular and vesicular components.

In these 3 cases, acute-phase serologic results were negative; convalescent-phase serologic results yielded an *R. australis* indirect fluorescent antibody (IFA) titer of 512. Illness resolved completely after treatment with doxycycline, 100 mg 2×/day, for 14 days.

Case 4

A 57-year-old man with gradual onset of fever (to 38.8°C) sought treatment 10 days after visiting a game park in South Africa. He did not recall a tick bite. Clinical examination showed an eschar on the torso, tender local lymphadenopathy, and generalized sparse rash characterized by papular and vesiculopustular lesions. Illness resolved completely after a regimen of doxycycline, 100 mg 2×/day. *R. australis* IFA acute titer was <128; titer for serum collected 44 days after fever onset was 128. *R. africae* antigen was unavailable for IFA. Acute-phase and convalescent-phase serum samples were negative for *R. typhi* and *Orientia tsutsugamushi* (all IFA titers <128).

Specimens on all 4 cases were collected as shown in the Table. Serologic analysis was performed by using the reference method of IFA testing using *R. australis* infected cells, according to Philip et al (*8*). Total antibody to *R. australis* was detected. A titer of 128 was regarded as borderline; titers >128 were regarded as positive. To confirm recent infection, a 4-fold rise in titer is preferre .

For PCR, specimens were collected by swab of eschar margin or, in the case of unroofed vesicular lesions, in the manner described by Rawls (*9*). Copan Amies Transport swab without charcoal and Copan Virus Transport swab (Interpath Services, Melbourne, Victoria, Australia) were used. Duplicates were collected from the same lesion in some cases; 1 specimen was collected using a dry swab, and another by using a sterile saline moistened swab.

Identification of *Rickettsia* spp. citrate synthase-encoding gene (*gltA*) PCR was performed based on the method of Roux et al. (*10*). DNA was extracted from the specimens by using the QIAamp DNA Mini kit (QIAGEN, Doncaster,

Table. PCR results by specimen, relation to clinical signs, and doses of doxycycline taken, Australia*

Case no.	Specimen type	Duration of lesion, d	Duration of fever, d	No. 100-mg doxycycline doses	PCR result
1					
EDTA	Blood, EDTA	NA	3	2	
Eschar	Eschar, Amies, dry	6	3	2	
Eschar	Eschar, virus, dry	6	3	2	+
Vesicle	Vesicle, virus, dry	6	3	2	+
Vesicle	Vesicle, virus, dry	7	4	4	–
2					
Blood	Blood, EDTA	NA	3	0	–
Eschar	Eschar, Amies, dry	6	3	0	+
Eschar	Eschar, virus, dry	8	5	0	+
Eschar	Eschar, virus, saline	10	7	4	+
Vesicle	Vesicle, Amies, dry	1	3	0	–
Vesicle	Vesicle, virus, dry	3	5	0	–
Vesicle	Vesicle, virus, saline	5	7	4	–
Palatal lesion	Lesion, virus, dry	5	7	4	+
3					
Blood	Blood, EDTA	NA	1	0	–
Eschar	Eschar, virus, saline	10	2	1	+
Vesicle	Vesicle, virus, saline	6	2	1	+
4					
Blood	Blood, EDTA	NA	5	2	–
Eschar	Eschar, virus, dry	10	5	2	+
Vesicle	Vesicle, virus, dry	4	5	2	+
Vesicle	Vesicle, virus, dry	4	5	2	+

*NA, not applicable.

Victoria, Australia) according to manufacturer's protocol. Positive control was *R. australis* DNA extracted from 3–5-day-old Vero cell culture, serially diluted from 1:10 to 1:105. Extraction blanks, consisting of water processed along with the specimens, were also included as negative controls.

Eppendorf DNA Thermalcycler (Eppendorf, North Ryde, New South Wales, Australia) was used for all PCR amplification. Five microliters of each DNA extraction was added to 45 μL of master mixture for each reaction. Final reagent concentration was 10 pmol for each primer, 200 μM for each deoxynucleotide triphosphate, 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Roche Molecular System, Inc., Indianapolis, IN, USA), and 1× PCR buffer. The following thermal cycler parameters were used with the primer pairs for the *gltA* gene RpCS.877p and Rp1273r primers, which amplify a 397-bp portion of the *gltA* gene from all *Rickettsia* spp.: 95°C (10 min), followed by 40 cycles of 95°C (30 s), 45°C (30 s), and 72°C (55 s), followed by an extension period (72°C, 10 min).

The amplicons were detected after electrophoresis on a 4% agarose gel stained with ethidium bromide and purified using the QIAquick DNA Purification kit (QIAGEN) for sequencing analysis. BLAST (www.ncbi.nlm.nih.gov/BLAST) was used for comparison and analysis of sequence data obtained. PCR-positive specimens on QTT cases yielded sequences 99% homologous with *R. australis* (Phillips strain). Amplification was unsuccessful in all negative controls. PCR results for herpes simplex and varicella zoster viruses were both negative on the swab of the palatal lesion. PCR-positive specimens on the ATBF case yielded sequences that were 99% homologous with *R. africae*. GenBank accession nos. for sequences are EU543436 (case 1 eschar); EU543438 (case 2 palatal lesion); EU543438 (case 3 vesicular lesion); and EU714268 (case 4 eschar).

PCR results for blood specimens were negative in all cases. For QTT cases, all eschars yielded positive PCR results for *R. australis*, irrespective of type of swab used. Vesicle swabs also yielded positive PCR results for *R. australis* but only in cases 1 and 3. In case 2, the palatal lesion yielded a positive PCR result for *R. australis*. For the ATBF case, all vesicular lesions and eschar swabs yielded a positive PCR result for *R. africae*.

Based on previous reports and recommendations, tissue collected from the eschar site is the most likely specimen to provide a positive result for rickettsia PCR or culture (*11,12*). Lepidi et al. (*13*) reported 8 cases of ATBF wherein age of eschars at biopsy ranged from 5 to 10 days. Of 8 eschar biopsies, positive results were obtained by immunohistochemical analysis (IHC) (6/8), serologic analysis (4/8), culture (4/8), regular PCR (6/8), and nested PCR (8/8). Although we did not use nested PCR, we still had 100% detection of rickettsiae in 4 eschars of similar ages and after up to 4 doses of doxycycline, 100 mg. Eschar scab

in scrub typhus yielded positive nested PCR result for *O. tsutsugamushi* in a child 7 days after commencement of treatment with azithromycin (*7*).

Accordingly, eschar specimens may permit characterization of the causative organism even after days of antimicrobial drug therapy. In the cases we studied, no blood specimens were positive by the PCR method used; swabs of all eschars and some vesicular skin lesions were positive. Rickettsemia may not be detected by any method because of variables such as stage of illness, effective antimicrobial drug therapy, individual variation in level of rickettsemia, and sensitivity of the detection method. We acknowledge that insufficient numbers of rickettsiae for detection by the PCR method used may have resulted in some false negative results. Real-time PCR methods likely offer greater sensitivity while also providing quantitative data. Additionally, PCR-negative lesions may have been positive by IHC, which when used in conjunction with PCR, has improved rickettsiae detection in skin biopsies (*12*).

Eschar or vesicular skin lesion swab has high patient acceptance because the test is simple and virtually painless. Although lesion biopsy enables IHC (and improved rickettsiae detection), our results justify further exploration of swab PCR to confirm diagnosis of QTT, ATBF, and other rickettsial spotted fevers.

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Oseltamivir- and Amantadine-Resistant Influenza Viruses A (H1N1)

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Surveillance of amantadine and oseltamivir resistance among influenza viruses was begun in Hong Kong in 2006. In 2008, while both A/Brisbane/59/2007-like and A/Hong Kong/2652/2006-like viruses (H1N1) were cocirculating, we detected amantadine and oseltamivir resistance among A/Hong Kong/2652/2006-like viruses (H1N1), caused by genetic reassortment or spontaneous mutation.

A high rate of oseltamivir resistance in seasonal influenza virus A (H1N1) infection was reported in Europe in the winter of 2007–08 (*1*). Of subtype H1N1 isolates tested in 18 European countries, 59 (13.5%) of 437 were resistant to oseltamivir and carried the substitution of histidine by tyrosine at residue 274 (H274Y) of the neuraminidase (NA) gene. Genetic analysis showed that all oseltamivir-resistant strains of subtype H1N1 virus remained susceptible to amantadine.

In Hong Kong Special Administrative Region, People's Republic of China, amantadine and oseltamivir are not commonly used to treat patients with influenza. Surveillance of amantadine and oseltamivir resistance among influenza viruses was begun in 2006 after a high rate of amantadine resistance was reported among subtype H3N2 viruses (*2*) and a stockpile of oseltamivir was purchased for pandemic preparedness. Oseltamivir resistance was first detected in January 2008. We report detection of subtype H1N1 virus isolates that became resistant to amantadine and oseltamivir because of genetic reassortment and spontaneous mutation.

The Study

As part of ongoing surveillance, respiratory samples are routinely collected from patients seeking treatment with respiratory illness in clinics and hospitals of Hong Kong. Virus isolation is performed according to a standard protocol (*3*). From January 2006 through June 2008, a total of 827 influenza viruses (H1N1) were tested for oseltamivir resistance by an enzyme inhibition assay (NA-STAR; Applied Biosystems, Foster City, CA, USA) or by nucleotide sequencing of the NA gene to detect the H274Y mutation. The isolates were also tested for resistance to amantadine by an in-house–designed PCR (protocol available on request) and nucleotide sequencing of the matrix (M2) gene segment. A 575-nt fragment of the NA gene and a 199-nt fragment of the M2 gene were amplified by the designed primers N1–1H and N1–2H, and MA 692 and MA 891 (available on request), respectively, and sequenced by using a Genetic Sequencer 3100 or 3130XL (Applied Biosystems). Sequences obtained were aligned by using Simmonics (*4*) and analyzed by MEGA version 2.1 (*5*). Increasing amantadine resistance, from 107 (48.0%) of 223 isolates in 2006 to 12 (85.7%) of 14 in 2007, was associated with clade 2C A/Hong Kong/2652/2006-like viruses (*6*); none were resistant to oseltamivir.

A subtype H1N1 virus resistant to oseltamivir, but sensitive to amantadine, was first detected in Hong Kong in January 2008, which coincided with the emergence of clade 2B A/Brisbane/59/2007-like viruses. From January through June 2008, 87 (12.5%) of 697 isolates tested were resistant to oseltamivir. They all carried the H274Y mutation in the NA gene and showed an ≈1,000-fold reduction in susceptibility to oseltamivir (50% inhibitory concentration values increased from 0.5 nmol/L to 500 nmol/L). Analysis of the M2 genes showed that 3 (3.4%) of 87 oseltamivir-resistant isolates also carried the S31N mutation associated with amantadine resistance. These 3 viruses were isolated from 2 infants and an elderly woman in different hospitals during 2008 (March, April, and June, respectively). To eliminate the possibility of mixed strains, we designed a real-time PCR using single nucleotide polymorphisms to detect antimicrobial drug–susceptible strains (with S31 and H274) and drug resistant strains (with S31N and H274Y) in the samples (method available on request). Results showed that the 3 isolates did not possess S31 and H274.

To further study the genetic mechanism of emergence of antiviral drug resistance among subtype H1N1 viruses, we performed nucleotide sequencing on a partial segment of the hemagglutinin (HA) gene on 84 (97%) of 87 oseltamivir-resistant and 37 (5%) of 697 oseltamivir-susceptible viruses isolated in our laboratory in 2008. PCR and DNA sequencing of the HA gene were carried out by using the primers H1–1 and H1–2, which flank a fragment of 612 nt of the HA segment (*7*). Sequences were obtained from 121 virus isolates (Table).

Of 37 oseltamivir-susceptible viruses, analysis of the HA, NA, and M2 genes showed that 20 were susceptible to amantadine and similar to the clade 2B A/Brisbane/59/2007 virus (GenBank accession no. CY030232) (*8*). Fourteen were similar to clade 2C A/Hong Kong/2652/2006 virus (GenBank accession no. CY031342), an amantadine-re-

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Table. Phylogenetic analysis of HA, NA, and M2 genes of influenza viruses A (H1N1) with different drug-resistance characteristics isolated in Hong Kong, January–June 2008*

Characteristics	No. isolates	HA clade	NA clade	M2 clade	Mutation
Oseltamivir resistant (n = 84)					
Amantadine susceptible	81	2B†	2B	2B	H274Y in NA
Amantadine resistant	1	2C†	2B	2C	H274Y in NA, S31N in M2
	2	2C	2C	2C	H274Y in NA, S31N in M2
Oseltamivir susceptible (n = 37)					
Amantadine susceptible	20	2B	2B	2B	None
Amantadine resistant	14	2C	2C	2C	S31N in M2
	3	2B	2B	2C	S31N in M2

*HA, hemagglutinin; NA, neuraminidase; M2, matrix.
†Clade 2B or clade 2C indicates the gene specified has a nucleotide homology >99% to that of A/Brisbane/59/2007 or A/Hong Kong/2652/2006 virus, respectively.

sistant virus isolated in our laboratory in 2006 which remained prevalent in Hong Kong in 2007 and 2008. Three isolates had HA and NA genes similar to those of clade 2B but carried an amantadine-resistant M2 gene similar to that of the A/Hong Kong/2652/2006 virus.

Among 84 oseltamivir-resistant strains, 81 remained susceptible to amantadine and had HA and M2 genes similar to those of clade 2B A/Brisbane/59/2007 virus but carried a single point mutation H274Y in the NA gene. The other 3 isolates were resistant to oseltamivir and amantadine; they had similar HA and M2 genes to those of clade 2C A/Hong Kong/2652/2006 virus but had the H274Y mutation in their NA gene. One of the viruses had an NA gene similar to that of the oseltamivir-resistant clade 2B A/Brisbane/59/2007 virus, whereas the other 2 viruses had NA genes similar to those of amantadine-resistant clade 2C A/Hong Kong/2652/2006 virus (Figure). These results suggest that the 3 isolates acquired amantadine and oseltamivir resistance by different mechanisms. A clade 2C A/Hong Kong /942/2008 virus acquired an NA gene carrying the H274Y mutation by reassortment with an oseltamivir-resistant clade 2B virus; the other 2 clade 2C viruses, A/Hong Kong /1052/2008 and A/Hong Kong /1313/2008, acquired oseltamivir resistance through a spontaneous H274Y mutation in the NA gene.

To study the effect of the resistant gene on the replication efficiency of the viruses, we conducted experiments using amantadine- and oseltamivir-resistant viruses in MDCK cells. Briefly, we placed in quadruplicate on MDCK cells one 50% tissue culture infectious dose per 0.1 mL of subtype H1N1 viruses with different antiviral drug–resistance patterns (amantadine-resistant only, oseltamivir-resistant

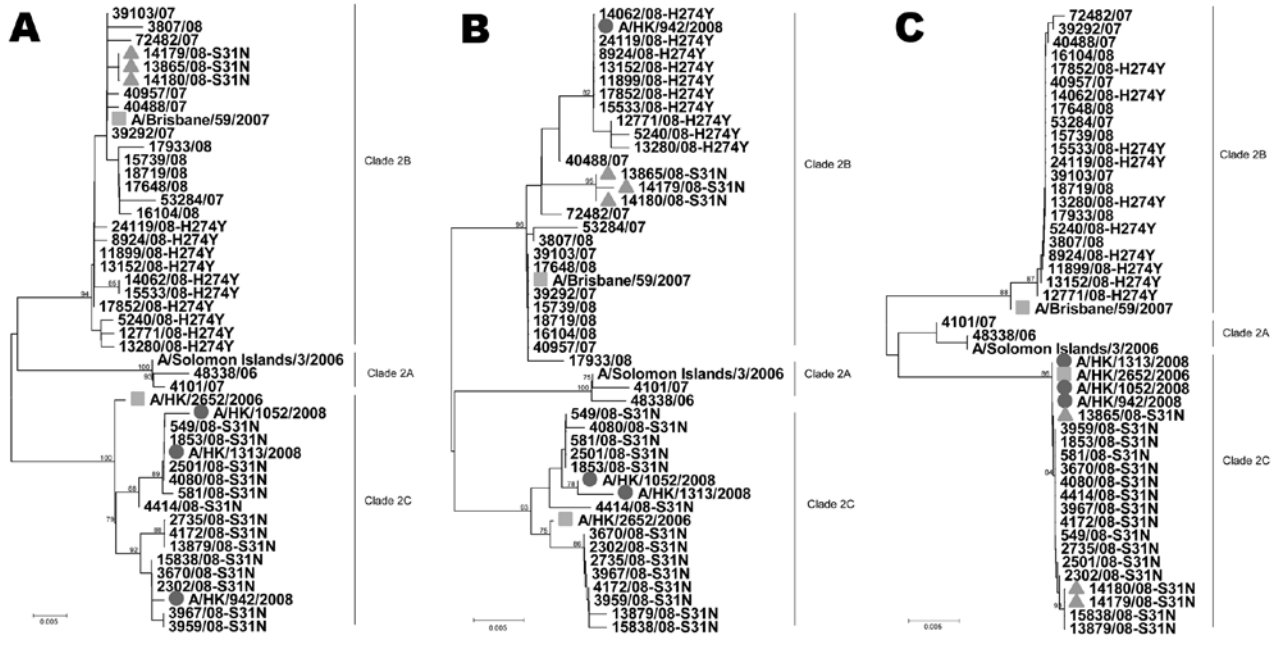

Figure. Phylogenetic analysis of the hemagglutinin gene (A), neuraminidase gene (B), and matrix gene (C) of influenza virus (H1N1). The 3 clade 2C viruses with double resistance are indicated by circles, the 3 clade 2B viruses with amantadine resistance are indicated by triangles, and A/Brisbane/59/2007 and A/Hong Kong/2652/2006 are indicated by squares. Scale bars indicate 0.5% difference in nucleotide sequence.

only, amantadine- and oseltamivir-resistant, and amantadine- and oseltamivir-susceptible), incubated the cultures at 33°C, and examined the cytopathic effect (CPE) daily for 7 days. By observing the timing of CPE appearance and relative progressive rate of CPE, we found no substantial difference in growth efficiency and similar replication patterns for the 4 different types of subtype H1N1 strains.

