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On the Cover

Clive Hicks-Jenkins (b. 1951)
The Prophet Fed by a Raven (2007)
Acrylic on panel (62 cm × 82 cm)

Courtesy of the artist, private collection, www.hicks-jenkins.com

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Influenza A (H5N1) Viruses from Pigs, Indonesia

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Pigs have long been considered potential intermediate hosts in which avian influenza viruses can adapt to humans. To determine whether this potential exists for pigs in Indonesia, we conducted surveillance during 2005–2009. We found that 52 pigs in 4 provinces were infected during 2005–2007 but not 2008–2009. Phylogenetic analysis showed that the viruses had been introduced into the pig population in Indonesia on at least 3 occasions. One isolate had acquired the ability to recognize a human-type receptor. No infected pig had influenza-like symptoms, indicating that influenza A (H5N1) viruses can replicate undetected for prolonged periods, facilitating avian virus adaptation to mammalian hosts. Our data suggest that pigs are at risk for infection during outbreaks of influenza virus A (H5N1) and can serve as intermediate hosts in which this avian virus can adapt to mammals.

A highly pathogenic avian influenza virus A (H5N1) was first recognized among geese in Guangdong Province, southern People's Republic of China, in 1996 (*1*). Within a year, this goose virus underwent reassortment with viruses circulating in other avian species. By 1997, the virus had become widespread among poultry in Hong Kong, and direct avian-to-human transmission of influenza A (H5N1) viruses was reported (*2,3*). Since late 2003, influenza A (H5N1) viruses have spread to domestic poultry in other Southeast Asian countries (*4*). Since mid 2005, they have been detected across Asia, Europe, and Africa, causing severe damage to the poultry industry and infecting >490 humans, resulting in a mortality rate of 60% (*5–8*). Indonesia has been particularly affected by these viruses; >160 cases of human infection (i.e., about one third of the total confirmed human influenza A (H5N1) infections worldwide) and a mortality rate >80% have been reported (*8*). Hence, understanding prevalence and adaptation of influenza A (H5N1) influenza viruses in Indonesia is crucial.

Influenza viruses attach to host cells by binding their hemagglutinin (HA) to cell-surface oligosaccharides containing a terminal sialic acid. The HA of avian influenza viruses preferentially binds to sialic acid linked to galactose by α-2,3 linkages (SAα2,3Gal); that of human viruses binds to SAα2,6Gal (*9*). Correspondingly, epithelial cells in the upper respiratory tracts of humans mainly bear SAα2,6Gal receptors (*10,11*), and those in duck intestines (the major replication site for duck viruses) mainly possess SAα2,3Gal (*12*). Virus receptor specificities and expression patterns of receptors on host cells are thought to be major determinants of the host range restriction of influenza viruses (*13*). Thus, the recognition of human-type receptors by avian viruses appears to be necessary for these viruses to replicate in the upper respiratory tract and be transmitted efficiently from human to human. Given that influenza A (H5N1) viruses isolated from humans are not transmitted efficiently despite their ability to recognize human-type receptors (*14*), mutations in the polymerase and other viral genes may also be needed for replication of influenza A (H5N1) viruses in the upper respiratory tract (*15*).

Traditionally, pigs have been considered as "mixing vessels" (*16–19*) because they support replication of avian

and human influenza viruses (*17*). Their tracheal epithelial cells reportedly bear SAα2,3Gal and SAα2,6Gal receptors (*18*). However, recent studies have shown that despite SAα2,3Gal and SAα2,6Gal receptors in pig respiratory tracts, SAα2,3Gal is found only in the smaller airways (bronchioli and alveoli) and not in the trachea (*20,21*). Kuchipudi et al. (*22*) found SAα2,3Gal and SAα2,6Gal receptors in the bronchi, bronchioli, and alveoli of chickens and ducks; however, SAα2,6Gal was dominant in chicken tracheal epithelium, and SAα2,3Gal, in duck trachea. Given that influenza A (H5N1) viruses have been transmitted directly from birds to humans, the central dogma of pigs as a mixing vessel may no longer stand. Moreover, under experimental conditions, pig susceptibility to infection with avian influenza A (H5N1) viruses is low (*23*). Nevertheless, the pandemic (H1N1) 2009 virus is a reassortant that originated from 4 genetically distinct viruses and appeared to be generated in pigs (*24*), suggesting their role in the generation of pandemic influenza viruses. Infection of pigs with influenza A (H5N1) viruses has been reported in Vietnam (*25*) and China (*26*); however, the infection status of pigs in Indonesia remains unknown. We, therefore, explored whether pigs in Indonesia had been infected with influenza A (H5N1) viruses and, if so, whether the viruses were transmitted multiple times and had acquired the ability to recognize human-type receptors.

Materials and Methods

Specimen Collection

Virologic and serologic surveillance was conducted during 3 rainy seasons during 2005–2009: January–February 2005, October–February 2007, and, November 2008–April 2009. Nasal, fecal, and serum samples were collected from apparently healthy pigs in various districts of Indonesia (Figure 1). The nasal and fecal samples were injected into 10-day-old embryonated eggs, and the allantoic fluid was tested for hemagglutination. Hemagglutination-positive allantoic fluid was subjected to reverse transcription–PCR by using H5 HA–specific and N1 neuraminidase–specific primers; only positive samples were tested further.

Serum was analyzed to estimate the prevalence of influenza virus A (H5N1) infection.

Cells and Virus Isolation

MDCK cells and an MDCK cell line that overexpresses the human β-galactoside α-2,6-sialyltransferase I gene (MDCK-ST6GalI) (*27*) were maintained in minimal essential medium (MEM) containing 5% newborn calf serum at 37°C in 5% CO_2. Virus isolation from specimens was performed by using 10-day-old embryonated chicken eggs, MDCK cells, or MDCK-ST6GalI cells in MEM containing 0.3% bovine serum albumin (BSA) (Sigma-Aldrich, Inc., St. Louis, MO, USA) (online Appendix Table, www.cdc.gov/EID/content/16/10/1515-appT.htm). Viruses isolated in MDCK cells were used whenever they were available.

Serologic Analysis

Swine serum samples were tested for neutralizing antibodies against influenza A/swine/East Java/UT6040/2007 (H5N1) and A/duck/Czechoslovakia/56 (H4N6) viruses. Subtype H4N6 was used as a negative control. Serum was mixed with 3 volumes of receptor-destroying enzyme (Denka Seiken Co., Ltd, Tokyo, Japan) overnight at 37°C and inactivated at 56°C for 30 min. A 2-fold serial dilution series of serum (1:4–1:512) was mixed with an equal volume of influenza virus at 100 $TCID_{50}$ (50% tissue culture infectious doses) and incubated at 37°C for 30 min. Viruses were inoculated to monolayers of MDCK cells for 1 h, washed 2×, and incubated with MEM containing 0.3% BSA for 2 d at 37°C in a 5% CO_2 incubator. Cytopathic effects were observed to determine the neutralizing activity of the test serum. The detection limit for the neutralizing antibody was <4 dilutions of serum.

Sequence Analysis

To characterize the swine influenza A (H5N1) viruses isolated in Indonesia, we sequenced the HA genes of 39 viruses isolated from pigs in Banten, East Java, North Sumatra, and South Kalimantan provinces and grouped them according to their genetic similarities. Viral RNA was extracted with ISOGEN (Nippon Gene, Tokyo, Japan) ac-
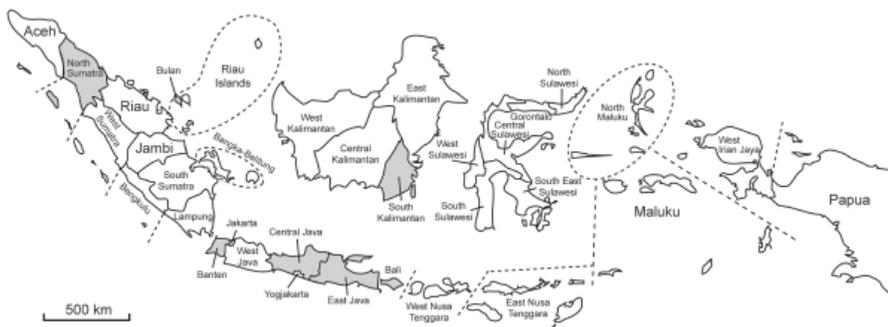
Figure 1. Provinces in Indonesia (gray shading) where surveillance for influenza A (H5N1) virus in pigs was conducted during 2005–2009.

cording to the manufacturer's instructions. Extracted RNA was reverse transcribed with SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and an oligonucleotide complementary to the 12-nt sequence at the 3′ end of the viral RNA and amplified by PCR with Pfu-ultra (Stratagene, La Jolla, CA, USA) or Phusion (Finnzymes, Espoo, Finland) high-fidelity DNA polymerase and primers specific for each segment of the influenza virus A (H5N1). Primer sequences are available upon request. The PCR products were separated by agarose gel electrophoresis, purified by using a MinElute Gel Extraction Kit (QIAGEN, Hilden, Germany), and then sequenced. The nucleotide sequences obtained in this study are available from GenBank, accession nos. HM440051–HM440154.