Conclusions

Reassortment of gene segments likely occurred among clade 2B and clade 2C subtype H1N1 viruses while they were cocirculating in Hong Kong. Clade 2B A/Brisbane/59/2007-like viruses also were cocirculating with clade 2C A/Hong Kong /2652/2006-like viruses in about the same proportion during the 2008 influenza season. We identified 3 clade 2B A/Brisbane/59/2007-like viruses (isolated from 1 outbreak) that were oseltamivir-sensitive in their HA and NA genes but carried an amantadine-resistant M2 gene that was similar to clade 2C A/Hong Kong/2652/2006 virus. We also identified 3 virus isolates that were resistant to both amantadine and oseltamivir. The HA, NA, and M2 gene sequences of the 3 oseltamivir- and amantadine-resistant subtype H1N1 isolates were deposited in GenBank (accession nos. FJ227684–FJ227689 and FJ581446–FJ581448).

All 3 isolates were clade 2C A/Hong Kong/2652/2006-like viruses, but they acquired oseltamivir resistance through spontaneous mutation or reassortment with a clade 2B A/Brisbane/59/2007-like virus. Gene sequencing results confirmed that no epidemiologic link existed among the 3 patients infected with these isolates.

Monitoring of antiviral resistance among influenza isolates showed that resistance pattern changed among subtype H1N1 viruses when different lineages were introduced. While oseltamivir resistance appears to be largely confined to clade 2B viruses and amantadine resistance to clade 2C viruses, oseltamivir- and amantadine-resistant viruses due to spontaneous mutation or reassortment were detectable when clade 2B and 2C viruses were cocirculating. If antiviral resistance markers are combined with sequence data of HA, NA, and M genes, systematic monitoring would make it possible to track the spread of influenza viruses globally and to clarify the underlying mechanism for the spread of such resistance.

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Clinical Recovery and Circulating Botulinum Toxin Type F in Adult Patient

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A 56-year-old woman in Helena, Montana, USA, who showed clinical signs of paralysis, received antitoxins to botulinum toxins A, B, and E within 24 hours; nevertheless, symptoms progressed to complete quadriplegia. On day 8, she began moving spontaneously, even though blood tests later showed botulinum toxin type F remained.

Botulism is a disease characterized by cranial nerve palsies and descending, symmetric, flaccid paralysis. Seven serologically distinct botulinum toxins, designated A through G, are known; virtually all human cases are caused by types A, B, E, and rarely, F (*1*). *Clostridium botulinum* produces all 7 toxin types (*2–4*). Toxin type E may also be produced by *C. butyricum* (*5*), and type F by *C. baratii* (*6–9*).

Botulism type F causes ≈1% of botulism cases in the United States (*10*). Two outbreaks have been reported, 1 in the United States, the other in Europe (*4*,*11*,*12*). A recent review described all 13 cases of botulism type F from the USA between 1981 and 2002 (*9*). Clinical signs were respiratory failure within 24 hours of symptom onset, complete or near complete quadriplegia by the fifth day, and neuromuscular recovery beginning on the eighth day. On average, patients received mechanical ventilation for 24 days and were hospitalized for 30 days. These features represent a more precipitous initial course than is typical for type A or type B botulism but a more rapid recovery. We describe a case of botulism type F in an adult who recovered despite the continued presence of toxin in the blood.

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The Case

In 2005, a 56-year-old woman sought treatment at the emergency department of a hospital in Helena, Montana, USA. She reported right-upper quadrant pain radiating to her back of 1 day's duration and shortness of breath. Her medical history included hypertension, hyperlipidemia, and gastroesophageal reflux. The abdomen was tender at the right upper quadrant. Routine blood test results were unremarkable; an abdominal radiograph showed copious stool and gas. The patient had respiratory arrest in the emergency department and was intubated. She had unresponsive pupils dilated to 5 mm, minimal extraoccular muscle motion, facial paralysis, normal palatal and gag reflexes, near-paralysis of proximal upper and lower extremities but near normal muscle strength in the hands and feet, and symmetric deep tendon reflexes. Results of computed tomographic scans of the brain, chest, and abdomen were unremarkable. Cerebrospinal fluid values were within normal limits. An electromyogram (EMG) showed normal sensory nerve function, low amplitude on motor stimulation, mild (10%) decremental response on repetitive low frequency, and incremental response on high-frequency nerve stimulation, consistent with botulism (*13*).

Antitoxins to botulinum toxin types A, B, and E were administered within 24 hours. Nevertheless, paralysis progressed after antitoxin administration; within 48 hours, the patient was quadriplegic with no voluntary muscle function or distal tendon reflexes. On hospitalization day 4, a repeat EMG study showed no response to repetitive nerve stimulation.

The first improvement in neurologic status occurred on hospitalization day 8, when she moved her eyebrows, shook her head horizontally and laterally, lightly gripped, and plantarflexed and dorsiflexed her feet. Heart rate, which had been notably fixed at ≈70 beats/min, began varying for the first time. The Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) provided heptavalent (anti-ABCDEFG) equine F(ab′)2 antitoxin, but because of progressive clinical improvement, it was surmised that no toxin remained in circulation, and the antitoxin was not administered. Her neurologic function gradually improved; cranial nerve function was substantially improved by hospitalization day 12, although the pupils remained fixed for much longer. The first bowel movement, with enema, occurred on day 13. Distal tendon reflexes were first noted on day 17. On day 25, the patient combed her own hair. Weaning from mechanical ventilation was completed on day 35.

The patient underwent 1 week of in-hospital physical therapy, 3 hours per day. This period was marked by intermittent lightheadedness, orthostatic hypotension, and fatigue. Complications during hospitalization were aspiration pneumonia, *Candida glabrata* urinary tract infection with fungemia, heart failure attributed to diastolic dysfunc-

tion, and otitis media. She was discharged in stable condition on day 47. After hospitalization the patient reported that she required help getting out of bed and bathing for 1 month, help dressing for 2 months, and help getting up from a seated position for >7 months. She reported attaining pre-illness health 10 months after discharge.

Botulinum toxin type F was identified by mouse bioassay (*2*) at the Utah Public Health Laboratories in serum samples drawn on hospital days 1 and 8. Testing was confirmed at CDC. Test results were reported 21 days after serum collection, by which time treatment with specific antitoxin was not deemed necessary in view of substantial neurologic improvement. No protection was conferred on mice by antitoxins to botulinum toxins A, B, or E. A stool sample collected on hospital day 1 tested negative for botulinum toxin but yielded *C. baratii* that produced botulinum toxin type F. Public health officials did not identify any suspect food or other potential source of exposure.

Conclusions

The clinical characteristics of this case closely resemble those in previously described adult botulism F patients (*9*). Our patient experienced respiratory collapse before her neurologic deficits were known, progressed to complete quadriplegia within 2 days, and showed the first signs of neurologic recovery on day 8. She was respirator dependent for 35 days and hospitalized for 45 days.

The patient had documented circulating botulinum toxin type F on hospital days 1 and 8. The assay's limit of detection is ≈1 50% mouse intraperitoneal lethal dose ($MIPLD_{50}$) per milliliter of patient blood. Therefore, estimating the patient's circulatory volume at 5,000 mL whole blood and 3,000 mL plasma, this patient had a minimum of 3,000 $MIPLD_{50}$ of type F toxin in circulation (apart from intracellular and bound toxin) on day 8 of hospitalization, the same day she demonstrated the first unequivocal signs of recovery from total quadriplegia. The type F toxin subcutaneous lethal dose in rhesus monkeys has been estimated at 25 $MIPLD_{50}$/kg and the oral lethal dose at $>4 \times 10^6$ $MIPLD_{50}$/kg (*3*). We cannot explain the patient's clinical improvement in the face of circulating active toxin.

We were unable to determine whether the patient had foodborne or adult colonization botulism. Gupta et al. reported that, of 13 adult type F cases, 2 had confirmed adult colonization and 1 may have had foodborne botulism, but the syndrome in the remaining patients was not known (*9*). Infant type F botulism, by definition a colonization condition, does occur (*14*,*15*).

The economic burden of illness in this case was substantial. Apart from hospital charges of US $230,000, the patient required constant care by a relative and could not work for at least 7 months.

The similarity of the clinical features of this case with those previously described (*8*) indicate a highly predictable course of illness for botulism type F intoxication. Early suspicion of type F intoxication, suggested by specimens producing positive mouse assay results without protection of mice by injection of antitoxins for botulism toxins A, B, or E, may facilitate timely treatment with experimental type-specific antitoxin, available from CDC. Stool samples should be cultured over an extended period to assess for possible intestinal colonization.

We describe circulation of botulinum toxin in a patient on the day she demonstrated the first signs of recovery from complete quadriplegia. When future type F cases are identified by the presence of type F toxin in clinical specimens, the patient should be promptly treated with the appropriate antitoxin. The possibility of type F illness should be suspected if onset is rapid and paralysis is severe, and the laboratory should conduct immediate, specific testing. Intensive care support and antitoxin treatment are the standards for botulism care (*2*). Suspected cases of botulism of any type should be immediately reported to the state health department's 24-hour emergency telephone number.

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Dr Sobel is a medical epidemiologist and the CDC Advisor to the Brazilian Ministry of Health's Field Epidemiology Training Program. His interests include training field epidemiologists and studying the epidemiology of infectious diseases.

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Murine Typhus in Child, Yucatan, Mexico

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A case of murine typhus in Yucatan was diagnosed in a child with nonspecific signs and symptoms. The finding of *Rickettsia typhi* increases the number of *Rickettsia* species identified in Yucatan and shows that studies are needed to determine the prevalence and incidence of rickettsioses in Mexico.

Murine typhus is a worldwide febrile illness caused by *Rickettsia typhi*, which is frequently associated with exposure to reservoir animals and their ectoparasites (*1*,*2*). Patients with murine typhus often have nonspecific signs and symptoms that mimic those of common febrile illnesses. Although the disease is generally self-limited, it sometimes has complications that require hospitalization and can even cause death if the appropriate timely treatment is not administered (*3*,*4*). Antimicrobial drug treatment dramatically reduces the symptomatic period as well as the economic effects by lowering expenses and minimizing loss of productivity.

Information about murine typhus in Mexico is scarce. Recently, a study of healthy adult blood donors in Mexico City showed a prevalence of antibodies against typhus group rickettsiae (using *R. typhi* antigen) of 14% (*5*).

The Study

In June 2007, a 3 year-old girl was brought to the emergency room of the public hospital with a fever of 3 days' duration. Her mother reported that the child had close contact with domestic animals (cat and dog), that mice were present in the house, and that the child had flea bites on her legs and arms 2 days before the onset of fever. The illness began with a high fever (39.8°C), abdominal pain, headache, fatigue, and myalgia. The patient's condition was diagnosed as viral pharyngitis and otitis media, and she was treated as an outpatient with nimesulide, amoxicillin, and trimethoprim; she did not respond to this treatment. Ten days after the first hospital visit, the girl was hospitalized with the diagnosis of *Salmonella typhi* infection because of serologic reactivity with O and H antigens (titers of 160 and 80, respectively). At time of admission, she had a high fever (39.8°C), arthralgia in the hands and ankles, and a petechial maculopapular rash on the thorax and extremities. She was treated with sulfonamide, cephalosporin, and acetaminophen without remission of the symptoms. Clinical laboratory evaluation showed the following: neutrophilic leukocytosis, hemoglobin 10.1 g/dL, hematocrit 30.3%, thrombocytopenia (40×10^6 platelets/L [reference range 140–440 $\times 10^6$ platelets/L]), elevated serum alkaline phosphatase (290 U/L [reference range 35–104 U/L]), elevated lactic dehydrogenase concentration (560 U/L [reference range 100–190 U/L]), and elevated antistreptolysin O and rheumatoid factor titers.

The diagnosis of murine typhus was established by PCR for *Rickettsia* 17 kDa and citrate synthase (*gltA*) genes as described previously (*6,7*). Positive controls were DNA of *Rickettsia felis*, *R. rickettsii*, *R. akari*, and *R. typhi*, and 1 reaction without DNA was used as a negative control. The DNA of the controls and the patient were handled separately to avoid contamination. *R. typhi* was identified as the causal agent by restriction fragment length polymorphism analysis (RFLP) of the amplified fragment of *gltA* (382 bp) and 17-kDa gene (434 bp) by using *Alu*I as described previously (*7*,*8*) (Figure) and by comparing the DNA sequences of the *gltA* and 17-kDa gene PCR amplicons using BLAST software of the National Center for Biotechnology Information (Bethesda, MD, USA) (*9*). The sequences showed 100% identity with the corresponding *R. typhi* genes (Table).

An indirect immunofluorescence assay (IFA) was performed for serologic diagnosis; *R. akari, R. rickettsii,* and *R. typhi* antigens were fixed on slides. (A positive human serum sample control and IFA slides were provided by the Rickettsial and Ehrlichial Diseases Research Laboratory, University of Texas Medical Branch at Galveston.) As a negative control, we used a serum sample (from a healthy donor) that was negative for *Leptospira* spp. (microscopic agglutination test and PCR); rickettsiae (IFA and PCR); HIV (microparticle enzyme immunoassay and PCR); hepatitis A, B, and C viruses (microparticle enzyme immunoassay); *Toxoplasma gondii* (ELISA); and *Mycobacterium tuberculosis* (ELISA, PCR).

We examined the serum specimens for immunoglobulin (Ig) G and IgM, assessing reactivity of γ-chain–specific and μ-heavy-chain–specific secondary conjugates, respectively, with rickettsial antigens. A serum sample collected 20 days after onset of the illness was serially diluted to 1:4,096 to determine the end-point titer. IFA showed antibody reactivity with *R. typhi*, *R. rickettsii*, and *R. akari* (Table). The child was treated with intravenous chloramphenicol, 75 mg/kg per day, for 7 days; symptoms were reduced in 48 hours.