Phylogenetic Analysis

We phylogenetically analyzed 13 representative swine influenza A (H5N1) viruses for all 8 viral genes and compared these sequences with publicly available sequences. All sequences were assembled and edited with BioEdit 7 software (28). Neighbor-joining tree analysis was conducted by using ClustalW (www.clustal.org). Estimates of the phylogenies were calculated by performing 100 neighbor-joining bootstrap replicates.

Receptor Specificity Assays

During replication in pigs, avian influenza viruses may adapt to recognize human-type receptors because such receptors are present in the epithelial cells of pig trachea (18). We therefore analyzed the receptor specificity of representative viruses from each of the 3 swine groups: A/swine/Banten/UT3081/2005 for the 2005 swine group, A/swine/East Java/UT6012/2007 for the 2006–07 swine (A) group, and A/swine/Banten/UT6001/2006 for the 2006–07 swine (B) group. We also analyzed A/swine/Banten/UT3062/2005 clone 6 and A/swine/Banten/3063/05 clone 1, each of which possesses a single amino acid change in HA that distinguishes it from other clones. The receptor specificity of these influenza A (H5N1) viruses was determined by use of an assay that measures direct binding to sialylglycopolymers possessing either SAα2,3Gal or SAα2,6Gal. We used this solid-phase binding assay with the sodium salts of sialylglycopolymers (poly α-L-glutamic acid backbones containing N-acetylneuraminic acid linked to galactose through either an α-2,3 or -2,6 bond (Neu5Acα2,3Galβ1,4GlcNAcβ-pAP and Neu5Aαc2,6Galβ1,4GlcNAcβ-pAP) as described (29,30). Briefly, microtiter plates (Polystyrene Universal-BIND Microplate, Corning, NY, USA, USA) were incubated with glycopolymer in phosphate buffered saline (PBS) at 4°C for 3 h and then irradiated under UV light at 254 nm for 2 min. After removal of the glycopolymer solution, the plates were blocked with 0.1 mL PBS containing 2% BSA (Invitrogen) at room temperature for 1 h. After being washed 5× with PBS, the plates were incubated in a solution containing influenza virus (128 hemagglutination units in PBS) at 4°C for 12 h. After 3 more washes with PBS, antibody to the virus was added to the plates, which were then incubated for 2 h at 4°C, washed 3× with ice cold PBS, and then incubated with horseradish peroxidase–conjugated protein A (Organon Teknika N.V; Cappel Products, Turnhout, Belgium; 2000-fold dilution in PBS) at 4°C. After being washed 4× with ice-cold PBS, the plates were then incubated with o-phenylenediamine (Sigma-Aldrich) in PBS containing 0.01% H_2O_2 for 10 min at room temperature, and the reaction was stopped by adding 0.05 mL of 1N HCl. Absorbance was determined at 490 nm.

Results

Virus Prevalence

Of 702 nasal swabs, 52 (7.4%) collected in 2005–2007 yielded influenza A (H5N1) viruses (Table 1; online Appendix Table); no virus was isolated from fecal samples of the same pigs. All 35 viruses isolated in 2005 were from 5 pig farms in the Tangerang District of Banten Province, near an area in which an influenza A (H5N1) outbreak among poultry had been confirmed in 2004 (31) and where the virus has since remained enzootic. Samples collected from a slaughterhouse in the Surabaya district of East Java Province were negative for influenza virus A (H5N1). In the subsequent surveillance period, October 2006–February 2007, we detected viruses in pigs on 4 farms in the Tangerang, Kediri, and Medan districts of Banten, East Java, and North Sumatra, respectively, and in slaughterhouses in the Surabaya and Banjarmasin districts of East Java and South Kalimantan; all sites were near previous outbreaks of influenza virus A (H5N1) infection among poultry. Pigs from which these viruses were isolated did not show any signs of influenza-like illness at the time of sample collection. During the November 2008–April 2009 surveillance period, virus was not isolated from any nasal swabs from 300 pigs tested. However, the 300 serum samples tested indicated that 3 (1%) pigs had neutralizing antibodies against a subtype H5N1 virus but not subtype H4N6, suggesting limited exposure to influenza A (H5N1) viruses. These positive samples were obtained from a farm in the Malang District of East Java Province; neutralizing titers were 4–16 (Table 1).

Virus Sequences

Among the 39 viruses sequenced, the first group comprised 24 isolates collected in Banten Province during January 2005–February 2005; the HA genes of these viruses were either identical or differed by no more than 2 nt. The second group comprised 9 isolates collected during October 2006–February 2007 and also differed by only 2 nt, al-

Table 1. Sites and prevalence of influenza A (H5N1) viruses isolated from pigs, Indonesia*

Surveillance period and location	No. samples/no. pigs†	Viruses isolated, no. (rate)	Management type	Distance from poultry
2005 Jan–2005 Feb				
Banten, Tangerang				
Farm A	41/500	12 (29)	Commercial	On site
Farm B	22/500	6 (27)	Commercial	On site
Farm C	13/50	0	Private	On site
Farm D	18/250	11 (61)	Commercial	On site
Farm E	13/50	0	Private	On site
Farm F	29/250	4 (14)	Commercial	On site
Farm G	23/100	2 (9)	Private	On site
East Java, Surabaya, SH	8/100	0	Private	2 km
Total	167/1,800	35 (21)	NA	NA
2006 Oct–2007 Feb				
Banten, Tangerang				
Farm H	34/150	4 (12)	Commercial	On site
Farm I	15/50	1 (7)	Private	On site
East Java				
Surabaya, SH	95/600–700	8 (8)	Government	1 km
Kediri, farm	30/150	1 (3)	Private	1 km
North Sumatra, Medan, farm	38/400	2 (5)	Commercial	0.5 km
South Kalimantan, Banjarmasin, SH	23/50	1 (4)	Private	On site
Total	235/1,400–1,500	17 (7)	NA	NA
2008 Nov–2009 Apr				
East Java				
Tulungagung				
Farm A	25/900	0	Commercial	1 km
Farm B	29/700	0	Commercial	2 km
Surabaya, SH	40/600–700	0	Government	1 km
Jember, farm	18/400	0	Private	2 km
Malang, farm	39/500	0‡	Private	1 km
Central Java, Solo, farm	15/100	0	Private	5 km
Bali				
Denpasar, SH	99/400	0	Government	2 km
Tabanan, farm	9/300	0	Commercial	0.2 km
Riau Islands, Bulan, farm	26/20,000	0	Commercial	No poultry farms on island
Total	300/23,900–24,000	0	NA	NA
Total	702/27,100–27,300	52 (7.4)	NA	NA

*SH, slaughterhouse; NA, not applicable.
†Numbers of pigs on farms are estimates.
‡Three samples were positive for A/swine/East Java/UT6040/2007 (H5N1); neutralizing titers were 4 for 1 sample and 16 for 2 samples.

though the viruses were collected in different provinces: Banten, East Java, North Sumatra, and South Kalimantan. The HA genes in these 2 groups differed from each other by 49–53 nt. The third group included 6 isolates collected in Banten, East Java, and North Sumatra during October 2006–February 2007; these HAs were identical except for 1 nt and differed from those of the first and second groups by 42–45 and 58–61 nt, respectively. Thus, the swine influenza A (H5N1) viruses collected in our surveillance study could be classified into 3 distinct groups on the basis of their HA gene sequences, irrespective of the province from which they were isolated, suggesting extensive movement of pigs among provinces.

Phylogeny

Phylogenetic analysis of the HA genes of the 13 representative viruses identified the same 3 groups described above. The HA genes of 4 viruses isolated in 2005 (2005 swine group) were placed in clade 2.1.1, and of the remaining 9 swine viruses isolated during 2006–2007, five were classified into the IDN/6/05-like sublineage (2006–07 swine [A] group) and 4 into clade 2.1.3 (2006–07 swine [B] group) (Figure 2, panel A). The most closely related strains of each swine virus group were chicken influenza A (H5N1) viruses: A/chicken/Indonesia/R60/2005 for the 2005 swine group, A/chicken/East Java/UT6016/2006 and A/chicken/East Java/UT6031/2007 for the 2006–07 swine (A) group, and A/chicken/East Java/UT6044/2007 for the 2006–07
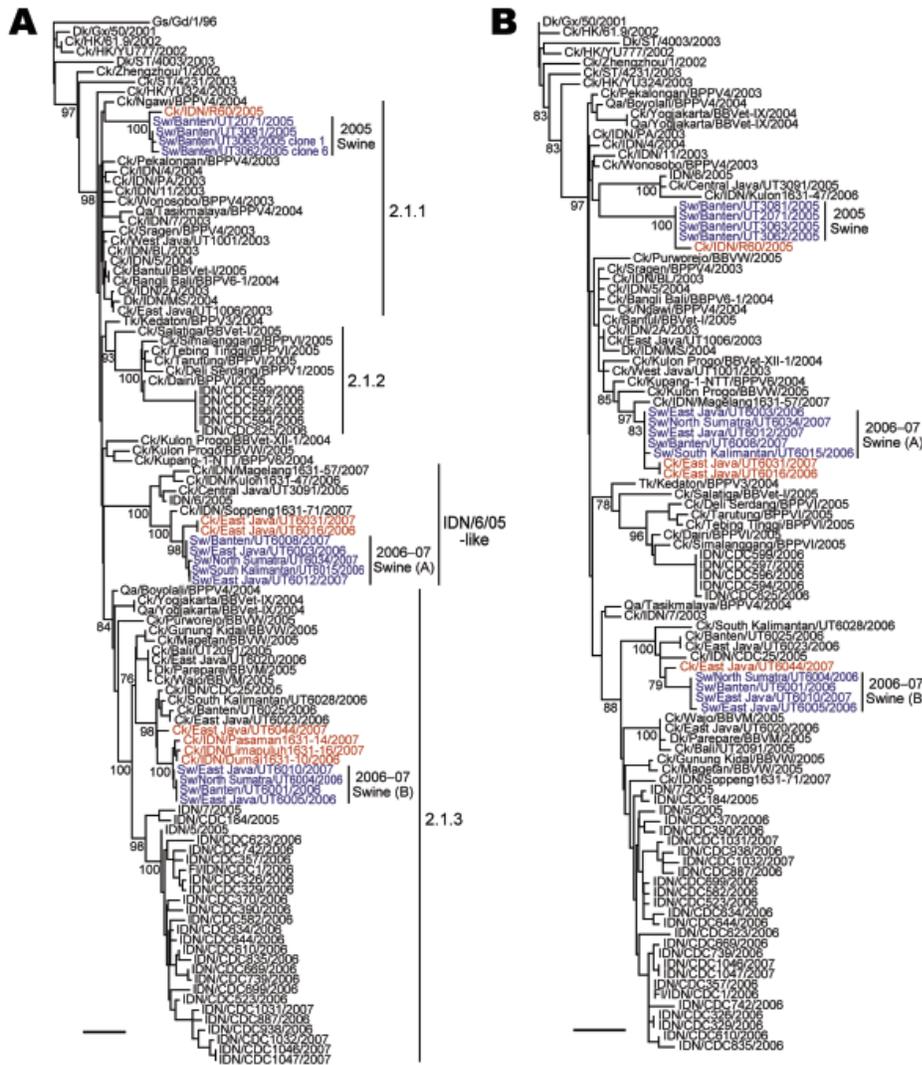
Figure 2. Phylogenetic relationships among the A) hemagglutinin (HA) and B) neuraminidase (NA) genes of influenza A (H5N1) viruses isolated in Indonesia. The numbers below or above the branch nodes indicate neighbor-joining bootstrap values. Analysis was based on nucleotides 281–1675 of the HA gene and 43–1037 of the NA gene. The HA and NA gene trees were rooted to A/goose/Guangdong/1/96 and A/duck/Guangxi/50/2001, respectively. Colors indicate swine viruses (blue) and chicken viruses (red) most closely related to swine viruses. Scale bars indicate 0.01 nt substitutions per site. Ck, chicken; Dk, duck; Fl, feline; Gd, Guangdong; Gs, goose; Gx, Guangxi; HK, Hong Kong; IDN, Indonesia; ST, Shantou; Sw, swine; Tk, turkey; Qa, quail.

swine (B) group. Analyses of the other 7 genes demonstrated that the phylogenetic relationships established for the HA gene were maintained; that is, the swine viruses in each group possessed nearly identical genes, and each group of swine viruses was most closely related to a chicken virus isolated near the site where the swine viruses were collected (Figure 2, panel B; online Appendix Figures 1–3, www.cdc.gov/EID/content/16/10/1515-appG1.htm, www.cdc.gov/EID/content/16/10/1515-appG2.htm, and www.cdc.gov/EID/content/16/10/1515-appG3.htm). Our results suggest that influenza A (H5N1) viruses were transmitted from avian species to pigs on at least 3 occasions.

Receptor Specificity

Sequence analysis of the PCR products of the HA genes of A/swine/Banten/UT3062/2005 and A/swine/Banten/UT3063/2005 indicated that nucleotides were heterogeneous at certain positions, prompting us to plaque purify the viruses in MDCK cells to obtain viral clones with distinct HA sequences (Table 2). We found that most of the swine influenza subtype H5N1 isolates bound to only SAα2,3Gal, whereas the plaque-purified clone 6 of A/swine/Banten/UT3062/05 bound to SAα2,3Gal and SAα2,6Gal (Figure 3), indicating that during their replication in pigs, avian influenza A (H5N1) viruses can acquire the ability to recognize human virus receptors.

Discussion

In contrast to the few reported cases of infection of pigs with highly pathogenic avian influenza A (H5N1) viruses (*17,25,26*), our surveillance study of 7 provinces in Indonesia during 3 periods shows that 7.4% of pigs surveyed during 2005–2007, but not 2008–2009, were infected with influenza A (H5N1) viruses. Phylogenetic analysis indicated that the viruses were transmitted to pigs on several different occasions, probably from poultry on

Table 2. Phylogeny of influenza A (H5N1) viruses isolated from pigs, Indonesia, 2005–2009

Hemagglutinin clade and virus	Group*	Chicken isolates with related genes
Clade 2.1.1		
A/swine/Banten/UT2071/2005	2005 swine	A/chicken/Indonesia/R60/2005†
A/swine/Banten/UT3062/2005‡		
A/swine/Banten/UT3063/2005§		
A/swine/Banten/UT3081/2005		
IDN/6/05-like clade		
A/swine/East Java/UT6003/2006	2006–07 swine (A)	A/chicken/East Java/UT6016/2006 and
A/swine/South Kalimantan/UT6015/2006		A/chicken/East Java/UT6031/2007
A/swine/North Sumatra/UT6034/2007		
A/swine/Banten/UT6008/2007		
A/swine/East Java/UT6012/2007		
Clade 2.1.3		
A/swine/Banten/UT6001/2006	2006–07 swine (B)	A/chicken/East Java/UT6044/2007
A/swine/North Sumatra/UT6004/2006		
A/swine/East Java/UT6005/2006		
A/swine/East Java/UT6010/2007		

*See Figure 2, panel A.
†Only hemagglutinin and neuraminidase genes are available through the public database.
‡Of 5 plaque-purified clones, 3 possessed serine at position 134 of the hemagglutinin molecule according to H5 numbering.
§Of 5 plaque-purified clones, 2 possessed tryptophan at position 145 of the hemagglutinin molecule according to H5 numbering.

nearby farms. According to the most recent classification of the HA gene (*32,33*), all avian and human influenza A (H5N1) viruses isolated in Indonesia belong to clade 2.1, which includes 3 well-defined lineages (clades 2.1.1–2.1.3) and a developing lineage termed IDN/6/05-like sublineage. In our study, all 24 viruses isolated during the first surveillance period belonged to the same cluster in clade 2.1.1 (2005 swine group) on the basis of recent HA classification (*32,33*). The 9 viruses collected during the second surveillance period belonged exclusively to the IDN/6/05-like sublineage, and the 6 remaining viruses collected during the same season were classified into clade 2.1.3; 2006–07 swine (A) and 2006–07 swine (B) groups, represented, respectively, by A/swine/East Java/UT6012/2007 and A/swine/Banten/UT6001/2006. Although no virus was isolated during the third surveillance period, 2008–09, a total of 3 (1%) pigs had neutralizing antibodies against influenza virus A (H5N1). These findings show that although influenza A (H5N1) viruses may not have been extensively circulating in pigs in Indonesia recently, these animals are susceptible to influenza A (H5N1) viruses and can serve as asymptomatic reservoirs for these viruses.

Because the phylogenetic relationships established for the HA gene extended to all viral genes, we conclude that the 3 groups of viruses identified in this survey were likely established independently, suggesting at least 3 separate avian-to-pig episodes of transmission of influenza A (H5N1) viruses during 2005–2009 in Indonesia. Our findings confirm sporadic reports of the susceptibility of pigs to influenza A virus (H5N1) infection in natural (*25,26*) and experimental settings (*23,34*) and suggest that when an outbreak of influenza A virus (H5N1) infection occurs

on poultry farms, pigs on nearby farms should be evaluated for infection.

We also found evidence of pig-to-pig transmission of influenza A virus (H5N1), particularly among animals sampled during the first surveillance period. Many viruses possessing almost identical genes were isolated from pigs on the same farms (Tables 1, 2). Pig-to-pig transmission would likely prolong the duration of influenza A (H5N1) virus infection within a pig population, thereby increasing the likelihood of adaptation and the subsequent generation of influenza A (H5N1) viruses that replicate efficiently in humans.

The lack of influenza-like signs in pigs infected with influenza A (H5N1) viruses has several public health implications. In Indonesia, pigs are transported to different locations according to market needs. This movement is reflected in our finding that clusters of swine viruses collected after 2006 were not consistent with those common to the sampling region. Indeed, viruses collected in North Sumatra, South Kalimantan, East Java, and Banten provinces showed identical or nearly identical genes, indicative of extensive transport of infected pigs throughout Indonesia. Thus, pathogenic influenza A (H5N1) viruses could easily evade detection as they spread through Indonesia in asymptomatic pigs being transported from province to province.