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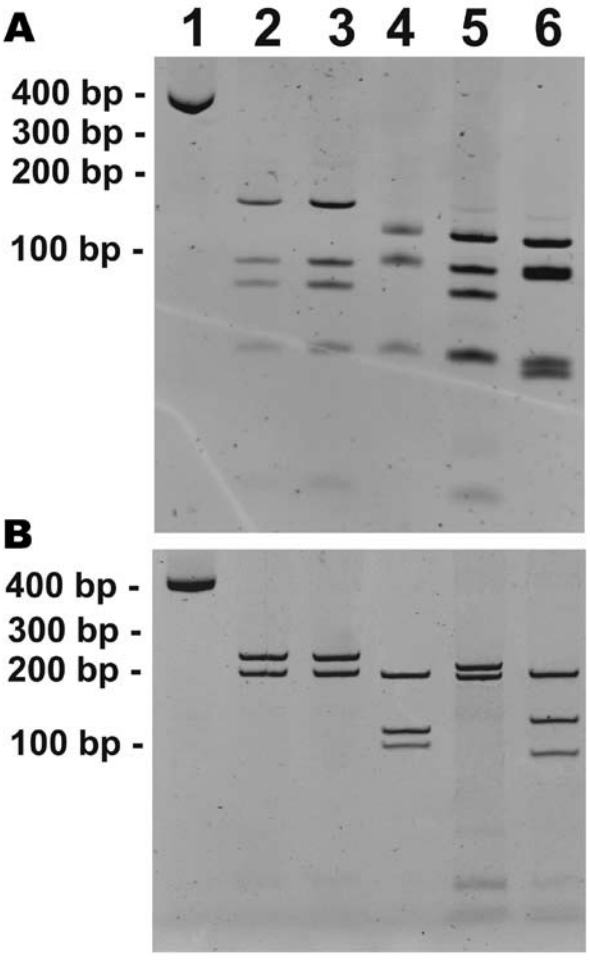

Figure. Restriction fragment length polymorphism patterns of *gltA* (A) and 17-kDa gene (B) PCR products digested with *Alu*I. Lane 1, undigested *gltA* and 17-kDa gene PCR amplicons (from blood sample of infected child); lane 2, human case; lane 3, *Rickettsia typhi*–positive control; lane 4, *R. felis*–positive control; lane 5, *R. akari*–positive control; lane 6, *R. rickettsii*–positive control.

Conclusions

In Yucatan state we have identified several cases of rickettsiosis caused by *R. felis* and *R. rickettsii*, some with fatal outcome, but not with *R. typhi* infection (*10*,*11*). Since we reported an emerging human rickettsiosis in Mexico 12 years ago, a surveillance system and educational efforts have been put in place to promote more timely diagnosis and treatment of rickettsioses. As a result of such efforts, we have also been able to detect new rickettsioses in the Yucatan Peninsula.

R. typhi is a bacterium that is broadly distributed around the world, and is the cause of many human infections every year (*12*,*13*). As with other febrile illnesses, children are a vulnerable population frequently exposed to this pathogen (*4*,*14*).

In Mexico, human proximity to domestic animals is common, and the habitats of both have a close relationship. In rural and suburban areas, for example, opossums, rats, and mice often inhabit backyards and houses. The potential transmission cycle of many vector-borne diseases, including rickettsioses, is evident through human exposure to ectoparasite vectors.

IFA showed serum IgM and IgG antibody reactivity with *R. typhi* and IgG antibody reactivity with *R. rickettsii* and *R. akari*. In our experience, IFA supports the diagnosis but sometimes is not conclusive because of cross-reactivity among species of the spotted fever group and the typhus group (*15*). However, we cannot exclude the possibility of other prior infections because of the living conditions and the presence of various rickettsial species in our environment in Yucatan. For that reason, we conduct PCR to diagnose human rickettsiosis. Our criteria for a confirmed diagnosis of any rickettsial infection are the same as those published by the US Centers for Disease Control and Prevention (Atlanta, GA, USA) for Rocky Mountain spotted fever: a clinically compatible case with a 4-fold change in IgG-specific antibody titer reactive with a rickettsial antigen by IFA between paired serum specimens or detection of rickettsial DNA in a blood, biopsy, or autopsy specimen through amplification of a specific target by PCR.

The identity of *R. typhi* was established not only by the RFLP pattern of the *gltA* and 17-kDa gene amplicons, but mainly by the sequence comparison with other rickettsial species. Identity was 100% for *R. typhi* (Table). Identification of the *Rickettsia* species is essential for determining the epidemiology and ecology of the transmission cycle and how the agent is maintained in nature. In addition, spe-

Table. Methods used to diagnose human *Rickettsia typhi* infection, Yucatan, Mexico, 2007*

Sample	PCR, % identity		IFA, titer†	
	Citrate synthase (*gltA*) (382 bp)‡	17-kDa gene (434 bp)§	IgM	IgG
Rickettsia typhi Wilmington strain	100 (AE017197.1)	100 (M28481.1), 99 (AE017197.1)	256	128
R. felis URRWXCal2	NR	89 (CP000053.1)	ND	
R. rickettsii Sheila Smith	92 (CP000848.1)	89 (CP000848.1)	Neg	128
R. akari Hartford strain	91 (CP000847.1)	96 (CP000847.1)	Neg	64

*IFA, indirect immunofluorescence assay; Ig, immunoglobulin; NR, not represented in the first 100 sequences with a significant alignment using BLAST (*9*); ND, not determined; Neg, negative.
†Titers refer to the reciprocal of the dilution.
‡Primers: RpCS.1258 5′-ATTGCAAAAA GTACCGTAAACA-3′ and RpCS.877 5′-GCCCCGCCGTGGGCAGGCCCCC-3′.
§Primers: 17-kDa 5′-GCTCTTGCAACTTCTATGTT-3′ and 5′-CATTGTTCGTCAGGTTGGCG-3′.

cies identification is useful for selecting the best preventive program appropriate for each region.

This finding of an autochthonous human case of murine typhus in Yucatan Mexico and the finding of *R. typhi* in Yucatan state increases the diversity of rickettsioses identified in this ecosystem. Because rickettsioses are treatable diseases, an educational program is critical to instruct the population about these infections and their transmission cycles, as well as to inform the medical community about the rickettsial diseases that must be included in the differential diagnosis of any acute febrile illnesses in the region.

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Murine Typhus and Leptospirosis as Causes of Acute Undifferentiated Fever, Indonesia

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To investigate rickettsioses and leptospirosis among urban residents of Semarang, Indonesia, we tested the blood of 137 patients with fever. Evidence of *Rickettsia typhi,* the agent of murine typhus, was found in 9 patients. Another 9 patients showed inconclusive serologic results. Thirteen patients received a diagnosis of leptospirosis. No dual infections were detected.

Fever is one of the main reasons for seeking medical attention in Indonesia. The cause of fever usually remains obscure because of limited laboratory diagnostic facilities and expertise in performing laboratory confirmation. Favorable environmental conditions mean that both rickettsiosis and leptospirosis are considered endemic in Indonesia and may result in clinically indistinguishable cases of acute undifferentiated fever (AUF). Serosurveys conducted on Java, Sumatra, and islands in eastern Indonesia identified antibodies to *Rickettsia typhi* (murine typhus), to *Orientia tsutsugamushi* (scrub typhus), and to members of the spotted fever group rickettsia (SFGR) in healthy persons (*1*–*3*). In addition, several investigations reported leptospirosis as cause of AUF in Indonesia (*4*,*5*).

Murine typhus and leptospirosis are likely to share routes of transmission in an urban setting where rats are abundant. The main vector for *R. typhi* is the Asiatic rat flea (*Xenopsylla cheopsis*). Humans usually become infected when *R. typhi*–infected flea feces contaminates excoriated skin or is inhaled. Leptospirosis is mainly spread by rats and other small mammals, which shed the bacteria through their urine into the environment. Humans are infected through mucous membranes, conjunctivae, or abraded skin.

The clinical features of mild leptospirosis and murine typhus are nonspecific. Generally, patients with murine typhus exhibit fever, headache, and a rash, although the latter is often absent. Renal failure, jaundice, and hemorrhages are the classic symptoms of severe leptospirosis; fever, headache, and myalgia may be the only exhibited symptoms of mild disease. Dual infections with murine typhus are reported to occur in Southeast Asia and may complicate treatment and clinical course (*6*,*7*). In this study, we attempted to find evidence for acute rickettsial disease, leptospirosis, and dual infections among patients with AUF in Indonesia, where risk factors for both diseases are present.

The Study

The study was based in Semarang, a large harbor city in central Java. Consecutive outpatients were recruited at 2 primary healthcare centers and hospitalized patients at a governmental referral center (Dr. Kariadi University Hospital, Department of Internal Medicine). All eligible AUF patients (≥5 years of age) were included who met the following criteria: fever ≥38°C (central) for <14 days with no apparent other disease. After informed consent was obtained, a blood sample was taken. A convalescent-phase sample was drawn after ≈14 days. The study was approved by the local medical ethical committee.

A specific microimmunofluorescent antibody (IFA) assay for *Rickettsia* spp. was performed in Marseille, France, by using whole-cell antigens of *O. tsutsugamushi*, *R. japonica*, *R. heilongjiangensis*, *R. slovaca*, *R. honei*, *R. conorii* subsp. *indica*, *Rickettsia* ATI, *R. helvetica*, *R. felis*, *R. typhi*, and *R. prowazekii* . The assay results were considered positive when 1) antibody titers were ≥256 for immunoglobulin (Ig) G and ≥64 for IgM, or 2) seroconversion was observed, or 3) a ≥4-fold increase in titers between the acute-phase and the convalescent-phase serum specimen was detected. Serologic analysis for leptospirosis was performed in Semarang, Indonesia. Crosschecks and PCR were performed in Amsterdam, the Netherlands. Convalescent-phase samples were screened with the LeptoTek Dri Dot (bioMérieux, Marcy l'Etoile, France). All positive samples were tested by the microscopic agglutination test (MAT) and IgG ELISA (*8*).

Additionally, a real-time PCR specifically targeting the *secY* gene of pathogenic *Leptospira* spp. (*9*) was performed on all samples. For the MAT, a panel of 31 serovars was used containing 28 pathogenic and 3 nonpathogenic serovars. For patient samples tested by ELISA or MAT, a titer ≥320 on a single sample was considered positive; also considered positive were those samples that showed sero-

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conversion or a ≥4-fold increase in titers between paired samples, as well as any patient sample with a positive PCR, irrespective of the serologic result. All samples were run in parallel.

From February 2005 through February 2006, 137 AUF patients were included: 67 hospitalized patients and 70 outpatients. A convalescent-phase sample was available for 106 (77%) patients. The main symptoms were headache (85%), myalgia (70%), nausea (64%), cough (44%), and abdominal pain (38%).

Murine typhus and leptospirosis were found to cause AUF in this clinical series (online Appendix Table, available from www.cdc.gov/EID/content/15/6/975-appT.htm). In total, 9 patients (7%) had evidence of an acute infection with *R. typhi*; none showed a rash. Murine typhus could be diagnosed in 6 (9%) of 67 hospitalized patients; 3 (4%) of 70 outpatients had acute murine typhus. Another 9 (7%) patients showed inconclusive *R. typhi* serologic results. One patient showed evidence of a past infection with *R. typhi* (IFA IgG/IgM titer 128/0 in both serum specimens). Evidence for acute infection with *O. tsutsugamushi* or SFGR was not found.

Leptospirosis was diagnosed in 13 (10%) of 137 patients; results for 2 of these patients were positive only by PCR. Eleven leptospirosis patients were recruited in the hospital; 2 patients were recruited outside the hospital. Consequently, the percentage of AUF caused by leptospirosis in hospitalized patients was 16% and in outpatients 3%. The most frequently identified serogroup by MAT was Bataviae (5 cases). No dual infections were detected; however, 3 (23%) leptospirosis patients showed titers in the *R. typhi* IFA assay.

Conclusions

We report that murine typhus and leptospirosis are important causes of AUF in Semarang, Indonesia. A previous study from rural Thailand identified both diseases in 2.8% and 36.9% of AUF cases, respectively (*6*,*7*). In Vientiane, the capital of Laos, *R. typhi* was reported to cause fever in 9.6% of investigated persons, results that closely resembling our data (*10*). Unfortunately, leptospirosis was not investigated.

In the present study, we expected leptospirosis to be a cause of AUF because the Dr. Kariadi University Hospital admits ≈50 severe cases each year. These cases were not included in the study because of the high clinical suspicion of leptospirosis on admission with jaundice, azotemia, and/or bleeding. A definite diagnosis of murine typhus and leptospirosis co-infections could not be made, but in 3 cases this scenario was plausible. We did not find evidence for scrub typhus, which we expected, because *O. tsutsugamushi* transmission occurs primarily in rural areas (*11*). Although SFGR have been reported in Southeast Asia and proof for their presence in Indonesia is accumulating (*2*,*12*), these rickettsia were not identified as a cause of AUF in the present study.

From an epidemiologic point of view, Semarang, Indonesia, seems to encompass environmental circumstances that are prerequisites for *R. typhi* and leptospirosis transmission. Previous studies have shown that murine typhus is particularly prevalent in tropical port cities where rats are abundant (*13*,*14*). In the Indonesian urban situation, *R. rattus* and *R. norvegicus* rats are likely to be the main hosts harboring *R. typhi*–infected *X. cheopsis* fleas (*12*,*15*). These rats are also likely to be the maintenance hosts for pathogenic *Leptospira* spp. in Indonesia. In fact, the identified serogroups are commonly associated with rats.

Although serologic analysis might be hampered by cross-reactions and test sensitivity issues, we believe that our data are representative for the area. The chosen cutoff values are unlikely to cause false-positive results in a disease-endemic setting. In regard to leptospirosis serologic analysis, we used a wide panel for the MAT. This panel included serovars recommended by the World Health Organization and serovars that were previously isolated in Indonesia. Moreover, most serogroups were represented in our panel, and cross-reactions are likely to detect missing serovars.

Because of nonspecific clinical features, both diseases are difficult to diagnose on clinical grounds only. Misdiagnosis can lead to aberrant use of antimicrobial drugs and other pharmaceuticals. Therefore, rapid, cheap, and reliable diagnostic tests are needed to support clinical decision making.

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etymologia

Typhus

[ti′ fəs]

From Greek τῖφος [*typhos*], meaning heavy stupor; also related to Greek *typhein*, to smoke. A disease known since antiquity, typhus has been described as follows: "A kind of continued fever, attended with great prostration of the nervous and vascular systems, with a tendency to putrefaction in the fluids and vitiation in the secretions; putrid fever. A genus of the order *Febres*, class *Pyrexia*, of Cullen's nosology" (J. Thomas, 1885).

Today, typhus refers to any of a group of acute infections caused by rickettsiae and transmitted to persons by the bite of arthropods such as fleas and lice. Epidemic typhus, caused by *Rickettsia prowazekii*, is characterized by headache, high fever, chills, rash, and, in serious cases, by stupor or lack of awareness of reality. Outbreaks usually occur in crowded or unsanitary environments.

Source: Dorland's illustrated medical dictionary, 31st ed. Philadelphia: Saunders; 2007; http://www.merriam-webster.com; Thomas J. A complete pronouncing medical dictionary. Philadelphia: JB Lippincott; 1885.

Religious Opposition to Polio Vaccination

To the Editor: In 1988, the Global Polio Eradication Initiative was formed, with the aim of reducing infection with poliomyelitis virus. Two decades later in 2008, a total of 1,625 children contracted acute flaccid paralysis caused by poliovirus infection (*1*). This finding represented a 150% increase over the number of cases in 2007 (*1*) and resulted in the reemergence of polio as one of the world's deadliest infections. As of 2009, polio remains endemic to 4 countries (India, Nigeria, Pakistan, and Afghanistan); in 2008, cases were also detected in 14 other countries.

Religious opposition by Muslim fundamentalists is a major factor in the failure of immunization programs against polio in Nigeria (*2*), Pakistan (*3*) and Afghanistan (*4*). This religious conflict in the tribal areas of Pakistan is one of the biggest hindrances to effective polio vaccination. Epidemiologists have detected transmission of wild poliovirus from polio-endemic districts in Afghanistan, most of which are located in the southern region of this country bordering Pakistan, to tribal areas of Pakistan (*4*). This transmission has resulted in new cases of polio in previously polio-free districts. The local Taliban have issued *fatwas* denouncing vaccination as an American ploy to sterilize Muslim populations. Another common superstition spread by extremists is that vaccination is an attempt to avert the will of Allah. The Taliban have assassinated vaccination officials, including Abdul Ghani Marwat, who was the head of the government's vaccination campaign in Bajaur Agency in the Pakistani tribal areas, on his way back from meeting a religious cleric (*5*). Over the past year, several kidnappings and beatings of vaccinators have been reported. Vaccination campaigns in Nigeria and Afghanistan have also been hampered by Islamic extremists, especially in the Nigerian province of Kano in 2003, which has resulted in the infection returning to 8 previously polio-free countries in Africa (*2*).

Before the Global Polio Eradication Initiative in 1988, a total of 1,000 persons/day were infected with a virus that would cripple them for the rest of their lives (*6*). To eradicate the disease, 1 major factor will be to gain support of those susceptible to fundamentalist propaganda. Islam is a progressive religion, and religious leaders should be asked to support polio eradication programs. The Imam of the Ka'aba and other influential religious figures should be asked to highlight the plight of children with polio. Vaccinators operating in conflict-ridden areas should be provided protection so that they are better able to perform their duties. Not only will children in these areas be safer, but the disease will not be exported to areas where wild polio transmission has been interrupted by vaccination. Further study of the attitudes of Muslim populations toward vaccination is needed.