Our analysis of viral receptor specificities showed that 1 plaque-purified clone of A/swine/Banten/UT3062/2005 bound to avian-type and human-type receptors. Serine at position 134 was responsible for the human-type receptor recognition. This position is located within the 130-loop structural component of the receptor-binding pocket (*35*). Hence, the amino acid change at this position may affect receptor binding. Because serine at position 134 is never seen in avian influenza A (H5N1) viruses (alanine is highly
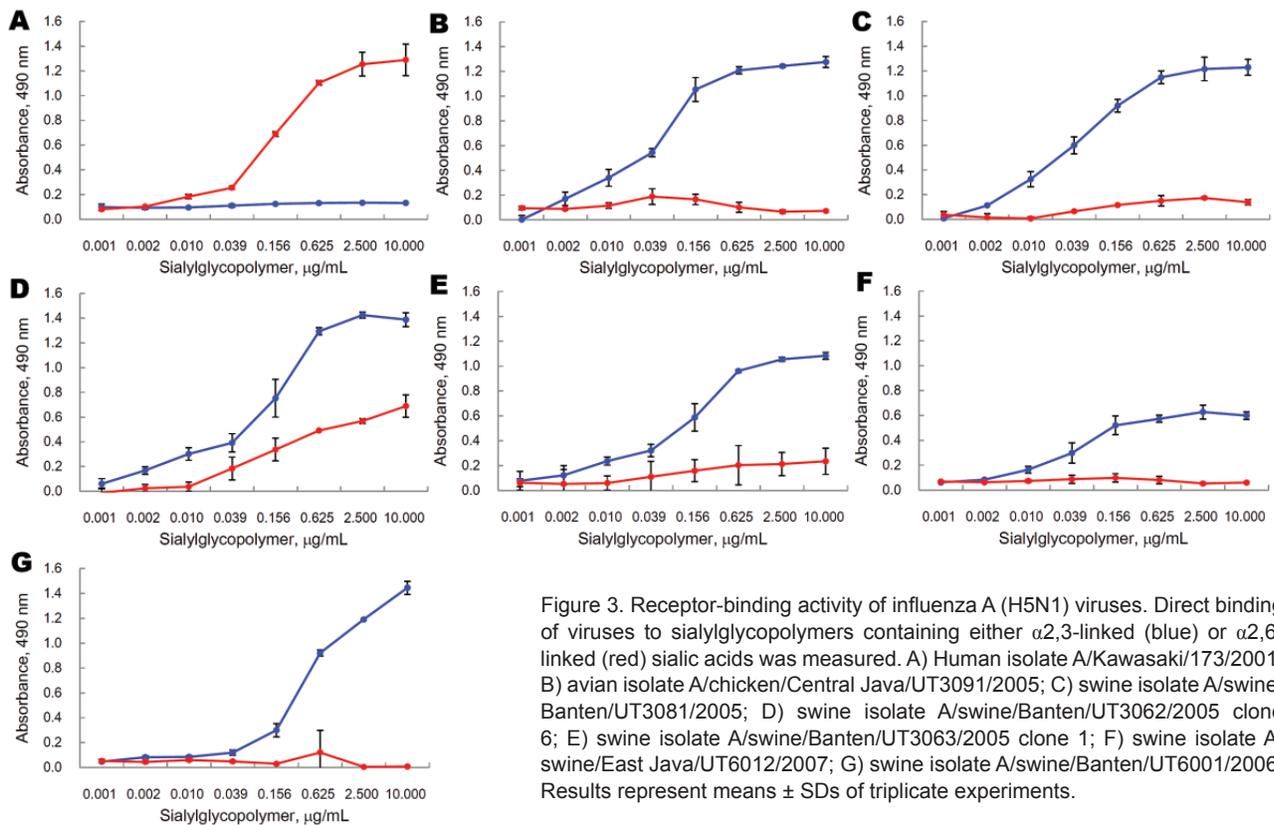
Figure 3. Receptor-binding activity of influenza A (H5N1) viruses. Direct binding of viruses to sialylglycopolymers containing either α2,3-linked (blue) or α2,6-linked (red) sialic acids was measured. A) Human isolate A/Kawasaki/173/2001; B) avian isolate A/chicken/Central Java/UT3091/2005; C) swine isolate A/swine/Banten/UT3081/2005; D) swine isolate A/swine/Banten/UT3062/2005 clone 6; E) swine isolate A/swine/Banten/UT3063/2005 clone 1; F) swine isolate A/swine/East Java/UT6012/2007; G) swine isolate A/swine/Banten/UT6001/2006. Results represent means ± SDs of triplicate experiments.

conserved at this position in avian influenza A [H5N1] viruses), the Ala134Ser mutation probably occurred during adaptation of the virus to pigs. According to a previous report (*36*), human isolates possessing valine at this position could also bind to the human-type receptor, although a mutation at position 129 (L129V) was also required for the human-type receptor recognition in this strain. Therefore, mutations at position 134 probably correlated with human-type receptor recognition and may serve as molecular markers for assessing the pandemic potential of influenza virus A (H5N1) isolates.

Although influenza virus A (H5N1) infection was not reported among swine workers in Indonesia while we were collecting our pig specimens, a previous cohort study showed that such workers, as well as their unexposed spouses, had increased levels of antibody to swine influenza A (H1N1) viruses (*37*), suggesting that humans are indeed susceptible to swine-adapted viruses (*38*). The recent swine-origin pandemic (H1N1) 2009 further demonstrates that pigs can be a potential source of virus capable of causing a human influenza pandemic (*24*). These findings suggest that as influenza A (H5N1) viruses spread among pigs and adapt to recognize human-type receptors, farmers, swine workers, and their families will be at greatest risk for infection by the newly adapted viruses.

In summary, we found that influenza A (H5N1) viruses have been transmitted multiple times to pig populations in Indonesia and that 1 virus has acquired the ability to recognize human-type receptors. Of particular concern is that pigs infected with influenza A (H5N1) viruses showed no significant influenza-like signs and were likely transported to and from different provinces in Indonesia. On the basis of our findings, we encourage the Indonesian government to control the transport of pigs within Indonesia. Otherwise, opportunities for this avian virus to adapt to mammals will increase, as will the risk for emergence of a new pandemic influenza virus.

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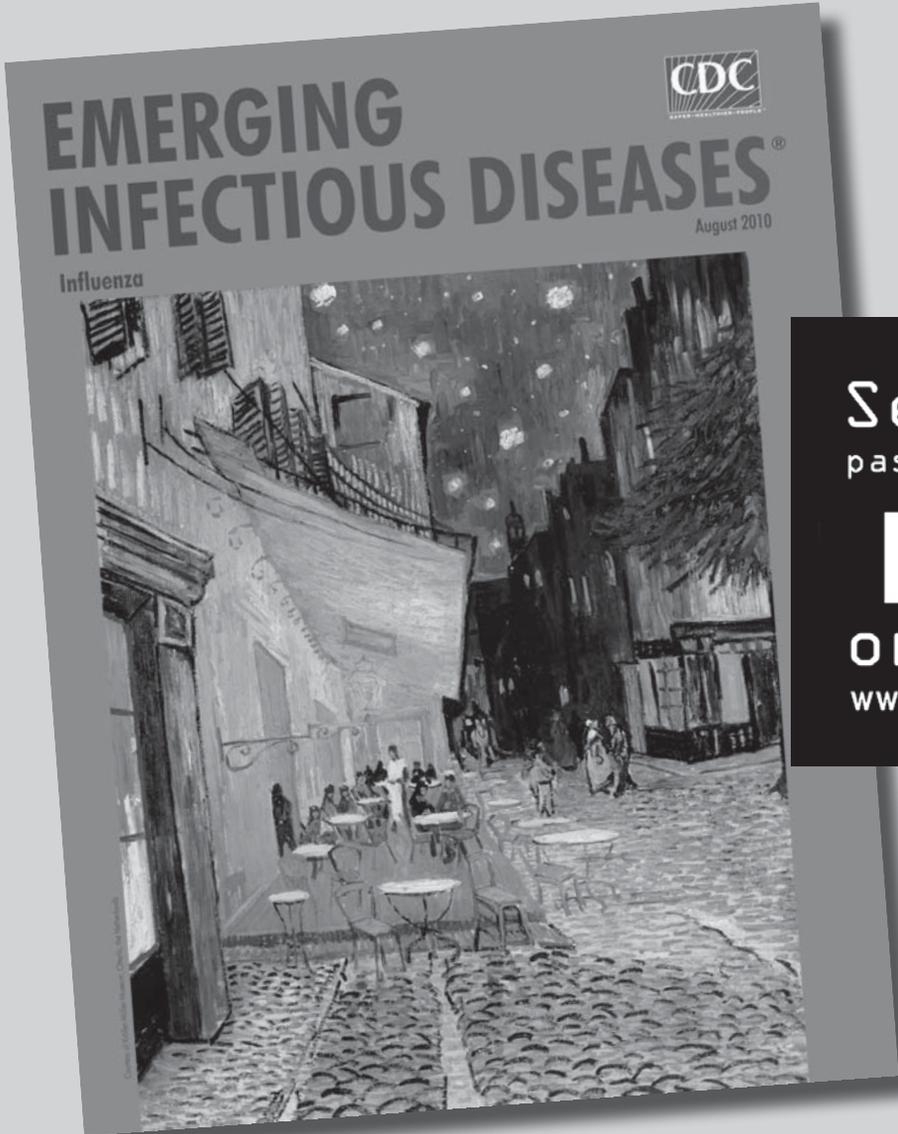
Mobile Phone–based Infectious Disease Surveillance System, Sri Lanka

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Because many infectious diseases are emerging in animals in low-income and middle-income countries, surveillance of animal health in these areas may be needed for forecasting disease risks to humans. We present an overview of a mobile phone–based frontline surveillance system developed and implemented in Sri Lanka. Field veterinarians reported animal health information by using mobile phones. Submissions increased steadily over 9 months, with ≈4,000 interactions between field veterinarians and reports on the animal population received by the system. Development of human resources and increased communication between local stakeholders (groups and persons whose actions are affected by emerging infectious diseases and animal health) were instrumental for successful implementation. The primary lesson learned was that mobile phone–based surveillance of animal populations is acceptable and feasible in lower-resource settings. However, any system implementation plan must consider the time needed to garner support for novel surveillance methods among users and stakeholders.