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Recurrent Human Rhinovirus Infections in Infants with Refractory Wheezing

To the Editor: Respiratory infections frequently cause illness among pediatric patients worldwide. Human rhinovirus (HRV) is a cause of acute respiratory tract infections (RTIs) (*1*); co-infections with other respiratory viruses such as respiratory syncytial virus (RSV) or influenza virus have also been reported. HRV strains have been subdivided into 2 genetic subgroups (HRV-A and HRV-B); a third genetic subgroup has been recently discovered (*2–7*). However, understanding of the epidemiology of novel HRV infection among Asian pediatric patients with respiratory illness (*4,5*) and association with recurrent wheezing and asthma has been limited (*8–10*).

We retrospectively analyzed 289 nasopharyngeal aspirates (NPAs) obtained from 286 pediatric patients admitted to Chulalongkorn Memorial Hospital in Bangkok, Thailand, during 2006–2007. The study was reviewed and approved by the Institutional Review Board, Chulalongkorn University,

Bangkok, Thailand. Each specimen was tested for common respiratory viruses such as RSV, HRV, parainfluenza 1–3, influenza A and B viruses, adenovirus, human metapneumovirus, and human bocavirus. On the basis of phylogenetic analysis of the VP4 region, we identified 2 patients who had been admitted with 5 episodes of acute RTIs and subsequent recurrent wheezing associated with HRV-A and HRV-C.

The first patient was an infant girl whose first episode of breathing difficulty was at 5 months of age; a diagnosis of RSV bronchiolitis was made. She was hospitalized with respiratory failure and required mechanical ventilation for 3 days. At 6 months, she had pneumonia and wheezing. At 14 months, she had a low-grade fever, mild cough, breathing difficulty, and wheezing. While she was hospitalized for 7 days, a novel HRV-C (FJ435240) was identified by seminested PCR, and RSV was detected by reverse transcription–PCR. Seven months later, she had recurrent wheezing and respiratory distress. Virologic analysis indicated that she was co-infected with a divergent HRV-C strain (FJ435256) and influenza A virus. Nucleotide sequence identity score between the 2 isolated strains of HRV-C indicated a different cluster (identity score 70.1%).

The second patient was an infant boy with a diagnosis of acute bronchiolitis at 7 months of age. His underlying condition was congenital heart disease and an allergy to cow's milk protein. Initial NPA showed HRV-A (FJ435274) and RSV by reverse transcription–PCR. Two months later, he had viral pneumonia and acute exacerbation of his reactive airway disease. He received systemic corticosteroids and a nebulized bronchodilator. His clinical course was complicated by 3 episodes of supraventricular tachycardia that were controlled with adenosine and cordarone. NPA was again positive for HRV-A (FJ435284). Three weeks later, he had upper respiratory tract symptoms, low-grade fever, and protracted cough; blood oxygen saturation was low and respiratory distress had rapidly increased. An NPA showed HRV-C (FJ435299). He received systemic corticosteroids and was discharged with corticosteroid inhalation. Comparison between 2 HRV-A strains isolated showed 82.5% nucleotide sequence identity. The sequence of the HRV-C strain also displayed 51.2% and 61.1% nucleotide identity to FJ435274 and FJ435284, respectively. Results of phylogenetic analysis are shown in the Figure.

A novel HRV-C infection in association with acute lower RTI was diagnosed in the first patient during her fourth and fifth hospitalizations. The 2 strains isolated are within the same genetic group and display 70% nucleotide similarity, which suggests that this infant was infected with 2 different virus strains. The second patient was infected with HRV-A during his first hospitalization. His condition subsequently progressed to refractory wheezing. Both patients were co-infected with RSV when a diagnosis of infection with lower RTIs was made. Two HRV-A strains detected in the

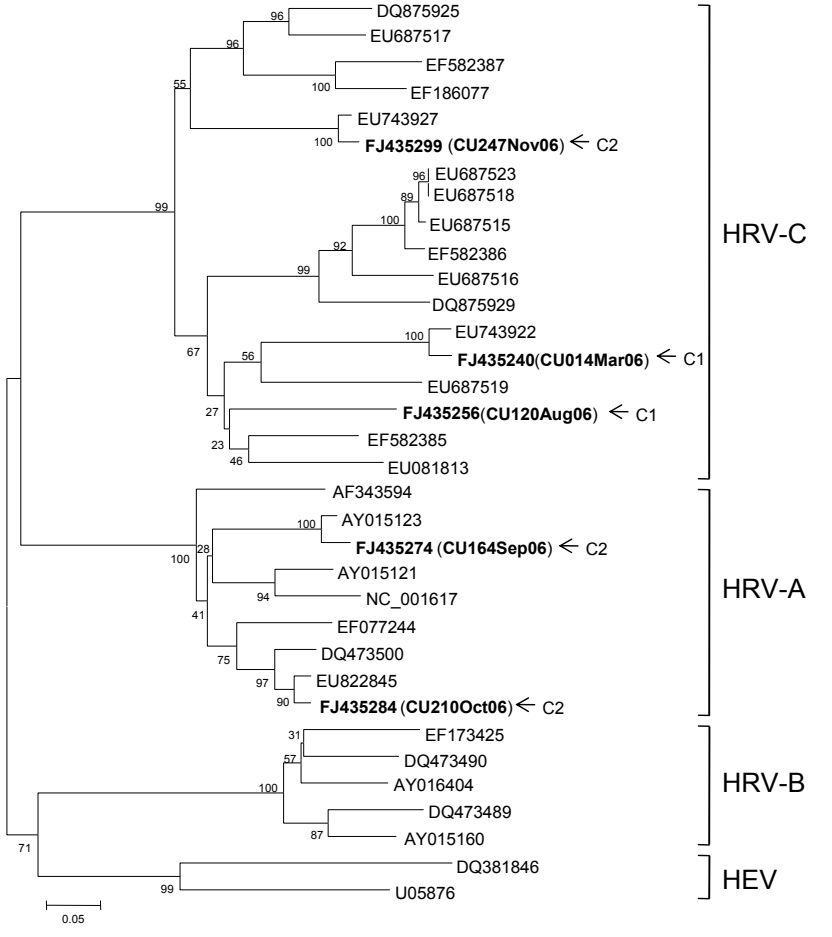

Figure. Phylogenetic analysis of nucleotide sequences of the virus capsid protein (VP4) region of 5 human rhinovirus (HRV) strains (shown in **boldface**) isolated from 289 nasopharyngeal aspirate specimens, including those of 2 infants with refractory wheezing (C1 and C2), on the basis of amplification of VP4/2 by seminested reverse transcription–PCR. The tree was constructed by using the neighbor-joining method and Kimura's 2-parameter distance with bootstrap replicated from 1,000 trees by using MEGA 4.0 (www.megasoftware.net). Scale bar indicates number of nucleotide substitutions per site. Human enterovirus (HEV) was used as an outgroup for comparison.

second patient were within the same subgroup, but similarity in nucleotide sequences was only 82.5%. This result suggests that this patient was infected with 2 different virus strains of HRV-A and a strain of HRV-C.

Comparison of the HRV-A strains with the HRV-C strain showed that they belonged to different subgroups and had low similarity for nucleotide sequences. The second patient had 3 distinct rhinovirus infections over 3 months, and each was associated with illness requiring hospitalization. Both patients had underlying diseases, reactive airway diseases, and repeated episodes of RTI that may have rendered them vulnerable to reinfection, compromising their immune responses.

Complete coding sequences of HRV-A and HRV-C have been determined (*4,7*). However, little is known about their involvement in the pathogenesis of recurrent wheezing in young children. According to recent reports, HRV-C has been detected in hospitalized children with lower RTI in the People's Republic of China (*5*). Possible association of novel infection with HRV and exacerbation of asthma in children has also been reported (*6*). We report HRV-A and HRV-C coinfections in conjunction with other respiratory viruses, such as RSV, as a potential cause of recurrent wheezing in infants with acute lower RTIs. Coinfections with HRV-A and HRV-C may contribute to increased virulence and subsequent pathogenesis of other respiratory viruses. Additional studies will be required to further explore the clinical role of novel HRVs.

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Extensively Drug-Resistant *Acinetobacter baumannii*

To the Editor: In the 1990s, patients infected with vancomycin-resistant *Enterococcus faecium* were successfully treated with new antimicrobial drugs. However, it is unlikely that new antimicrobial drugs will be available in the near future to treat patients infected with gram-negative pathogens such as *Acinetobacter baumannii* (*1*). No new antimicrobial drugs active against this organism are currently in clinical trials (www.clinicaltrials.gov). We report a patient infected with *A. baumannii* that lacked susceptibility to all commercially available antimicrobial drugs.

The patient, a 55-year-old woman, had a prolonged stay in an intensive care unit at the University of Pittsburgh Medical Center (Pittsburgh, PA, USA) after undergoing lung transplantation. In the tenth postoperative week, ventilator-associated pneumonia developed, which was caused by *A. baumanni* that lacked susceptibil-

ity to all antimicrobial drugs tested except colistin (MIC 0.5 μg/mL). Therapy with colistin and tigecycline was begun. Colistin was administered intravenously and by inhalation. Although the pneumonia showed radiographic response to the antimicrobial drug therapy, *A. baumannii* continued to be isolated from respiratory secretions on numerous occasions. Despite another course of therapy with colistin and cefepime, the patient never recovered from respiratory failure. She eventually died of sepsis caused by vancomycin-resistant *E. faecium*. An *A. baumannii* isolate obtained just before she died lacked susceptibility to all commercially available antimicrobial drugs (Table).

Multidrug-resistant *A. baumannii* has emerged as a substantial problem worldwide (*2*). Such strains are typically resistant to all β-lactams and fluoroquinolones and require salvage therapy with colistin, amikacin, or tigecycline. Unfortunately, notably high-level resistance to colistin and amikacin was found in the isolate we have described (Table). Tigecycline, a newly available glycylcycline antimicrobial drug, showed intermediate susceptibility. No randomized trials have been performed to specifically evaluate combination antimicrobial drug therapy for treatment of infection with *A. baumannii*.

Considerable media attention has been paid to extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (*3*). Infections with XDR strains are extremely difficult to treat and pose considerable infection control issues. We recently proposed that gram-negative bacilli lacking susceptibility to all commercially available antimicrobial drugs also be referred to as XDR because no therapeutic options are available (*4*).

Numerous outbreaks of *A. baumannii* infection have been reported worldwide (*5*). Unfortunately, multidrug-resistant *A. baumannii* strains have become endemic in some institutions. Experimental and clinical isolates lacking susceptibility to colistin, often considered the drug of last resort, are increasingly being reported (*6*–*8*). Therefore, we alert healthcare workers to the need for stringent care in adhering to infection control precautions when caring for patients infected with XDR *A. baumannii*. Use of contact isolation precautions, enhanced environmental cleaning, removal of sources of infection from the hospital environment, and prudent use of antimicrobial drugs can contribute to control of such outbreaks (*5*). Fortunately, no spread of the XDR strain affecting this patient occurred. A crisis is looming should XDR *A. baumannii* become established pathogens in hospitals.

Table. MICs and antimicrobial drug susceptibility for an extensively drug-resistant strain of *Acinetobacter baumannii**

Drugs	MIC, μg/mL	Interpretation
Carbapanems		
Imipenem	>32	Resistant
Meropenem	>32	Resistant
Penicillins		
Ampicillin/sulbactam	32	Resistant
Piperacillin/tazobactam	>256	Resistant
Cephalosporins		
Ceftazidime	48	Resistant
Cefepime	16	Intermediate
Aminoglycosides		
Gentamicin	>256	Resistant
Tobramycin	>256	Resistant
Amikacin	>256	Resistant
Others		
Ciprofloxacin	>32	Resistant
Tigecycline	2	Intermediate
Colistin	>1,024	Resistant

*Susceptibility testing was performed by using the Etest (AB Biodisk, Solna, Sweden), except for colistin, for which the standard agar dilution method was used. Interpretation was according to breakpoints provided by the Clinical and Laboratory Standards Institute (CLSI; Wayne, PA, USA). No tigecycline breakpoints for *Acinetobacter* spp. are provided by the CLSI, the European Committee on Antimicrobial Susceptibility Testing (Basel, Switzerland), or the US Food and Drug Administration (Silver Spring, MD, USA). Breakpoints of the British Society for Antimicrobial Chemotherapy (Birmingham, UK) are indicated for tigecycline.

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Cryptosporidium Pig Genotype II in Immunocompetent Man

To the Editor: Protozoan parasites from the genus *Cryptosporidium* have been described as a cause of diarrheal disease in immunodeficient and immunocompetent humans worldwide. Although *C. hominis* and *C. parvum* (cattle genotype) cause most infections, humans can be infected by several other *Cryptosporidium* species or genotypes: *C. meleagridis*; *C. felis*; *C. canis*; *C. suis*; *C. muris*; *C. andersoni*; *C. hominis* monkey genotype; *C. parvum* (mouse genotype); and *Cryptosporidium* rabbit genotype, deer genotype, skunk genotype, horse genotype, and chipmunk genotype I (*1*–*4*). Wild and domestic animals are sources of infection for humans (and other animals) and important contributors to contamination of food and drinking water; many nonhuman *Cryptosporidium* species or genotypes are detected in untreated water (*5*). We examined the diversity of *Cryptosporidium* spp. in immunocompetent persons in South Bohemia in the Czech Republic.

Diarrheal fecal samples (n = 457) from 203 anonymous immunocompetent patients ≤69 years of age with suspected cryptosporidiosis (at least 2 samples/patient/3-day period) were obtained from local health departments and public hospitals in South Bohemia during 2005–2007. Samples were examined for *Cryptosporidium* oocysts by using aniline-carbol-methyl violet staining and light microscopy at × 1,000 magnification (*6*). The microscopically positive samples were confirmed by DNA sequencing of the small subunit (SSU) rRNA gene. Total DNA was extracted from 200–300 mg stool by using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions, after previous homogenization and disruption of oocysts with the Mini-BeadBeater (Biospec Products, Bartlesville, OK, USA). An ≈830-bp fragment of the SSU rRNA gene was amplified by nested PCR according to Jiang et al. (*7*). Purified PCR products were sequenced in both directions on an ABI3130 sequencer analyzer (Applied Biosystems, Foster City, CA, USA) by using the secondary PCR primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were assembled by using Chromas Pro (www.technelysium.com.au/chromas.html) and aligned with reference sequences using ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX). The BLAST server (www.ncbi.nlm.nih.gov/BLAST) was used for DNA database searches. The SSU rRNA gene partial sequences of the 7 patient isolates have been submitted to GenBank (Table).

Of the 203 patients, 7 (3.4%) (6 children and 1 adult) had positive results for *Cryptosporidium* spp. Moreover, all samples obtained from these persons during the 3-day period were *Cryptosporidium* spp. positive; partial sequences of the *Cryptosporidium* SSU rRNA gene were obtained from all positive samples identifying 3 different species or genotypes of *Cryptosporidium*. Five were *C. parvum* (bovine genotype), 1 was *C. hominis,* and 1 contained the *Cryptosporidium* pig genotype II (Table). *Cryptosporidium* pig genotype II was found in stool samples from a 29-year-old man who also was infected with *Giardia intestinalis* (assemblage A) (data not shown).

Only *C. parvum* (bovine genotype), *C. hominis*, and *Cryptosporidium* rabbit genotype have been implicated in waterborne outbreaks of cryptosporidiosis in humans. Further studies are needed to determine the potential of other cryptosporidia of animal origin. Recent genetic and biologic characterization studies have identified 2 distinct host-adapted cryptosporidia in pigs, *C. suis* and *Cryptosporidium* pig genotype II. Furthermore, both above-mentioned cryptosporidia have been identified in untreated water (*8*). Pigs could be sources of *Cryptosporidium* water and food pollution and a consequent risk to public health.