Emerging infectious diseases in animals and humans are being identified more frequently, many in low-income tropical countries, and this trend is expected to continue (*1*). Because ≈75% of these diseases in humans have originated in animals (*1*), interest has increased considerably in the utility of animal health surveillance for prediction of human health risks (*2–5*). The Canary Database, an online database named after the canary in the coal mine analogy, demonstrates the broad interest in this idea; it contains >1,600 articles related to animal sentinels of zoonotic, environmental, and toxic effects on human health (*6*). However, in practice, establishing links between animal and human health data has been difficult because data from animal and human health surveillance systems are obtained at different resolutions and scales and for different purposes. Human health surveillance is often based on aggregated diagnoses data obtained from standardized electronic medical records. Animal health surveillance systems vary widely (*7*). Where electronic veterinary records are kept, data can be extracted to central databases and analyzed. However, in lower-resource settings, electronic recording of veterinary services is often not feasible.

In many human health projects in resource-challenged areas, mobile technologies have emerged as a promising solution for obtaining, transmitting, and analyzing human health information in a timely fashion (*8–11*). In Peru, a mobile phone–based surveillance system has been used for early detection of infectious disease outbreaks in the Peruvian Navy (*12*). In Africa, the Satellife project has been using mobile data collection devices for >2 decades in human health surveys, and a project is under way that uses mobile phones and wireless technology for disease surveillance in Uganda (*13*). Many United Nations health and development projects in Africa now use mobile phones for obtaining field data (*14*). However, we are not aware of any examples of mobile phone–based disease surveillance that supports an animal-based emerging infectious disease system in the developing world.

In response to these challenges, we have developed the Infectious Disease Surveillance and Analysis System

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(IDSAS), a mobile phone–based surveillance system specific for animal populations in lower-resource settings. A pilot version of this system was implemented in January 2009 in partnership with the Department of Animal Production and Health (DAPH) in Sri Lanka. The objective of this system is to obtain animal health information from field veterinarians in a timely fashion to establish baseline patterns in animal health. By establishing these baseline patterns through regular electronic surveillance, we aim to build capacity to detect changes that may facilitate early detection of changing risks for emerging infectious diseases. We describe the design and implementation of the system, present preliminary data on submission patterns, provide examples of data that are being obtained, and discuss obstacles and opportunities encountered during the first 9 months of operation. This report highlights and generalizes some of the lessons learned during the planning and implementation of the IDSAS in Sri Lanka.

Materials and Methods

Veterinary Services in Sri Lanka

Veterinary services in Sri Lanka are provided largely by the DAPH, a national-level body responsible for control of livestock diseases, livestock research, animal breeding, and education in animal husbandry. Veterinary services are delivered through provincial-level DAPH councils and field offices. Provinces are made up of districts, which are further divided into divisional secretariat. Each divisional secretariat is assigned a field veterinarian who is responsible for providing animal health services within that division.

System Structure

Forty field veterinarians were recruited to pilot the IDSAS in 4 districts in separate provinces. The districts (Nuwara Eliya, Anuradhapura, Matara, Ratnapura) were selected to capture variation in livestock practices, climate, and environment (Figure 1).

Capacity for electronic collection and submission of data was developed in the IDSAS to decrease the time from detecting to reporting animal health events from that of the existing method of mailed written reports. Internet access is limited in many parts of Sri Lanka, but the cellular phone network is extensive. Mobile phones (Palm-Centro Smartphones; Palm, Inc., Sunnyvale, CA, USA) were used as the data collection platform. Animal health surveys were developed by using EpiSurveyor, a free and open-source software package developed for obtaining public health data (www.datadyne.org). EpiSurveyor has been used extensively for human health data collection in Africa.

Surveys were filled out in remote areas without cellular service and transmitted when the user returned to an area of
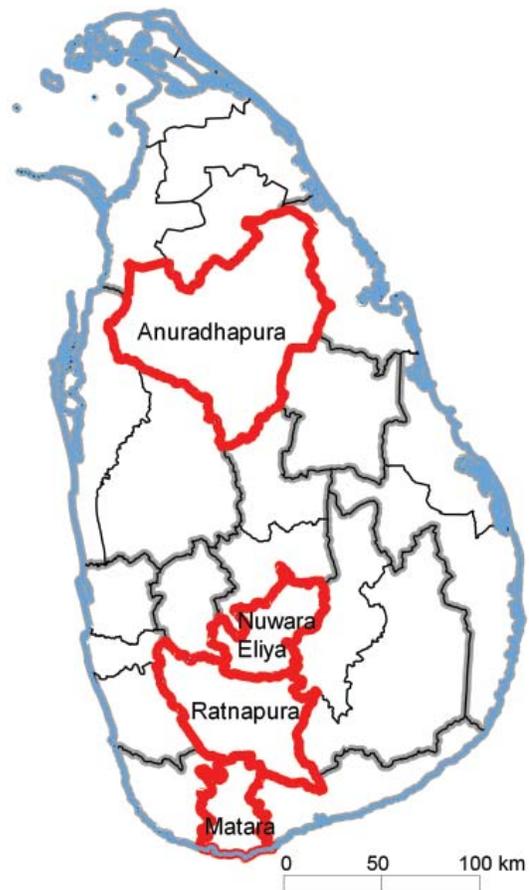
Figure 1. Study districts in Sri Lanka where field veterinarians participated in the Infectious Disease Surveillance and Analysis System and obtained data on animal health during their daily work activities. Study districts are indicated by red outlines; provincial boundaries are indicated in gray, and district boundaries are indicated in black.

reception. Decoupling data collection from transmission-capable locations greatly expanded the geographic range of the surveillance system. The location of each survey was also collected with global positioning system (GPS) software and an external receiver connected to the phone by Bluetooth (www.bluetooth.com/English/Pages/default.aspx). Field veterinarians obtained data from their daily working activities (clinic and farm visits). Survey and GPS data were encoded and transmitted to a central database by email at the end of each day. A schematic overview of the IDSAS is shown in Figure 2.

Information Structure

The pilot study was restricted to health issues in chickens, cattle, and buffalo. Every time a field veterinarian visited a farm or saw a case involving 1 of these 3 species, a survey was completed by using EpiSurveyor, and the
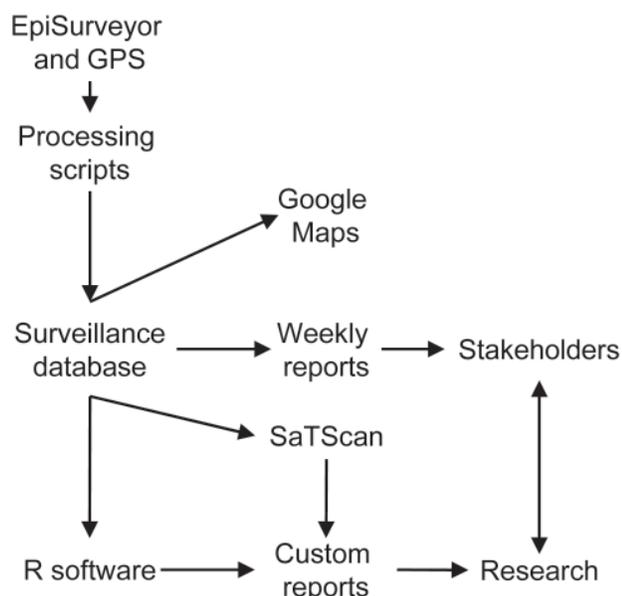
Figure 2. Schematic overview of major components of the Infectious Disease Surveillance and Analysis System, Sri Lanka. GPS, global positioning system; stakeholders, groups and persons (field veterinarians, administrators, and researchers) whose decision-making and actions are affected by emerging infectious diseases and animal health.

location (for farm visits) was recorded. Although we sought daily submissions, our minimum target submission rate was 2 surveys/field veterinarian/wk. This value was based on an estimate of the number of cases in chickens, cattle, and buffalo seen on average by field veterinarians and on work-related disruptions that could interfere with data submission (training, sick days, holidays).

The first draft of the survey was based on the Alberta Veterinary Surveillance Network Veterinary Practice Surveillance initiative (15). In the second stage, the survey was reviewed with several field veterinarians and government employees within the DAPH to ensure that it was applicable to veterinary practice in Sri Lanka. Most questions were single-answer, multiple-choice questions, although additional comments were allowed in a free-text field. The survey was designed to minimize the time required to fill out each survey, reduce the number of data entry errors, and enable easier and automated data analysis.

Data for each case included date, location, type of operation, nature of visit (routine/nonroutine), age and sex of affected animal, number on farm, number affected, clinical syndrome, clinical diagnosis, laboratory testing if applicable, and other animals on the premises. A survey could contain up to 3 cases if all 3 species were present on a farm. Field veterinarians selected from clinical syndromes shown in Table 1. Within EpiSurveyor, each syndromic grouping was linked to a list of clinical diagnoses.

Reporting and Data Analysis

Data reported represent the experience of the IDSAS during January 1–September 30, 2009. Weekly surveillance reports were disseminated to project partners containing a list of cases. These reports documented the following details pertaining to each case submitted during the previous week: date, species, reported syndrome, suspected clinical diagnosis, number of animals affected, number of animals on farm, number of dead animals, and a flag indicating whether samples were submitted to a laboratory.

Data Completeness and Submission Patterns

Measures of data completeness used for the IDSAS at the planning and early implementation stages followed the guidelines of Lescano et al. (16). In the planning stage, assessment of the workload of data collectors is essential to determine whether data can be obtained with existing resources. The IDSAS data collection procedure involved separate software programs for animal health surveys and GPS data collection. These data were linked by a common identifier entered by field veterinarians at the time of survey completion. To explore the link between survey and GPS data, we report completeness for surveys, GPS points, and linked survey–GPS records. We also report the percentage of surveys with a linked GPS point. Because field veterinarians worked 6 days per week, we expected a day-of-the-week effect and therefore examined variation in survey submission by day of the week. Finally, we examined

Table 1. Syndrome groupings in animal health surveys in the Infectious Disease Surveillance and Analysis System, Sri Lanka, January 1–September 30, 2009

Species	Syndrome grouping
Buffalo and cattle	Abortion/birth defect
	Ambulatory lameness
	Decreased feed intake/milk production
	Gastrointestinal signs
	Neurologic signs
	Recumbency
	Peripheral edema/miscellaneous swelling
	Reproduction/obstetrics problems
	Respiratory
	Skin/ocular/mammary
	Sudden or unexplained death
	Urologic
	Vesicular/ulcerative
	Other
Chickens	Ambulatory
	Decreased egg production, weight gain, and appetite
	Neurologic/recumbent
	Peripheral edema/miscellaneous swelling
	Respiratory
	Skin/ocular
	Sudden or unexplained death
	Other

weekly submission counts to determine temporal patterns and fitted a linear trend model to weekly counts to determine the average change in submissions per week.

Statistical Surveillance

Digital storage of data that otherwise might not be captured enables more sophisticated statistical analysis. To demonstrate how the IDSAS database could be used in an outbreak detection context, we present an example of statistical surveillance by using the total number of weekly surveys submitted by participating field veterinarians as an indicator for unusual animal health events. We used these data in a prospective temporal surveillance cumulative sum (CUSUM) statistic implemented in the statistical software package R (*17*). The CUSUM measures accumulations of extra variance in a sequential framework, and alarms are signaled when the statistic exceeds a specified threshold. Parameters are required for the expected value, the reference value *k*, and the alarm threshold *h*. We estimated values for *k* and *h* on the basis of an expected false-positive rate of 1 every 52 weeks to detect a change that was 2 SD above the reference value. We evaluated 2 baseline scenarios: the mean of the first 14-week period, and a set value of 100 surveys per week. Data were analyzed weekly beginning at week 14 until the end of the study.

Caseload and Case Profile

The distribution of cases is presented as divided by species and district. We also present the frequency of the 5 most commonly reported syndromes for each species.

Assessing System Implementation

The experience of implementing the IDSAS provides lessons for future surveillance projects in lower-resource settings. We synthesize some of the key lessons learned during this phase of the IDSAS on the basis of technical, financial, political, and ethical/societal/cultural considerations (*18*).

Results

Data Completeness and Submission Patterns

The IDSAS operated for 273 days. During this period, 3,981 unique surveys were submitted to the system by participating field veterinarians. This value corresponds to ≈99 surveys/field veterinarian over a 9-month period (11 per month); our intended submission target was a minimum of 2 submissions/field veterinarian/week. During this period, 96% of days had ≥1 conducted survey. A total of 1,650 unique GPS points were submitted, of which 1,172 (71%) were linked to an associated survey. For the total days under surveillance, GPS data were collected for 76%, and GPS and survey data were recorded for 64%. Informal

discussions with many field veterinarians showed that it took ≈1 minute to complete an animal health survey and 1 minute to obtain a GPS point once the IDSAS had been in place for 6 months.

A generally increasing overall trend in temporal patterns in submissions (Figure 3) was evident. The linear trend model showed a significant weekly increase in submissions of 1.65% (p<0.001, $R^2 = 0.31$). The trend was also characterized by large variation (coefficient of variation 3.01), with a large decrease (39 surveys) in submissions during week 14. As expected, day-of-the-week variation was present in submissions, and weekly survey counts were 306 on Saturdays and 326 on Sundays. During the week, totals ranged from 515 to 695.

Statistical Surveillance

On the basis of the parameters described, the reference value *k* was ≈2.6 and the threshold value *h* was ≈4.1. Using week 14 as a baseline, we expected 84 weekly visits, which in the CUSUM analysis flagged an alarm at week 26 and weeks 30 through the end of the study (week 38). Using the expected value of 100 weekly visits, we determined that alarms were signaled during weeks 31–38.

Caseload and Case Profile

Of 3,981 surveys submitted during the 9 months of operation, 3,150 cases were reported (i.e., reported an animal health issue). Most (83%) cases were in cattle, followed by chickens and buffaloes (Table 2). These cases were mostly from an area known to contain a large number of dairy cattle operations. Production-related syndromes were the most commonly reported across all species, with decreased feed intake/milk production most prevalent in cattle and buffaloes, and decreased egg production/weight gain/appetite in chickens (Figure 4). In buffaloes, markedly higher gastrointestinal and lameness submissions were noted relative to
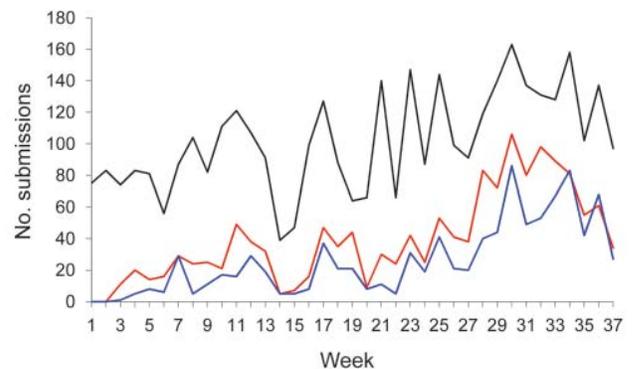
Figure 3. Number of survey (black line), global positioning system (red line), and linked survey–global positioning system (blue line) submissions to the Infectious Disease Surveillance and Analysis System, by week, Sri Lanka, January 1–September 30, 2009.

Table 2. Cases in animals in the 4 study districts covered by the Infectious Disease Surveillance and Analysis System, Sri Lanka, January 1–September 30, 2009*

District	No. cattle cases	No. buffalo cases	No. chicken cases	Total no. cases
Ratnapura	548	106	146	800
Matara	388	62	55	505
Nuwara Eliya	1,095	16	11	1,122
Anuradhapura	596	70	57	723
Total	2,627	254	269	3,150
*Cases are defined as animal health issues.				

other syndrome groupings. Gastrointestinal signs were common in Anuradhapura across all species. Cases in chickens were found predominantly in Ratnapura, where there are a large number of poultry operations. Syndrome profiles for chickens were similar across all districts (Figure 4).
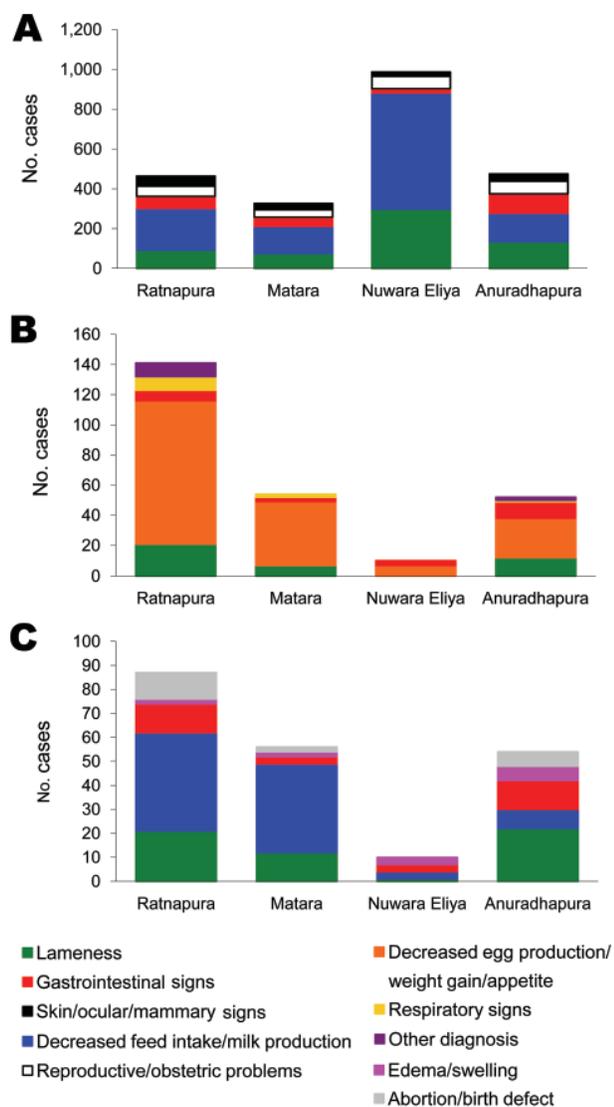
Figure 4. Frequency of syndrome groups seen by field veterinarians in cattle (A), buffalo (B), and chickens (C) in 4 study districts as part of the Infectious Disease Surveillance and Analysis System, Sri Lanka, January 1–September 30, 2009.