Although human infection with *C. suis* has been previously described (*9*), human infection with *Cryptosporidium* pig genotype II has been never reported. This genotype was found in diarrheal stool of 1 adult patient in this study. However, onset of diarrhea could have been caused by co-infection with *G. intestinalis* (assemblage A), which recently also has been described in pigs (*10*). Contact with infected animals and ingestion of contaminated food or water could be

Table. *Cryptosporidium* genotypes identified by using sequencing of partial sequences of the small subunit rRNA gene in the stool samples of immunocompetent humans, Czech Republic

Patient no.	Age, y/sex	Examination year	*Cryptosporidium* species/genotype	Infection intensity*		GenBank accession no.
				Sample 1	Sample 2	
H15	9/M	2005	*C. parvum*†	56	78	EU331237
H23	10/M	2005	*C. hominis*	77	121	EU331242
H98	10/F	2005	*C. parvum*†	43	25	EU331238
H101	11/M	2006	*C. parvum*†	11	5	EU331239
H132	8/M	2006	*C. parvum*†	150	62	EU331240
H158	11/M	2007	*C. parvum*†	26	85	EU331241
H199	29/M	2007	*Cryptosporidium* pig genotype II	38‡	27‡	EU331243

*Numbers of oocysts per 30 fields at ×1,000 magnification, unless otherwise indicated.
†Bovine genotype.
‡Numbers of oocysts per whole slide at ×1,000 magnification.

the source of both *Cryptosporidium* and *Giardia* infection in the *Cryptosporidium* pig genotype II–positive patient. The passage of oocysts can be excluded because of the number of oocysts detected in repeat samples (Table). Moreover, identification of the infection in an immunocompetent patient underlines the zoonotic potential of this pig genotype and possible presence of risk factors in rural areas with poor water treatment or inadequate biosecurity in pig units. Further evidence of the zoonotic potential of this *Cryptosporidium* genotype is needed to show its pathogenic potential in immunocompetent patients as a cause of gastroenteritis (in the absence of *Giardia* spp. and other established enteropathogens) and to demonstrate invasive tissue stages. The use of molecular techniques to identify *Cryptosporidium* spp. probably will show more zoonotic species or genotypes in humans.

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Crimean-Congo Hemorrhagic Fever, Southwestern Bulgaria

To the Editor: Crimean-Congo hemorrhagic fever virus (CCHFV) causes a severe multisystem disease characterized by profuse bleeding with a case-fatality rate as high as 30%. The infection is endemic to the Balkans (*1,2*). In Bulgaria, most cases are reported from the central and eastern parts of the country (*3,4*). We report a cluster of cases observed in early spring 2008 in southwestern Bulgaria, an area considered at low risk for CCHF outbreaks.

The index case-patient was a 49-year-old man in whom fever, severe myalgia and joint pain, diarrhea for 1 day, cough, and weakness developed on March 20. Three days before, while not using hand protection, he removed ticks from cows. On March 25, severe epistaxis developed and he was hospitalized. His condition rapidly deteriorated; leukopenia, thrombocytopenia, and elevated levels of liver enzymes developed, and he died on March 26. The autopsy found hemorrhages in the lungs but not in the hypophysis or gastrointestinal tract. Immunoglobulin (Ig) M antibodies against CCHFV were detected in the serum sample.

The second case-patient was a 34-year-old man who had removed ticks from cows from the same herd as the index case-patient. Symptoms developed on March 23 and he was hospitalized on March 26 with fever, diarrhea, and bloody sputum. Laboratory findings showed moderate leukopenia and thrombocytopenia. His condition improved within 1 week. IgM antibodies against CCHFV were detected in a serum sample collected during the acute phase of the disease.

The third confirmed case-patient was a 52-year-old woman (nurse) who cared for the index case-patient after his hospital admission. Although she reported wearing gloves, she was extensively exposed to the patient's blood and vomit and received immunoprophylaxis (specific hyperimmune gamma globulins). On March 28, a mild disease characterized by fever, headache, weakness, and maculopapular rash with petechiae developed; she was hospitalized on April 2. She had leukopenia, thrombocytopenia, and normal levels of liver enzymes. The serum sample collected during the acute phase of the disease was IgM positive, and a 4-fold increase was present in the IgG titer in a sample collected during the convalescent phase (from 160 to 640). Blood and serum samples taken during the acute phase of the disease were positive for CCHFV by real-time PCR (*5*) and reverse transcription–nested PCR (*6*). Purified PCR product was sequenced; the nucleotide sequence was submitted to GenBank (accession no. FJ160262). Viral load was 3.88×10^7 copies/mL.

The fourth confirmed case-patient was a 50-year-old woman, the wife of the index case-patient. She was hospitalized April 10 with fever, headache, myalgia, weakness, stomach pain, and nausea. She reported exposure to her husband's blood before hospital admission. Thus, hyperimmune gamma globulins against CCHFV were administered. She had leukopenia, thrombocytopenia, and elevated levels of aspartate aminotransferase and alanine aminotransferase. The symptoms lasted only 7 days. CCHFV was detected by both PCRs (*5,6*) in a serum sample taken on day 3 of the disease; sequence of the PCR products was submitted to GenBank (accession no. FJ445749).

A phylogenetic tree including sequences from the third and fourth cases was constructed (Figure). The 2

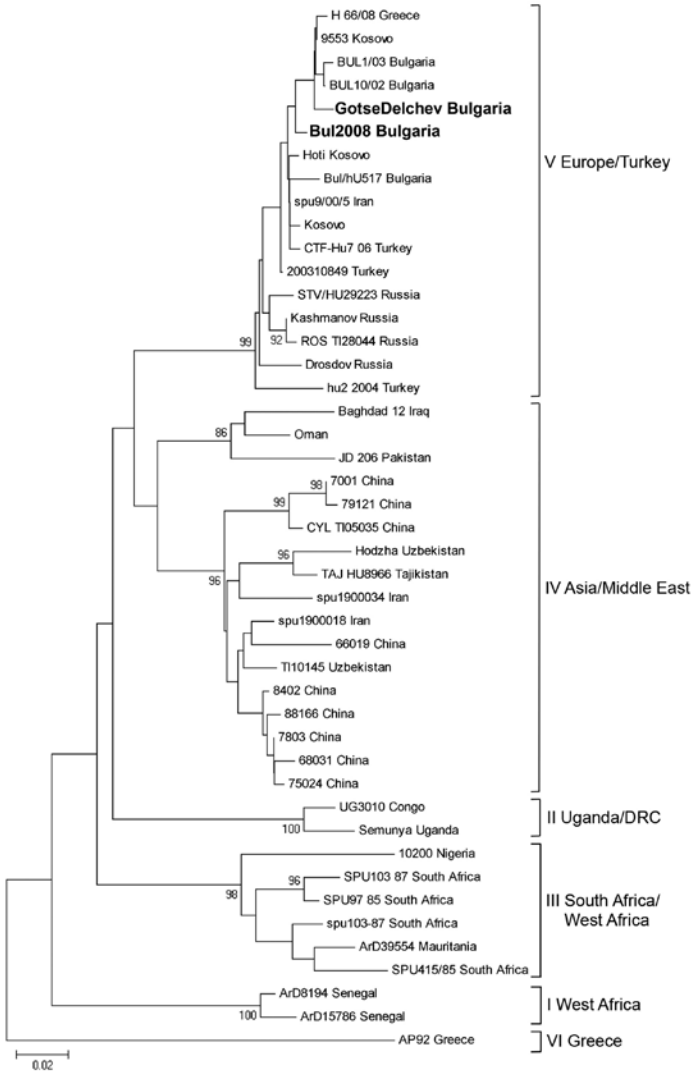

Figure. Phylogenetic tree of partial sequence (256 bp) of Crimean-Congo hemorrhagic fever (CCHF) virus nucleoprotein gene. CCHF virus sequences are listed as viral strain name and country of origin. Sequence of case 3 is designated in the tree as "Gotse Delchev Bulgaria," and sequence of case 4 is designated as "Bul 2008 Bulgaria" (in **boldface**). Strain AP92, Greece, was used as an outgroup. Numbers at the nodes represent bootstrap values. Scale bar indicates number of nucleotide substitutions per site.

sequences clustered within the Europe/Turkey clade. The genetic distance between the 2 strains was 1.15%, but the 2 sequences were identical at the amino acid level. Sequences from the present study showed 96.4%–98.8% similarity with respective CCHFV sequences from Bulgaria from a former study (BUL10/02 and BUL1/03) (*3*) but differed from the Kosovo 9553/2001 strain by 0.8%–2.0% and from the Greek 66/08 strain by 1.2%–2.4%.

Two additional suspected CCHF cases occurred in the same area, on March 30 and April 9 (*7*). Both persons were negative for CCHFV infection. All 119 ticks of various species (*Hyalomma marginatum*, *Dermacentor marginatus*, *Rhipicephalus bursa*, *Ixodes ricinus*) collected from the area and tested by reverse transcription–nested PCR were negative for CCHFV.

This cluster of CCHF cases has several important highlights. First, it occurred in a region that was considered to have low CCHF endemicity; however, the area is only a few kilometers from Greece, where a human fatal case was observed in June 2008 (*8*). The index case was observed earlier in the year than in previous years, and clinical manifestations of the cases were unusual (absence of craniopharyngeal syndrome and bleeding from gastrointestinal tract that are typical for CCHF patients from Bulgaria); in the fatal case, autopsy of the patient showed hemorrhages only in the lungs. Two cases were attributable to tick exposure, whereas the other 2 were most likely secondary cases attributable to contact with the index case-patient (in this regard, CCHFV sequences of the secondary cases were almost identical). Finally, the longer incubation period of the wife of the index case-patient might be associated with administration of hyperimmune gamma globulin against CCHFV.

In conclusion, CCHF emerged in southwestern Bulgaria near the border with Greece. Person-to-person transmission emphasizes the need for rapid diagnosis of CCHF, especially in cases with atypical clinical manifestations.

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Wohlfahrtiimonas chitiniclastica Bacteremia in Homeless Woman

To the Editor: In May 2006, a 60-year-old homeless woman with a history of alcoholism was admitted to the emergency department of the Conception Hospital, Marseille, France. Firefighters had just found her in an abandoned container in the outskirts of the city, beside the body of her companion, who had died several days earlier. She described no symptoms other than fatigue. On examination, she was found to be dirty and covered with thousands of body and hair lice; dozens of insect larvae were in her hair. She was mildly febrile (38°C) and had widespread excoriations but no sign of localized bacterial infection. Head shaving exposed superficial ulcers on her scalp but no maggots. Blood analysis showed marked neutropenia

(0.44 $\times 10^9$/L), thrombocytopenia (28 $\times 10^9$/L), a marked but well-tolerated iron deficiency anemia (hemoglobin 6.8g/dL), and a C-reactive protein level of 182 mg/L. Louse infestation was treated with a single dose of ivermectin (12 mg), and the woman was hospitalized. On day 3, she was still febrile. Louse-borne borreliosis had been ruled out by a negative blood smear, and results of serologic testing and molecular screening of lice for the other 2 louse-transmitted bacteria, *Rickettsia prowazekii* and *Bartonella quintana* (*1*), were negative.

In contrast, 2 cultures of blood taken at the time of admission grew gram-negative rods susceptible to amoxicillin, ceftriaxone, imipenem, ciprofloxacin, amikacin, and trimethoprim/sulfamethoxazole. However, phenotypic tests failed to identify this bacterium with accuracy. Intravenous therapy with ceftriaxone at 2 g/d was initiated, and the patient's fever, neutropenia, and thrombocytopenia improved. Scalp wounds healed with local care. Using 16S rRNA gene amplification and sequencing as previously described (*2*), we identified the bacilli as *Wohlfahrtiimonas chitiniclastica* and determined its similarity to be 99.5% with strain E43 (GenBank accession no. AJ517825). The 16S rRNA sequence obtained from the patient's strain was deposited in GenBank under no. EU484335. The strain was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR; World Data Center for Microorganisms 875, http://ifr48.timone.univ-mrs.fr/portail2/index.php?option = com_content&task = view&id = 96&Itemid = 52) under reference CSUR P16.

W. chitiniclastica is a recently described γ-proteobacterium isolated from larvae of the parasitic fly *Wohlfahrtia magnifica* (*3*). Although the pathogenicity of this new species for humans is as yet undescribed, it is phylogenetically close to *Ignatzschineria larvae*, another bacterium associated with *W. magnifica* larvae (*4*), which cause severe wound myiasis in cattle (*5*). Because of its strong chitinase activity, *I. larvae* may play a role in the metamorphosis of its host fly, as has been observed for other fly symbionts, and thus may be a symbiont of *W. magnifica* flies (*6*). The bacterium was later discovered in swine waste in Quebec (*7*). In 2007, three publications renewed researchers' interest in *I. larvae*. First it was reclassified as the only species within the genus *Ignatzschineria* (*4*). Then 2 case reports demonstrated that it plays a role as a human pathogen (*8*,*9*). Both described an *I. larvae* bacteremia in adults with myiasis in southeastern France. The first patient was an elderly farmer with diabetes and myiasis of the leg, scrotum, and anus (*8*). The second patient was a middle-aged homeless man with a history of alcoholism who also had foot wound myiasis (*9*).

We report *W. chitiniclastica* bacteremia also in a homeless woman from southeastern France. Although we did not test body lice for *W. chitiniclastica*, we believe that the bacteremia originated from the patient's scalp maggots. Unfortunately, as previously reported for cases of *I. larvae* bacteremia, the maggots had been rapidly discarded, permitting neither bacterial analysis nor entomologic identification. However, these larvae may have been from *W. magnifica* flies. These flies are present in southern France, and although they are not typically found at low altitude and in a semiurban environment, their distribution is known to be progressively expanding, in part because of their broad adaptation capacities. Animal hosts for *W. magnifica* flies are numerous, but humans can also be infected; >10 cases of this myiasis in humans have been reported in Europe, Asia, Morocco, and Egypt. The scalp was affected in 2 of these patients (*10*).

Among homeless persons, ectoparasitism is very common; body lice (*Pediculus humanus humanus*) are of particular interest because they transmit 3 bacterial bloodstream infections: trench fever (*B. quintana*), epidemic typhus (*R. prowazekii*), and louse-borne relapsing fever (*Borrelia recurrentis*) (*1*). Myiasis should also be considered as a relevant type of ectoparasitism in homeless and hygiene-deficient persons. In addition, like body lice, ticks, and fleas, fly larvae should also be regarded as another potential source of specific arthropod-borne bacterial systemic infections.

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Serologic Screening for *Neospora caninum*, France

To the Editor: In the June 2008 issue of Emerging Infectious Diseases, McCann et al. (*1*) reported on serologic screening for *Neospora caninum* antibodies and reported a lack of serologic evidence for *Neospora* infection in humans in England, where prevalence of infection with the closely related parasite *Toxoplasma gondii* also is low. Only limited data are available on human exposure to *Neospora*. We investigated the seroprevalence of *N. caninum* in humans in France, where *Toxoplasma* spp. seroprevalence is high.

Our study comprised 500 serum samples from healthy women, followed at the Cochin–Port Royal University Hospital in 1997 within the framework of toxoplasmosis surveillance during pregnancy, and 400 serum samples from HIV-infected patients. All serum samples were submitted to anti-*Toxoplasma* antibody testing by using indirect immunofluorescence (IIF; Toxo-spot IFI; bioMérieux, Marcy l'Etoile, France) and ELISA (Platelia Toxo IgG and IgM; BioRad, Hercules, CA, USA). An in-house microplate IIF test previously validated in cattle was used for simultaneous detection of anti-*Neospora* and anti-*Toxoplasma* immunoglobulin (Ig) G on the same microplate. All samples were screened at dilutions of 1:20 and 1:80, as is usually done in anti-*Toxoplasma* IIF assays in humans. Correlation between the anti-*Toxoplasma* IIF commercial test and in-house IIF was excellent (kappa coefficient = 0.98) and allowed us to compare the antibody titers against both parasites. Forty (8%) samples from immunocompetent persons and 21 (4%) from immunocompromised persons yielded a weak fluorescence when diluted 1:20. All but 4 had significant titers of anti-*Toxoplasma* IgG (>200 IU/mL in 77% of cases), which suggests low-level cross-reactions. Whereas titers of >200 and >320 are considered sufficient to diagnose neosporosis in dogs and cattle, respectively (*2*), positivity threshold was difficult to resolve in the absence of a positive human control. We decided on a positivity threshold of 1:80, which is similar to the threshold defined by others in further studies using an indirect fluorescence antibody test in humans (*3*,*4*). None of the 500 samples from immunocompetent persons were positive for *Neospora* antibodies when assessed at a dilution of 1:80. Within the group of immunocompromised persons, 3 were positive for *Neospora* antibodies at a titer of 80, and 1 was positive at a titer of 160. Three of these 4 HIV-infected patients had high titers of anti-*Toxoplasma* IgG (>2,000 IU/mL), suggesting *Toxoplasma* serologic reactivation. We found no evidence of *Neospora* infection or exposure in immunocompetent persons but could not exclude possible *Neospora* infection associated with *Toxoplasma* infection or reactivation in immunocompromised persons.