Alerts Identified by the IDSAS

In 1 instance, suspected cases of black quarter (*Clostridium chauvoei* bacterial infection) were identified at the time of review of the weekly report review. Because the DAPH was made aware of the cases shortly after they were identified by the field veterinarian, it was able to confirm that the field veterinarian obtained tissue samples for diagnostic testing. This increased information flow would not have been possible in the DAPH surveillance program because written reports of suspected cases from field veterinarians are received monthly, and each must be reviewed individually to identify suspected cases of a particular disease of interest. Additional statistical alerts generated by analysis could be evaluated because part of the objective of the IDSAS is to establish the baseline caseload in areas under surveillance.

Assessment of System Implementation

Technical Considerations

Technical barriers were a major challenge during implementation of the IDSAS (Table 3). The system introduced new data collection requirements for field veterinarians. Use of cell phones for data collection required training and ongoing technical support.

Financial Considerations

The main costs of the system were associated with data collection hardware. Each phone and GPS extension set cost ≈Can$500. This cost may have been reduced if phones were available for purchase locally. Proprietary software options with different hardware requirements were available but rejected because recurring licensing costs could not be sustained and hardware was a 1-time expense. Although data plans are an ongoing cost, the size of files generated by the IDSAS is typically <1 kilobyte. The cost of data transmission per user per month in Sri Lanka is <Can$5. Investments in hardware and human resources for data collection can be quickly recouped because these resources are extendible to many other fields in which the government of Sri Lanka is involved (e.g., human epidemiology, environmental assessment, disaster planning).

Table 3. Lessons learned in planning and implementing surveillance systems, Sri Lanka, January 1–September 30, 2009*

Consideration for surveillance in lower-resource settings	IDSAS experience	Generalized lessons
Technical	Cell phones permitted timely collection and transmission of data to the surveillance system. Touch screen interfaces were new technology for field veterinarians.	Use of familiar technologies such as basic cell phones will minimize training time. Cell phones enable timely data collection and transmission.
	Ongoing training was essential. A local research assistant made training more effective, in particular because field veterinarians could learn the system in their native languages.	Developing local expertise at the project outset is invaluable for ensuring sustained technical and logistical support.
Financial	Hardware required for data collection was relatively inexpensive but much more expensive than hardware available in Sri Lanka. Importing cell phones for the project was challenging.	Where possible, hardware that is locally available should be used.
	Open-source software was used when possible, eliminating licensing as a recurring cost but requiring more training and technical skills to maintain.	Open-source software options should be selected over proprietary options to reduce costs and generate technologic capacity.
Political	External funding covered the initial hardware and software costs.	Obtaining external financial support to cover the initial investment required will make implementation more feasible.
	Support at the provincial level was critical for engagement of field veterinarians.	Garnering support at all levels of government is critical at the early implementation phase.
	Engagement of key political stakeholders was essential to alleviate fears about potential for harm caused by novel types of surveillance data.	Early in the design process it is important to discern what the outputs of the system will be and their added value.
Ethical, societal, and cultural	Government officials were initially concerned about data security.	Build appropriate data security into all components of the system.
	It was late in the implementation phase when government stakeholders recognized the potential for additional data uses.	Examples of additional uses of data obtained will generate support for new surveillance initiatives.
	At the onset of the project, field veterinarians were skeptical about the usefulness of data generated by the IDSAS. However, over time they envisaged how the outputs could be used in disease surveillance and in improving their daily veterinary duties.	Adoption of novel surveillance methods requires user acceptance and new technical skills. Time and experience will enable this transition to occur.
	Many farms are geographically isolated making access to field veterinarians difficult.	Quality and quantity of data from surveillance systems are affected by the ability of an animal owner to access animal health services.

*IDSAS, Infectious Disease Surveillance and Analysis System.

Political Considerations

Political support has been the most important factor in the successful implementation and operation of the IDSAS. Animal health reporting standards set by the World Animal Health Organization require member countries to report on a group of animal diseases. The introduction of a new surveillance system as part of a research project resulted in initial confusion about how such a system could fit within existing surveillance networks. A major challenge in the implementation of the IDSAS was drawing the distinction between the IDSAS as a research project and the national animal disease reporting system of the DAPH. Negotiating this challenge was possible with support from key figures in the government and the University of Peradeniya.

Ethical, Societal, and Cultural Considerations

During the design and early implementation of the IDSAS, concerns around privacy and data security were addressed promptly as they arose. No information pertaining to animal owners was collected. No personal identifiers from field veterinarians were linked to survey submissions.

Discussion

The IDSAS was developed on the premise that monitoring animal health can provide information for early warning of emerging infectious diseases and changing disease patterns. Our preliminary results demonstrate enhancement of existing technologic infrastructure. Equipping field veterinarians with the necessary means of communication enabled timely submission of cases, and the skills to make use of these tools helped to build further capacity in animal health surveillance. Weekly reports document increased knowledge and information flow between animal health stakeholders (groups and persons [field veterinarians, administrators, and researchers] whose decision-making and

actions are affected by emerging infectious diseases and animal health) in Sri Lanka. Finally, through the IDSAS, major progress was made toward establishing baseline patterns of suspected diagnoses and syndromes in cattle, buffaloes, and chickens.

Uptake of the IDSAS over its initial 9 months of operation resulted in data generation for ≈4,000 interactions between field veterinarians and reports on the animal population. Increasing use of the IDSAS over time was also illustrated by a positive linear trend in submissions. Statistical surveillance of the number of surveys submitted by field veterinarians showed that an upward shift in submissions occurred at approximately week 30. The overall trend was likely caused by field veterinarians acquiring competency with the technology. The shift was likely caused by a combination of reduced number of submissions in weeks 14–16 related to training and examinations, the final stages of the civil war in weeks 19–21, and retraining in week 23. The alarms signaled by the CUSUM analysis illustrate the need for modeling the expected value when surveillance statistics are used.

The distribution of cases highlights 1 of the challenges with this type and many types of surveillance data, which is how to interpret variability in cases in the absence of data on the population at risk. The high number of cattle cases in Nuwara Eliya was expected given prior knowledge of the large number of milk-producing cattle in that region. However, the distribution of cases would be expected to reflect only the true disease incidence in the population if the likelihood of a veterinarian seeing a case in a given species was proportional to the underlying disease distribution in the 3 species in each area. For example, in Nuwara Eliya, cattle raisers might be more inclined to call their veterinarian in the event of a sick cow than a sick chicken. The solution to this problem, if the aim is to establish a predictive, prospective disease surveillance system, is establishing standard patterns of case submission for the population. For this solution to be realized, this system (and others) must be maintained over an extended period within the same geographic areas.

One of the barriers to implementation of the IDSAS in its current form is the cost of hardware and the need for a server administrator. However, since the pilot project was originated in Sri Lanka, a new version of EpiSurveyor has been released. Several major changes have been made: the software now runs on a wide range of standard mobile phones, data can be uploaded to servers administered (by datadyne.org) and analyzed on cell phones, and GPS data can be obtained in EpiSurveyor. These changes drastically reduce the costs of implementing mobile surveillance; the cost per mobile phone unit is reduced substantially, and governments do not need to purchase and administer their own database.

At this time, the DAPH has decided to incorporate the IDSAS into its ongoing disease surveillance efforts, and the system is being run on 2 parallel servers, 1 at the DAPH and the original server that hosts the IDSAS. After this transition period, the system will continue to run only on the DAPH server and may be modified to suit additional surveillance priorities (e.g., goats, swine). The DAPH will not be providing incentives to field veterinarians for participation. Solicitation of further review by field veterinarians (once the system has been transitioned) and monitoring of submissions long term would be valuable.

Beyond data obtained by the IDSAS, this research demonstrates that through developing social capital and technologic capacity, novel surveillance methods can be implemented that are feasible and acceptable in lower-resource settings. These considerations are supplemented with lessons for planning and implementation of surveillance systems. It is hoped that by disseminating the results of this initiative, other governments will tailor the IDSAS to their particular animal health surveillance needs. The collaboration and relationships established in this project should yield further benefits through technical training and pooling of human and physical resources for sustaining and promoting veterinary public health in Sri Lanka. Additionally, the advantages of electronic health surveillance that uses mobile data collection afforded by the IDSAS are immediately known to administrative persons who can affect change in other areas of animal and human health policy and planning.