Taken together, our data agree with data from other studies conducted in European countries (*1*,*5*), which suggest that neosporosis in healthy humans is unlikely. However, the *Neospora* spp. seropositivity of some HIV-infected patients, although weak compared with the level of seropositivity in cattle or dogs, could suggest circulation of the parasite within immunocompromised hosts, a hypothesis supported by Lobato et al. (*3*). However, our observation of a strong serologic reactivation against *T. gondii* in 3 of 4 patients with anti-*Neospora* titers >80 mostly favors cross-reactivity involving homologous antigens of both parasites and nonspecific antibody binding from polyclonal stimulation of the immune system. Finally, one should keep in mind that the positive predictive value of a serologic test used in screening in low-prevalence populations is low. Large-scale studies are needed to more precisely determine the potential role of this parasite in immunodeficient humans and to isolate the parasite or detect *Neospora* DNA in such patients.

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Escherichia coli and *Klebsiella pneumoniae* Carbapenemase in Long-term Care Facility, Illinois, USA

To the Editor: *Escherichia coli* harboring *Klebsiella pneumoniae* carbapenemases (KPCs) are now rarely being reported. Worldwide, KPC-2 has been detected in Israel and the People's Republic of China (*1*,*2*). Within the United States, carbapenem-resistant *E. coli* carrying bla_{KPC} has been isolated in New Jersey (*3*) and Cleveland, Ohio (*4*), and 7 carbapenem-resistant *E. coli* isolates were obtained from 3 different hospitals in Brooklyn, New York (*5*). Urban et al. (*6*) recently reported 9 KPC-2 and KPC-3 carbapenemases in urinary *E. coli* isolates from 7 long-term care facilities. We report such an isolate from a resident of a long-term care facility.

This case involved a 68-year-old female resident of a long-term care facility in Centralia, Illinois, who had multiple chronic medical problems, including cerebral palsy, a seizure disorder, and recurrent urinary tract infections. A urine culture grew $>10^5$ CFU/mL of *E. coli* susceptible to amikacin, gentamicin, tobramycin, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, imipenem, and nitrofurantoin. Tigecycline susceptibility was not determined. Trimethoprim/sulfamethoxazole therapy was initiated. Follow-up urine culture almost 3 weeks later again grew $>10^5$ CFU/mL of *E. coli*, now susceptible to amikacin, gentamicin, tobramycin, nitrofurantoin, and tigecycline. The isolate was resistant to imipenem and meropenem. A modified Hodge test demonstrated production of a carbapenemase (*7*), and the bla_{KPC} gene was detected by PCR at the Centers for Disease Control and Prevention (CDC). The patient was treated with a 10-day course of nitrofurantoin, 100 mg by gastrostomy tube 2× per day. Chart review indicated that contact precautions were instituted only after discovery of the second *E. coli* isolate.

Seventeen days later, a repeat urine culture grew $>10^5$ CFU/mL of *K. pneumoniae* susceptible only to amikacin, gentamicin, tobramycin, and tigecycline. No treatment was given. Follow-up urine culture grew $>10^5$ CFU/mL of *K. pneumoniae* again with a similar resistance pattern. The modified Hodge test result was positive (*7*) and was confirmed as bla_{KPC} positive by PCR at CDC. The resident was transferred to an acute care facility for further evaluation and was treated with amikacin. At completion of therapy, a repeat urine culture was negative for organisms.

Our case, like that of Urban et al. (*6*), involved a urinary isolate from a resident of a long-term care facility. As increasing numbers of resistant gram-negative rods colonize such patients, the patients may acquire a bacterium carrying a KPC plasmid conferring broad-spectrum resistance as described in our patient. These plasmids may then be laterally transferred to other gram-negatives, which may have occurred in this case.

Our case underscores the gravity of the evolutionary process of emergent, multidrug–resistant enterobacteriaceae. Even though *E. coli* strains that harbor carbapenemase genes are not ubiquitous, additional therapeutic interventions are needed to prevent the spread of these bacteria, which are likely to infect increasing numbers of patients.

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Bedbugs and Healthcare-associated Dermatitis, France

To the Editor: Bedbugs (*Cimex lectularius*) are hematophagous insects. Adults are 4–6 mm long, flattened, oval and wingless, and brown to brownish–red (Figure, panel A) (*1*). They may feed in the wild on birds or bats (*2*), but they are mainly associated with human dwellings and can be found on furniture and clothing (*3*). Because bedbugs are nocturnal and feed painlessly only in the dark, while humans sleep, initial bedbug proliferation usually goes unnoticed until several weeks later when the patient discovers a pruritic cutaneous eruption of unknown origin (*4*). Decades ago, bedbugs were frequently found worldwide, but reports of cases in industrialized countries have progressively declined, probably the result of improved living conditions (*3*). They nonetheless remain a pest in less-developed countries and in the wild (*5*). The past 10 years have seen the revival of this insect in industrialized countries (*3*,*6*,*7*). Increasing reports describe isolated cases or bedbugs spreading throughout a single building (*8*). We report an outbreak of healthcare-associated dermatitis caused by bedbugs in a hospital nursing home in Cannes, French Riviera.

In July 2007, Mrs. Q arrived, with her bed and mattress, for admission to a single room in a hospital nursing home. This facility has 112 rooms located on 2 floors, each having A and B wings. Mrs. Q's first lesions, diagnosed as insect bites, appeared in October 2007. Concomitantly, Mrs. T, a long-term resident of the room across the hall (1.5 m away), developed similar lesions. Examination of Mrs. Q's room led to the discovery of an aggregation of 200 *C. lectularius* bedbugs beneath her mattress. In Mrs. T's room, 15 bedbugs were identified (Figure 1, panel B). Suspected insect excreta were also found in another nearby room. A private company conducted a nonspecific pest-control intervention in these 3 rooms.

In November 2007, another 2 residents in rooms located 3 and 6 m away from Mrs. Q's had insect-bite dermatitis: 15 bedbugs were found in each room. Over a 3-week period, the nursing home staff performed the second pest-control intervention in these 2 infested rooms and also treated 10 adjacent rooms. They disassembled furniture and applied insecticides to furniture, room corners (imiprothrine and cypermethrine), and clothing (esdepallethrine and piperonyl butoxide).

No additional skin lesions occurred during the next 4 months, and no new resident was admitted. In March 2008, a new long-term resident developed similar bedbug-dermatitis lesions (Figure, panel C); 12 *C. lectularius* bedbugs were found in his room (33 m from Mrs. Q's room, same floor, wing B). This time, a specialized private company conducted the pest-control intervention over a 2-month period in the 56 rooms on the second floor (wings A and B); they treated furniture and clothing and placed silicone sealer around doors and floorboards to obstruct potential pest refuges. All furniture was removed, disassembled, and washed. When no bedbugs or eggs were found, bendiocarb was applied preventively; otherwise, curative *d-trans*-tetramethrin was applied (*3*). No further infestation has been observed.

Three pest-control interventions were required to eliminate these infestations. The first was not specific for bedbugs, and the second was not sufficiently extensive. Only specific and extensive insecticide application achieved elimination. The temporal–spatial distribution of dermatitis in this facility suggests 2 types of transmission: during the first 2 waves, spontaneous movement of the bedbugs is the most likely hypothesis because

Figure. A) Adult bedbug (*Cimex lectularius*); B) mattress infested with bedbugs (an adult, eggs, and dejecta); C) dermatitis caused by bedbug bites.

infested rooms were located near one another. During the last wave, bedbugs were most likely transported on clothing and/or furniture moved from room to room because affected rooms were 32 m from each other and no new resident had moved into the infested rooms or adjacent rooms (*3*).

Clusters of bedbug-infestation cases are well known in various communities, especially where living conditions are poor or in urban environments (*3*,*5*). This outbreak of bedbug dermatitis occurred in a nursing home. Because this type of outbreak in a medical facility can be considered healthcare associated, medicolegal implications must be considered and appropriate control measures adapted.

Increased worldwide travel (*9*) and insecticide resistance (*6*) contribute to the resurgence of bedbug dermatitis. Because the cockroach co-inhabits with bedbugs in the same biotope, as demonstrated by Émile Brumpt in 1936 (*10*), recent changes in pest-control techniques (i.e., use of selective cockroach-attracting traps that spare bedbugs) could be another factor enabling bedbug reemergence. At this time, healthcare facilities provide a welcoming environment for future bedbug-dermatitis outbreaks.

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Angiostrongyliasis in the Americas

To the Editor: We read with special interest the article by Hochberg et al. about angiostrongyliasis in Hawaii (*1*). *Angiostrongylus cantonensis* meningitis in the Americas was reported by Aguiar et al. in Cuba in 1981 (*2*), and we have studied this zoonosis during the ensuing 25 years. We agree with the authors about the difficulty in obtaining a specific immunoassay for detection of antibodies to *A. cantonensis* antigens. In Cuba, as in Hawaii, no other cause of eosinophilic meningitis was identified.

To improve accuracy of the diagnosis we investigated immunoglobulin (Ig) E intrathecal synthesis during the first diagnostic lumbar puncture. We also confirmed this synthesis as either a 2-class response (IgG + IgA) or a 3-class response (IgG + IgA + IgM) that appeared 8 days later in cerebrospinal fluid (*3*).

Since 1991, our records show that the major incidence of the disease is during the second quarter of the year. We detected 32% of the cases during the rainy season when rats come into houses in rural and semirural areas and snails and slugs appear more often in gardens and yards where children play. Ethnicity data show that 52% of those affected were Caucasian and 32% were African. The median interval from onset of symptoms to lumbar puncture was 1–3 days. Although no children died, 6 (23%) of 26 adult patients died. The clinical signs and symptoms of the Cuban patients are similar to those in Hawaii (*4*,*5*). We congratulate the authors for systematically determining incidence rates of *A. cantonensis* meningoencephalitis, a severe but preventable infection.

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Increase in Group G Streptococcal Infections in a Community Hospital, New York, USA

To the Editor: Identified in 1935 by Lancefield and Hare, group G streptococci (GGS) are part of the normal flora of the pharynx, gastrointestinal tract, genital tract, and skin (*1–3*). However, previous case reports have indicated that GGS also could cause complicated infections, including cellulitis, osteomyelitis, septic arthritis, meningitis, endocarditis, and bacteremia (*3–6*). Since the mid-1980s, several studies worldwide have reported an increasing incidence of GGS bacteremia (*1,5–8*), but no recent study has been conducted in the United States to determine the incidence of overall GGS infection.

We noticed that an increasing number of patients with GGS have been admitted to Long Island College Hospital in Brooklyn, New York, USA, during the past few years. To better understand the trend of GGS infection in our institution, we retrospectively reviewed charts of patients admitted from January 2003 through December 2007 who had microbiologically proven GGS infection. Inclusion criteria were clinically and microbiologically documented GGS infection in patients who received appropriate antimicrobial drugs and were ≥18 years of age. Lancefield GGS were identified in the laboratory by latex agglutination test; resistance profiles were not done for GGS.

A total of 73 persons with GGS were admitted to the hospital during the 5-year study period; the number of patients admitted increased yearly (Figure). Mean age of patients was 53 years; most (77%) were <65 years of age; 52% were women, and most (61%) patients were African American. Thirty (41%) patients had polymicrobial infections; other identi-

fied organisms included methicillin-susceptible *Staphylococcus aureus* (8 [11%]), methicillin-resistant *S. aureus* (MRSA) (9 [12%]), and gram-negative or anaerobic organisms (13 [18%]).

The spectrum of GGS infections ranged from mild skin and soft tissue infection (34 [46%]) to invasive diseases, including urogenital infection (7 [10%]); lower respiratory tract infection (7 [10%]); pharyngitis (6 [8%]); endocarditis and catheter infection (5 [7%]); and others (14 [19%]), such as peritonitis, pelvic abscess, rectal abscess, and septic arthritis. Four of the 6 persons with pharyngitis were assumed to be colonized with the organism. Eight (24%) of 34 skin and soft tissue infections were associated with bacteremia, 5 (15%) with osteomyelitis, and 20 (59%) with polymicrobial infections. Six persons with lower respiratory tract infections and 1 each with endocarditis, genital tract infection, pelvic abscess, and dental abscess also had polymicrobial infections.

Eighteen persons had bacteremia, the trend of which also increased yearly. Of these, 8 had skin and soft tissue infections, 4 had endocarditis, 2 had urinary tract infections, 1 had possible spontaneous bacteria peritonitis, and 1 had hemodialysis catheter infection; 2 were of unknown source. Of the patients with endocarditis, 2 had vegetations on the native valves, 1 had a pacemaker infection, and 1 had prosthetic valve vegetation. One case of native valve endocarditis occurred in a tricuspid valve in an injection drug user. Another case occurred in a patient in which an epidural abscess was associated with an aortic valve vegetation.

Most of the patients had underlying medical conditions; 34% had diabetes mellitus. In contrast to previous reports, which stated that malignancy was the most common underlying disease (*2,3*), only 7 (10%) of the patients in our study group had underlying malignancy, of whom 4 had active malignancy and the rest had had previous malignancy. Nine patients with a history of injection drug use and 5 with HIV infection were identified; the patient with bacteremia secondary to hemodialysis catheter infection had a history of both HIV and intravenous drug use.

Three (4%) patients died; their deaths were unlikely to be attributable to GGS because all were elderly (78–92 years) and had underlying coexisting conditions and co-infections. All 4 persons with endocarditis and the patient with the catheter infection survived. Five patients who were co-infected with MRSA were treated with vancomycin or daptomycin; the remainder were treated with β-lactam antimicrobial drugs and had the sources of infection (catheter or pacemaker) removed. When infections caused by gram-negative or anaerobe organisms were identified, they were also treated with appropriate antimicrobial drugs. The overall average length of stay for all patients with GGS was 9.4 days, with longer stays for those with underlying diabetes mellitus (14.6 days) than for those without diabetes (6.7 days).

GGS was an important etiologic agent for a wide spectrum of infections. Its impressive increase in our institution during the past 5 years raises concerns because other types of β-hemolytic streptococcal infection have increased recently. Group A and B (*9,10*) increased substantially during the 1980s. A multicenter analysis may confirm GGS as an emerging human pathogen and may help us better understand the reason for this increase.

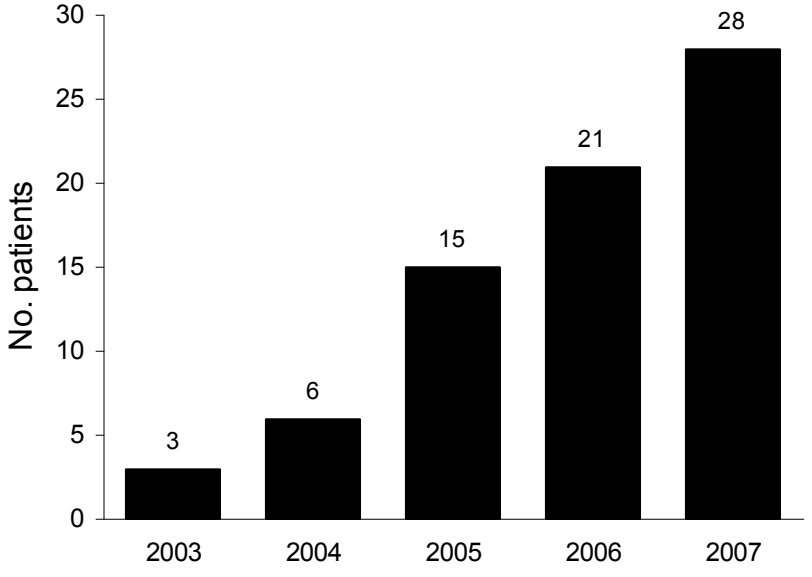

Figure. Annual number of patients with group G streptococcal infections admitted to Long Island College Hospital, Brooklyn, New York, USA, 2003–2007.

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New Saffold Cardiovirus in Children, China

To the Editor: A new member of the genus *Cardiovirus*, termed *Saffold virus* (SAFV), was discovered recently in stool specimens and nasopharyngeal aspirate samples from patients with fever of unknown origin, respiratory symptoms, or gastroenteritis; these have been considered the first documented reports of cardiovirus infection in humans (*1*–*4*). However, the epidemiologic characteristics and pathogenic role of the virus are not fully understood.

From July 2006 through June 2008, stool specimens were collected from 631 hospitalized children with diarrhea and 161 asymptomatic controls in Lanzhou, People's Republic of China. All children were <5 years of age (median age 8 months, range 0–60 months). Diarrhea was defined as ≥3 loose stools in the previous 24–72 h. Controls were asymptomatic children who had been brought to the First Hospital of Lanzhou University Pediatric Primary Care Center for a routine checkup and had not had fever, diarrhea, vomiting, or a respiratory illness in the previous 3 weeks. The stool specimens were then transported to the Chinese Center for Disease Control and Prevention, Beijing, to undergo screening for common enteric viruses. The specimens were tested for rotavirus by using a commercially available ELISA kit (IDEIA Rotavirus; DAKO, Glostrup, Denmark), and PCR and reverse transcription PCR (*5*) were used to screen for other common enteric viruses, including norovirus, sapovirus, astrovirus, and adenovirus.

Viral RNA and DNA were extracted from 140 μL of 10% fecal suspension in phosphate-buffered saline by using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany); viral RNA and DNA was supposed to be extracted simultaneously, according to the manufacturer's instructions. Extracts of nucleic acid were tested for SAFV by a nested PCR that targeted the 5′ untranslated region (UTR) gene as described by Drexler et al. (*4*). The viral protein 1 (VP1) gene from positive samples was amplified as described by Chiu et al. (*3*). Positive bands were cloned and sequenced in both directions.

By confirming sequences of the 5′ UTR gene, 3 (0.5%) specimens from the 631 children with diarrhea (LZ50419, LZ52903, LZ53879) and 1 (0.6%) from the 161 asymptomatic children (LZ53010) were found to be positive for SAFV. Of the 4 positive specimens, 2 were collected in October, 1 in September, and 1 in June. The median age of the 4 patients with positive specimens was 6 months (range 2–25 months). Viral co-infection was detected in the 3 children with diarrhea who had SAFV-positive specimens; 2 were co-infected with rotavirus and 1 with norovirus. No co-infection was detected in the asymptomatic child with SAFV-positive results.

The 0.5% detection rate of SAFV in children with diarrhea in our study is lower than the 1.2% reported by Chiu et al. (*3*). One possible reason could be that the patients in our study were younger (median age 8 months), but in other studies, the median age was 20 months for all patients with confirmed cases. Nonetheless, the seasonal distribution of the positive cases in our study is in accordance with the result of Drexler et al. (*4*), namely, in late summer and early fall.

The 5′ UTR sequences of the 4 positive samples were deposited in GenBank (accession nos. FJ586238, FJ586239, FJ610244, and FJ623968). After several trials, only the VP1 sequence of sample LZ50419 was amplified (accession no. FJ586240). The obtained sequences were analyzed by using the DNASTAR software package (DNASTAR, Madison, WI, USA). A BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) demon-

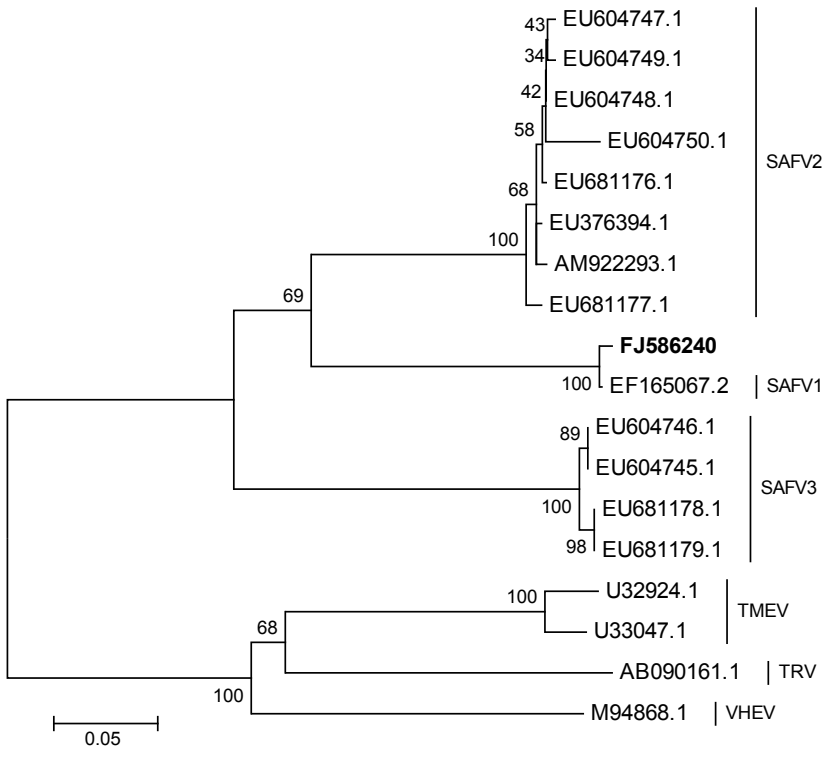

Figure. Phylogenetic relationships of deduced partial viral protein 1 amino acid sequences. Phylogenetic analyses using MEGA version 3.1 (www.megasoftware.net) and the neighbor-joining algorithm calculated by the Poisson correction model were based on alignment of 289 amino acids. The new strain from this study is shown in **boldface**. The scale bar indicates the genetic distance of 0.05 substitution/site. SAFV, Saffold virus; TMEV, Theiler's murine encephalomyelitis virus; TRV, Thera virus; VHEV, Vilyuisk human encephalomyelitis virus.

strated that that 5′ UTR sequences of the 4 positive samples— LZ50419, LZ52903, LZ53010, and LZ53879— had nucleotide identity of 96%, 91%, 96%, and 95% to the SAFV prototype strains (SAFV 1 California/81, EF165067.2), respectively; the VP1 sequence of sample LZ50419 had a nucleotide identity of 87% and amino acid identity of 97% with the SAFV1 California/81. Phylogenetic analysis (Figure) showed that the SAFV found in China clustered with the strain isolated in the United States in 1981. This finding suggests that the 1981 VP1 lineage was still circulating. More study is needed to address genetic variation of this lineage.

Our finding of SAFV in children hospitalized with diarrhea in China suggests that the virus is distributed worldwide. The detection of the virus in the asymptomatic control in our study is also noteworthy. Fisher exact test results showed no significant difference in the detection rate between the case group and the control group. The concurrent detection of SAFV and other enteric viruses raises concern over a causative role of SAFV in human gastroenteritis. The current study does not suggest that SAFV has any association with acute enteritis based on a statistical analysis. Also, as with several other novel viruses discovered recently, SAFV has not been associated with any clinically relevant disease in humans, although it has been isolated from cell cultures (*4*). Thus, we presume that SAFV is only a gastroenteric passenger because most picornaviruses are associated with enteric infection and with a known fecal–oral route of transmission. More comprehensive studies are needed to ascertain whether SAFV has any clinical relevance.

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Methicillin-Resistant *Staphylococcus aureus* USA400 Clone, Italy

To the Editor: In the past 30 years, methicillin-resistant *Staphylococcus aureus* (MRSA) has been the leading cause of nosocomial infections throughout the world. Healthcare-associated MRSA (HA-MRSA) isolates are resistant to multiple antimicrobial drugs. This resistance severely hampers treatment options. During the past decade, MRSA isolates have also emerged as major pathogens in the community, first in the United States and later worldwide. Community-associated MRSA (CA-MRSA) isolates are usually more susceptible to antimicrobial drugs but are more virulent than HA-MRSA isolates. Among various determinants involved in the pathogenesis of CA-MRSA infections, special attention has been focused on the Panton-Valentine leukocidin (PVL), which has a strong epidemiologic link with CA-MRSA clones (*1*).

It has been suggested that CA-MRSA might move to healthcare settings, blurring the line between HA- and CA-MRSA (*2*). Nevertheless, CA-MRSA isolates are increasingly being reported as pathogens in the general population in persons with no risk factors for HA-MRSA acquisition. These pathogens are generally associated with skin and soft tissue infections, but also with more severe infections such as necrotizing pneumonia or septicemia. CA-MRSA strains usually harbor a staphylococcal cassette chromosome (SCC) *mec* (type IV or V) that is smaller than the type I–III SCC*mec* elements commonly found in HA-MRSA strains. To date, 5 major CA-MRSA clonal lineages from diverse genetic backgrounds have been recognized by pulsed-field gel electrophoresis and multilocus sequence typing; certain clones predominate in specific areas of the world (*1*).

The most common lineages in the United States are sequence type (ST) 1 (USA400) and ST8 (USA300), which usually carry type IV SCC*mec* and PVL-encoding genes. Over the past few years, ST8 (USA300) has become predominant in the United States (*3*), also emerging as a major cause of nosocomial infections (*4*). In Europe, data are more limited, but the situation appears to be more varied: the predominant CA-MRSA clonal lineage is ST80 (*5*), although single cases or small clusters caused by ST8 (USA300) have increasingly been reported (*6–8*). In contrast, the ST1 (USA400) clone is still rare in Europe (*9*,*10*). We describe the importation of ST1 (USA400) into Italy and its isolation in the country. The organism was isolated from an Italian woman with a skin infection that she contracted in the United States.

In late November 2007, a 36-year-old Italian woman was seen at Pordenone Hospital (northeastern Italy) for spider-bite–like skin lesions on the face, characterized by rapid evolution to furuncles and small abscesses. The infection had started ≈1 month earlier in California, where she had spent several months on business (wine import-export), and where she had been treated empirically with amoxicillin/clavulanate for 10 days (1 g, 3×/day), with no clinical improvement.

Culture of the pus from the abscesses yielded an MRSA isolate that was resistant to oxacillin and susceptible to all non-β-lactam antimicrobial drugs tested by Vitek 2 AST-P536 card (bioMérieux, Marcy l'Etoile, France). Such a particular susceptibility pattern and the community origin of the infection prompted molecular investigation and typing by established methods, which confirmed the isolate to be CA-MRSA and identified it as belonging to the USA400 clone (ST1, type IVa SCC*mec*, presence of PVL genes, *agr* type III, *spa* type t128). Notably, t128 is the *spa* type found in MW2, the highly virulent prototype strain of USA400. Treatment with oral levofloxacin for 7 days (500 mg, 1×/day) led to complete resolution of the infection. After more than a year, the patient has experienced no recurrences.

All 3 previously reported cases of CA-MRSA infection in Italy were caused by type IV SCC*mec*, PVL-positive strains, none of which, however, belonged to the ST80 clonal lineage that predominates in Europe (*7*). The first case (in 2005) was a necrotizing pneumonia caused by an ST30 isolate; the 2 other cases (2006) were severe invasive sepsis and a neck abscess, both caused by ST8 (USA300) isolates. The case we note here documents the importation of a US pathogen into a country in Europe, from an area where the pathogen is widespread and has been highly virulent since the late 1990s, to an area where its penetration in the past has been poor.

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Meningitis and Radiculomyelitis Caused by *Angiostrongylus cantonensis*

To the Editor: *Angiostrongylus cantonensis* infection is endemic in regions such as Southeast Asia, China, the Pacific Basin, and the Caribbean, but international travel has spread the disease elsewhere, including Europe (*1–10*). Dissemination of the parasite to many regions has also occurred because of the ship-borne international migration of rats and the diversity of potential intermediate hosts. The target organ in humans is the central nervous system in which an eosinophilic reaction develops in response to dying larvae. We report a case of eosinophilic meningitis and lumbosacral myeloradiculopathy caused by *A. cantonensis* and present a review of cases of *A. cantonensis* infections from Europe.

A 47-year-old merchant seaman was admitted to the University Hospital of Infectious Diseases, Zagreb, Croatia, in March, 2006 on the 17th day of illness because of fever, headache, vomiting, and constipation. At the end of the first week of illness, paresthesias developed in his feet; on the 10th day of illness, he also noticed difficulties with urination. He had returned from a 1-month trip to Southeast Asia (Malaysia and Singapore) 35 days before the onset of symptoms and recalled eating vegetables and salads. He also consumed shrimp, but he believed that they were from salt water. On physical examination, we noticed increased muscle tone, tremor of the tongue and upper limbs, and decreased deep tendon reflexes of the lower limbs. He experienced urinary retention, and catheterization was required. Saddle anesthesia was observed. There was no neck stiffness, and the results of the rest of the physical examination were normal.

His blood leukocyte count was 11.5 × 10^9/L with 80% neutrophils, 12% lymphocytes, 4% monocytes, 2% basophils, and 2% eosinophils. Cerebrospinal fluid (CSF) analysis showed 320 cells/μL with 6.5% eosinophils (21 eosinophils/μL). Results of CSF testing by PCR for herpes simplex virus 1 (HSV-1) and HSV-2 DNA were negative, as were cultures for bacteria, mycobacteria, and fungi. Results of serum and CSF antibody tests for *Borrelia burgdorferi*, *Treponema pallidum*, HSV-1, HSV-2, tick-borne encephalitis virus, *Toxoplasma gondii*, *Taenia solium, Toxocara* spp., and *Trichinella* spp. were also negative. Results of stool examination for *Ascaris lumbricoides*, *Trichuris trichiura, Taenia* spp., *Giardia intestinalis*, *Strongyloides* spp., and *Entamoeba histolytica* were negative. The patient was also negative for HIV by ELISA. Magnetic resonance imaging scans of the brain and spine were unremarkable. *A. cantonensis* infection was diagnosed by immunoblot testing at the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok. Antibodies against *A. cantonensis* 31-kDa antigen were detected in serum and CSF of the patient; antibodies against *Gnathostoma spinigerum* were not detected.

Treatment was symptomatic; to lessen the headache, 4 lumbar punctures were performed. After 1 month, the patient's general condition was greatly improved; however, minor symptoms such as diminished concentration, slow thinking, and mild headache persisted. Urinary retention lasted for 38 days, and the patient had occasional mild headaches and paresthesia in his feet for the next 5 months.

Because large numbers of persons from Europe travel to destinations where angiostrongyliasis is endemic, it is somewhat surprising that the infection has been rarely described in Europe. In a Google and Medline

Internet literature search, we identified 9 additional case reports and 1 report on a cluster of 5 *A. cantonensis* infections. The first report was in a 14-month-old child born in Tahiti who became ill in France in 1988 (*1*). Eight cases, as did our case, involved travelers returning to Europe after a visit to disease-endemic areas (*2–9*) (Table). Not all cases were serologically confirmed, most likely because antibody tests for *A. cantonensis* infection have not been widely available. In a retrospective cohort study, 5 French policemen, who returned from French Polynesia with severe headache and blood eosinophilia, were believed to have eosinophilic meningitis caused by *A. cantonensis* (*10*). However, CSF examination was performed in only 1 patient, and none of the cases were serologically confirmed.

Definitive diagnosis of angiostrongyliasis would require identification of larvae or young adults in human tissue, such as the brain, CSF, and eye chamber, which is rarely achieved. Thus, the diagnosis is usually made on the basis of serologic test results. Specific *A. cantonensis* antigens (29 kDa, 31 kDa, and 32 kDa) were identified; antibodies against these antigens can be detected by ELISA, dot-blot ELISA, or Western blot.

In most patients, *A. cantonensis* causes a benign and self-limiting disease; treatment is usually symptomatic. Data are limited, but mostly favorable, on the use of steroids, albendazole, and mebendazole. However, the administration of antihelmintics without steroids is not recommended because such treatment might elicit deleterious inflammatory responses to dying worms within the nervous system or ocular structures.

In summary, the presence of headache, fever, and paresthesias in travel-

Table. Epidemiologic and clinical findings from 11 reported case-patients with *Angiostrongylus cantonensis* infection, Europe*

Country, year of report	Patient age, y/ sex	Possible country of origin	Suspected food	Clinical features	Diagnosis	Treatment	Ref.
France, 1988	1/F	Tahiti	Not reported	Eosinophillic meningoencephalitis, lumbosacral myeloradiculitis, tetraplegia coma, hypertensive hydrocephalus, paresthesias	IFA	Thiabendazole, steroids, ventriculoperitoneal catheter	(*1*)
Switzerland, 1995	46/F	Tahiti	Freshwater shrimp	Eosinophilic meningoradiculits	Clinical	Supportive	(*2*)
France, 1996	25/F	Tahiti	Raw fish	Eosinophilic meningitis, paresthesias (left lower leg)	Clinical	Supportive	(*3*)
France, 2002	16/M	Tahiti	Not reported	Eosinophilic meningoencephalitis, cranial palsy (n. abducens), cerebellar syndrome, paresthesias	IFA	Ivermectin	(*4*)
Switzerland, 2004	26/M	Cuba	Not reported	Eosinophilic meningitis, generalized hyperesthesias	WB	Supportive	(*5*)
Germany, 2006	27/F	Dominican Republic	Not reported	Eosinophilic meningitis, paresthesias (right elbow, right thigh)	WB	Albendazole, steroids	(*6*)
Italy, 2007	30/M	Dominican Republic	Freshwater shrimp	Eosinophilic meningitis, generalized paresthesias	Clinical	Mebendazole,† steroids	(*7*)
UK, 2007	30, F	Thailand	Snails	Eosinophilic meningitis, cranial nerve palsy (right n. abducens), altered sensation (lateral border of right leg)	Serologic‡	Supportive	(*8*)
Belgium, 2008	22/F	Costa Rica, Ecuador, Chile, Argentina, Fiji Islands	Sashimi, ceviche (raw fish), salads	Eosinophilic meningitis, paresthesias (left hemithorax, feet)	WB	Albendazole, steroids	(*9*)
France, 2008§	26–36/M	French Polynesia	Uncooked freshwater prawns	Eosinophilic meningitis¶	Clinical	Ivermectin (1 patient) or albendazole; steroids (1 patient)	(*10*)
Croatia	47/M	Malaysia, Singapore	Vegetables, salads, shrimp#	Eosinophilic meningitis, lumbosacral myeloradiculitis (conus medullaris syndrome), generalized paresthesias	WB	Supportive (repeated lumbar puncture)	This study

*Ref., reference; IFA, immunofluorescent antibody assay; WB, Western blot.
†One dose only.
‡Method not specified.
§Five French policemen.
¶Diagnosis confirmed by cerebrospinal fluid analysis in only 1 patient.
#The patient thought he consumed saltwater shrimp but was uncertain.

ers returning from disease-endemic areas should alert clinicians to the possibility of eosinophilic meningitis caused by *A. cantonensis*. With growing international travel, physicians may encounter *A. cantonensis* infection more frequently.

Acknowledgment

We thank our patient for his consent to publish this case report.

Tomislav Maretić, Marta Perović, Adriana Vince, Davorka Lukas, Paron Dekumyoy, and Josip Begovac

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Sex, Sin, and Science: A History of Syphilis in America

John Parascandola

Praeger Publishers, Santa Barbara, CA, USA, 2008
ISBN-10: 0275994309
ISBN-13: 978-0275994303
Pages: 224; Price: US $49.95

At of end his book, John Parascandola writes, "It is my hope that the reader ... acquired a broader appreciation of disease as a social as well as a medical construct and of the way in which social and cultural factors influence our understanding of and reaction to any given disease." The social context of syphilis is nicely summarized by the title, Sex, Sin, and Science: A History of Syphilis in America. Because syphilis is sexually transmitted, it is often considered as a moral issue, and thus people who have syphilis have sinned. This perception is both absurd and insightful.

Parascandola's history of syphilis is compelling from the beginning. "Because many believed that the disease first made its appearance in the French troops besieging Naples, it was often called (especially by the Italians) *morbus gallicus* ('French disease') ... The French, on the other hand, preferred to call it the 'Neapolitan disease' blaming it on the city of Naples."

Syphilis was so sinful that it could not be discussed by name. "This continued hesitancy to discuss sexual matters is reflected in the terminology used in newspapers and other public media of the early twentieth century." "Social evil" meant prostitution. Syphilis and gonorrhea were "social diseases," and the effort to combat them was the "social hygiene" movement. We learn that in 1911 California became the first state to require physicians to report cases of venereal disease and that, to assure confidentiality, reporting was done by number rather than by name.

Some of those involved in the social hygiene movement were more interested in preventing sex than in preventing disease. A Public Health Service (PHS) advisory committee recommended changing an educational film so that "'some attention be given to the influence of moral standards on the spread of disease' because if no reference was made to moral issues, it might appear to some that the PHS was 'condoning sexual promiscuity.'"

There was fear that penicillin might offer "complete freedom to indulge in licentiousness..." or "if extramarital sex did not lead to significant illness, only a 'few intangibles of the spirit' would remain to guide people into moral paths." Parascandola notes, "Social hygienists had always been at least as interested in moral as in health issues, and so their fight would not end with the defeat of venereal disease."

Parascandola's book informs readers that in 1953, "the Eisenhower administration proposed eliminating the PHS venereal disease program because its job was essentially done." But syphilis came back, along with gonorrhea, herpes, chlamydia, and AIDS.

There is considerable scientific evidence that HIV causes AIDS. Nonetheless, just as with syphilis, others think the cause is sin. Absurd. Yet the social construct Parascandola describes remains so pervasive that it continues to affect us all.

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Novel and Re-emerging Respiratory Viral Diseases: Novartis Foundation Symposium 290

Gregory Bock and Jamie Goode, editors

John Wiley & Sons, Ltd, Chichester, UK, 2008
ISBN: 978-0-470-06538-9
Pages: 174; Price: US $170.00

This slim booklet, the product of a Novartis Foundation symposium held April 23–27, 2007, at Singapore's Institute of Molecular and Cell Biology, primarily highlights scientific issues concerning influenza and severe acute respiratory syndrome (SARS). The booklet comprises a mere 12 chapters of about 12 pages each of reports presented and discussed by only 29 participants. This may be its strength—the book's usefulness derives from its focus on only 2 diseases covered by multidisciplinary participants in a cross-cutting fashion and relative depth.

The book contains little new material. Rather, the chapters are brief but state-of-the-art reviews that, perhaps surprisingly, fit together well. For example, Gabriele Neumann and Yoshi Kawaoka discuss broad aspects of pandemic influenza; other scientists discuss the transmission and pathogenicity of influenza viruses A (H5N1), their genetic and antigenic characteristics, general antigenic associations between human and swine viruses, and the molecular aspects of viral membrane fusion. Reading for less than half an hour yields an awareness of many key issues surrounding influenza emergence, well reviewed by international experts. The chapters about SARS are equally strong, with presentations by teams from both Taiwan and Singapore highlighting their real-world experiences in an epidemic

crisis. More general presentations by Larry Anderson and Suxiang Tong (characterization of novel viruses) and by Malik Peiris and Yi Guan (the animal–human interface) provide excellent background and balance. A particularly interesting and strong chapter by Eddie Holmes reviews viral evolution and emergence, analyzing viral host-switching and the theoretical frameworks used to study it.

The chapters are all well written, well edited, succinct, and readable, apparently aimed at scientists—readers familiar with both research and public health aspects of emerging viral diseases. A bonus is inclusion of discussions by the participants at the end of each chapter. Although the value of these varies, in many cases the discussion provides additional perspectives that otherwise would be missed.

The book is a pleasant surprise: modest, succinct, authoritative, readable, and enjoyable. It is particularly valuable for scientists and advanced students who either work with the 2 diseases in question or who work with issues of viral disease emergence.

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Vincent van Gogh (1853–1890) The Potato Eaters (1885) (detail) Oil on canvas (81.5 cm × 114.5 cm) Van Gogh Museum, Amsterdam, the Netherlands (Vincent van Gogh Foundation)

"Sometimes the naked taste of potato reminds me of being poor"

—Leonard Nathan

Polyxeni Potter

"I want to paint men and women with that something of the eternal which the halo used to symbolize and which we seek to convey by the actual radiance and vibration of our coloring," wrote Vincent van Gogh in one of his celebrated letters. Hundreds of these were written mostly to his brother Theo, an art dealer in Paris who provided him with financial and emotional support throughout his brief but brilliant career. The letters lay out the artist's philosophy of life and reveal ample literary inclinations as well as spiritual depth. "Saying a thing well is as interesting and as difficult as painting it," he wrote.

Van Gogh was born in Zundert, the Netherlands, and was raised in a religious albeit not always harmonious household. "Father cannot understand or sympathize with me …. I too read the Bible … as I read Michelet or Balzac or Eliot … and what Father in his little academic way gleans from it I cannot find in it at all." Nonetheless, Vincent tried to follow in his father's evangelical footsteps, but his youthful zeal and empathetic ministry were misinterpreted by the hierarchy of the Dutch Reformed Church. They rejected him, ending his studies in theology and his tenure as missionary to a coal mining community in Belgium.

"Even in that deep misery," he wrote about his rejection, "I felt my energy revive, and I said to myself, in spite of everything I shall rise again: I will take up my pencil, which I had forsaken in my discouragement, and I will go on with my drawing." Van Gogh began his artistic career at age 27, while still in Belgium, by painting peasants, whom he perceived as closer to nature than other people, in the manner of his contemporary Jean-François Millet. And with as much zeal as he had pursued his religious mission, he now tried to capture the divine in everyday life.

The Potato Eaters, on this month's cover, was van Gogh's first major work. This painting of a family gathered around the table for the evening meal reflected his preoc-

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cupation with the plight of the poor, whose lives he had experienced from close up. "The point is," he wrote to Theo, "I've tried to bring out the idea that these people eating potatoes by the light of their lamp have dug the earth with the self-same hands they are now putting into the dish, and it thus suggests *manual labor* and a meal honestly *earned*."

Depicting night scenes was a creative outlet, a way to test technical innovations and explore the relationship between the cycles of nature and rural life. He drew from the traditions of the 17th-century Dutch masters, particularly Rembrandt, and the Barbizon school landscape painters Charles Daubigny and Jules Dupré. He was also influenced by the impressionists, the pointillists, and Japanese printmakers Hiroshige and Hokusai. Before van Gogh's untimely death at age 37, these diverse influences had culminated in a unique style, the blend of striking colors and riveting brushstrokes.

"When weavers weave that cloth which I think they call cheviot, or those curious multicolored Scottish tartan fabrics," van Gogh wrote in reference to the coloring in the Potato Eaters, "then they try, as you know, to get strange broken colors and grays into the cheviot and to get the most vivid colors to balance each other in the multicolored chequered cloth so that instead of the fabric being a jumble, the … pattern looks harmonious from a distance."

The somber hues and harsh texture of The Potato Eaters went against convention, as did the exaggerated features of the peasants. "I've held the threads of this fabric in my hands all winter long and searched for the definitive pattern," he wrote, "and although it is now a fabric of rough and coarse appearance, the threads have nonetheless been chosen with care and according to certain rules. And it might just turn out to be a *genuine peasant painting. I know that it is*."

But despite van Gogh's efforts, this night scene was not well received. It was perceived as not realistic enough, awkward, even technically incorrect. His artistic goals were not understood. "What I try … is not to draw a hand, but the gesture, not a mathematically correct head, but the general expression …." And his preliminary work was not appreciated. He had done extensive drawings and visited a local family regularly, sketching while they ate. "By continually observing peasant life, at all hours of the day, I have become so involved in it that I rarely think of anything else."

The life of the poor was also on the literary minds of van Gogh's day. In 1884–85, naturalist author Émile Zola wrote Germinal, his novel about a coal miners' strike in northern France in the 1860s. This famous account of poverty and oppression struck a nerve even if it did not end mining strikes or the misery that brought them about. This "wholly different way of life from ours" continued. "According to official statistics just made public for the last six years," the New York Times reported in 1901, "an average of 150,000 persons have yearly died in France from consumption, while in Paris alone the total for that period has been 83,274 deaths …. All classes have suffered from the disease, but it has been particularly fatal in those sections of the city occupied by working families."

When he "took up his pencil" against the values of industrial society, van Gogh made no effort to sugarcoat anything. He knew squalor. "Miners, men and women, going to the shaft in the morning through the snow, by a path along a hedge of thorns," after a day of exhausting labor, they had the color of a "very dusty, unpeeled potato," their postures showing isolation and resignation.

Poverty, with its attendant malnutrition and crowding, so well captured by van Gogh in The Potato Eaters and by Zola in Germinal, always has been a hotbed of emerging infections. No longer referred to as consumption, TB is still a killer, its rates disproportionately high among the poor. Lice and other pests thrive among the homeless, spreading trench fever and other infections. And proximity to domestic animals and rodents in crowded areas expands the range of influenza, spotted fevers, and plague. But public health efforts to prevent and control the effects of poverty persist. This hope recalls the message of Germinal, "Beneath the blazing sun, in that morning of new growth, the countryside rang with song, as its belly swelled with a black and avenging army of men, germinating slowly in its furrows, growing upwards in readiness for harvests to come, until one day soon their ripening would burst open the earth itself."

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

Factors Affecting Incidence of Human Infection with Puumala Virus, Germany

Azole-Resistant *Asperigillus fumigatus* and Treatment Failure

Replacement of North American Influenza Clade with Eurasian Avian Influenza Virus Subtype H6

Relapse Associated with Active Disease Caused by Beijing Strain of *Mycobacterium tuberculosis*

Lymphocytic Choriomeningitis Virus in Wild Rodents, Italy

Effect of Species Diversity on Prevalence of Sin Nombre Virus

Tick-borne Relapsing Fever and *Borrelia hermsii*, California, USA

Sylvatic Epidemic Typhus Cases Associated with Flying Squirrels, 2004–2006

Multiple Origins of Foot-and-Mouth Disease Virus Serotype Asia 1 Outbreaks, 2003–2007

Spread of Beijing Genotype Strains of Multidrug-Resistant *Mycobacterium tuberculosis*, Europe

Co-infections with Chikungunya Virus and Dengue Virus, India

Case of Eczema Herpeticum that Meets Clinical Criteria for High Risk of Smallpox

Recurrent Lymphocytic Meningitis Positive for Herpes Simplex Virus Type 2

Rickettsia slovaca and *R. raoultii* in Tick-borne Rickettsioses

Latent Tuberculosis and Disease Rates among the Homeless, New York, NY, USA

Influenza Virus A (H1N1) in Giant Anteaters

Genetically Diverse Coronaviruses in Wild Bird Populations, Northern England

Human T-lymphotropic Virus 1 among Blood Donors, Israel

Intergenogroup Recombinant Sapovirus in Japan, 2007–2008

Chinese-like Strain of Porcine Epidemic Diarrhea Virus, Thailand

Complete list of articles in the July issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

June 4–5, 2009
Drug-resistant and Vaccine-escape Hepatitis B Virus Mutants: Emergence and Surveillance
Atlanta, GA, USA
http://www.cdc.gov/hepatitis/hbvsymposium2009

June 18–21, 2009
26th International Congress of Chemotherapy and Infection
Sheraton Centre Toronto Hotel
Toronto, Canada
http://www.icc-09.com

June 21–23, 2009
6th International Congress on Bartonella as Medical and Veterinary Pathogens
Queen Hotel
Chester, United Kingdom
http://www.liv.ac.uk/university/vets/cpd/documents/All_information_bartonella.pdf

June 28–July 1, 2009
18th ISSTDR: International Society for STD Research
QEII Conference Centre
London, United Kingdom
http://www.isstdrlondon2009.com

August 3–4, 2009
UC Berkeley CIDER SUMMIT 2009: "Advances in the Control and Epidemiology of Emerging Infectious Diseases"
Sheraton Gateway Hotel
Burlingame, CA, USA
http://www.idready.org/CIDERSummit2009.html

August 10–21, 2009
11th International Dengue Course
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Article Title

Past, Present, and Possible Future Human Infection with Influenza Virus A Subtype H7

CME Questions

1. Which of the following best describes the anticipated attack rate of a pandemic influenza virus on the basis of attack rates in past pandemics?

A. 10% to 15%
B. 25% to 35%
C. 40% to 50%
D. 55% to 65%

2. Which of the following is least likely to be an early clinical manifestation of H7 influenza virus?

A. Pneumonia
B. Conjunctivitis
C. Coryza
D. Encephalitis

3. Which of the following is the most likely reason for expectations of future human infection with the H7 avian influenza virus?

A. Increased detection in nonpoultry farm animals
B. Increased frequency of human and poultry infection
C. Increased detection of human infection in the African continent
D. All of the above

4. Which of the following best describes the difference between infection with H5N1 and H7 subtypes of the avian influenza virus in humans?

A. H5N1 manifests most frequently as neurologic disease
B. H7 most frequently manifests as conjunctival disease
C. H7 manifests only rarely as respiratory disease
D. The 2 infections are indistinguishable clinically

5. Which of the following strategies is considered the best protection of humans against avian influenza viruses?

A. Antiviral agents
B. Quarantine and slaughter of infected poultry
C. Vaccination of humans
D. Handwashing hygiene measures

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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Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief 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").

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Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. 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.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Name, publisher, number of pages, other pertinent details should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted online only, depending on the event date.)

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only. Summaries, which should contain 500–1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.