Developing surveillance capacity in Sri Lanka has generated valuable human resources and relationships that, when coupled with technology, may be the key to early detection of emerging infectious diseases. Field veterinarians are developing a valuable technologic skill set for remote data collection. The data obtained from the IDSAS offers DAPH stakeholders and field veterinarians a new perspective on disease within the animal population and creates new opportunities for dialogue and mutual understanding. Increased communication through training, surveillance reporting, and regular meetings has been a major aspect of improving veterinary public health awareness and is a key result of the IDSAS project. Social capital, although difficult to measure, is a major precursor to successful surveillance in the developing world (19). Building social capital under any circumstances takes considerable time. Future development of similar surveillance programs should take this temporal component of project development into consideration to help ensure that new initiatives gain momentum over time. Field veterinarians have indispensable local knowledge about animals in their division. Leveraging this awareness by regular electronic surveillance is a first step toward formalizing this knowledge store to improve surveillance of emerging infectious diseases in Sri Lanka.

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Oral Fluid Testing during 10 Years of Rubella Elimination, England and Wales

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Surveillance of rubella in England and Wales has included immunoglobulin M testing of oral (crevicular) fluid from reported case-patients since 1994. The need for laboratory confirmation to monitor rubella elimination is emphasized by poor sensitivity (51%, 95% confidence interval 48.9%–54.0%) and specificity (55%, 95% confidence interval 53.7%–55.6%) of the clinical case definition. During 1999–2008, oral fluid from 11,709 (84%) of 13,952 reported case-patients was tested; 143 (1.0%) cases were confirmed and 11,566 (99%) were discarded (annual investigation and discard rate of clinically suspected rubella cases was 2,208/100,000 population). Incidence of confirmed rubella increased from 0.50 to 0.77/1 million population when oral fluid testing was included. Oral fluid tests confirmed that cases were more likely to be in older, unvaccinated men. Testing of oral fluid has improved ascertainment of confirmed rubella in children and men and provided additional information for assessing UK progress toward the World Health Organization elimination goal.

In 1970, rubella vaccination was introduced in the United Kingdom for prepubertal girls and nonimmune women of childbearing age to protect them from the risks for rubella during pregnancy. Although this selective vaccination policy effectively reduced the number of cases of congenital rubella syndrome (CRS) and terminations of pregnancy, rubella during pregnancy continued to occur (*1*). In 1988, measles, mumps, and rubella (MMR) vaccine was introduced for universal vaccination at 13–15 months of age with the goal of eliminating circulating rubella.

A considerable decrease in rubella in young children followed, but in 1993, clinically diagnosed and laboratory-

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confirmed rubella increased; the increase occurred predominantly in older men who had previously not been offered a rubella-containing vaccine (*2*). Therefore, in November 1994, rubella vaccine was included in a school catch-up campaign to prevent a predicted measles epidemic (*3*). Approximately 92% of children 5–16 years of age received combined measles–rubella vaccine. In 1996, to maintain measles control, a second dose of MMR was recommended for children 5 years of age.

For any disease in the elimination phase, accurate surveillance is necessary to identify reservoirs of infection and susceptible groups (*2*). In 2005, the World Health Organization (WHO) European Region adopted a resolution to eliminate indigenous rubella by 2010 (elimination goal of confirmed rubella incidence <1 per 1 million population) (*4*). WHO has developed a clinical case definition for rubella (*5*), but identification of cases based on clinical suspicion alone becomes less reliable as disease incidence decreases. Therefore, for countries trying to eliminate rubella, laboratory confirmation of all suspected cases is recommended (*4*).

Before 1994, surveillance of laboratory-confirmed rubella in England and Wales was based mainly on detection of immunoglobulin (Ig) M against rubella in serum. However, because rubella infection is usually mild, physicians are reluctant to obtain blood samples for serum confirmation, especially from young children. There is also some reluctance to obtain serum from men because the diagnosis is not of major clinical significance. Oral or crevicular fluid is a noninvasively obtained clinical specimen that is likely to be more acceptable, especially for children, and is safe and easy to obtain (*6–9*). Transudates from the capillary bed situated beneath the margin between the tooth and gum are obtained by rubbing an absorptive device between the gum and the cheek. These samples, which are distinguishable

from saliva samples, contain mucosal cells that enable detection of the rubella virus by PCR. Methods for obtaining, extracting, and storing oral fluid samples are well established (7,10–13). Detection of rubella IgM in oral fluid has been validated and shown to be ≈90% sensitive and 99% specific compared with detection in serum (2). Samples are also suitable for genome detection (14,15). Therefore, since late 1994, the enhanced surveillance program in England and Wales has relied on oral fluid testing to provide laboratory confirmation for clinically diagnosed cases of measles, mumps, and rubella (however, serum testing is still recommended for confirmation of infection during pregnancy).

An additional increase in rubella incidence occurred during 1995–1998. Reports of rubella peaked in 1996 (a total of 9,081 clinically diagnosed cases were reported) (16). This situation offered an opportunity to evaluate the sensitivity and specificity of the WHO clinical case definition for rubella. In addition, we describe the added value of oral fluid testing during the subsequent 10 years of rubella elimination (1999–2008).

Methods

Since 1988, physicians in England and Wales have been required by law to report clinically suspected cases of rubella to the proper officer at the local health authority (usually a public health consultant in a Health Protection Unit [HPU]). Since late 1994, when a report is received, the HPU sends an oral fluid kit to the primary-care physician or patient for confirmatory testing. The kit is then returned by prepaid envelope to the Virus Reference Department at the Health Protection Agency Centre for Infections, for analysis. A request form contains vaccination history and, until July 2003, some brief clinical features (presence of a rash, fever, conjunctivitis, cough, and lymphadenopathy [type not specified]). Oral fluid testing was also used to test cases that were not formally reported as part of outbreaks in 3 universities associated with imported virus from Greece in 1999 (17). A similar process is used for measles (and mumps) (18,19). If there is a strong clinical or epidemiologic suspicion of rubella in samples tested for measles and for measles in samples tested for rubella, dual testing is performed.

Oral (crevicular) fluid specimens, obtained by wiping a specially designed sponge swab (oral test kit [Oracol; Malvern Medical Developments, Worcester, UK]) around the gum margins, were tested for rubella-specific IgM initially by using a solid-phase IgM–antibody capture radioimmunoassay (20). After 2002, an in-house assay for rubella IgG was introduced (21), and after 2003, all samples taken within 1 week after symptom onset that were negative for rubella IgM and rubella IgG were tested by reverse transcription–PCR for rubella virus (14). In 2006, the rubella solid-phase IgM–antibody capture radioimmunoassay was replaced by a commercial enzyme immunoassay (22).

Results of testing were sent to the reporting physician, and copies were sent to the relevant HPU. Confirmed cases were defined as samples positive for rubella-specific IgM in oral fluid or detection of rubella virus genome by PCR in persons without a history of receipt of rubella vaccine in the previous 6 weeks. These cases were reconciled with confirmed rubella infections (positive for rubella IgM in serum) reported to the Health Protection Agency Centre for Infections, from laboratories in England and Wales, and duplicates are removed. Since 1999, residual samples from cases reported by local laboratories are requested to be sent to the national reference laboratory for confirmation by an alternative IgM assay (with or without avidity testing) (23). Cases with negative results for the second IgM test or with high avidity are then excluded from the confirmed total. Further details (including travel and contact history) are requested, and vaccination status is checked for all confirmed case-patients.

Data obtained during January 1995–July 2003 were analyzed to estimate the sensitivity and specificity of a clinical case definition. The WHO definition of a case of rubella is a generalized maculopapular rash and fever and arthralgia/arthritis or cervical, suboccipital, or postauricular lymphadenopathy (5). Because information for arthritis was not routinely obtained, the accuracy of a modified case definition based on rash, fever, and lymphadenopathy was calculated against the standard of presence or absence of rubella-specific IgM in an oral fluid sample. The sensitivity, specificity, and positive predictive value were compared by patient age, sex, and year of report.

Data obtained during January 1999–December 2008 from 3 sources (clinically reported cases, confirmation in oral fluid samples, and laboratory reports) were then compared with respect to age, sex, vaccination status, and region. Rates were calculated by using 2001 population estimates from the Office for National Statistics. The incidence rate for cases confirmed positive by oral fluid was adjusted for the proportion of reported cases tested in each region and compared with the rate from laboratory reports alone. All analyses were conducted by using Stata/SE version 9.2 (StataCorp LP, College Station, TX, USA). Proportions were compared by using the χ^2 test.

Results

Accuracy of Clinical Case Definition

During January 1995–July 2003, of 29,825 reported case-patients, oral fluid from 17,042 (57%) was tested for IgM; complete clinical information was obtained for 12,220 (72%) patients who submitted oral fluid samples. The overall sensitivity of the clinical case definition of maculopapular rash and fever and lymphadenopathy was 51% (95% confidence interval 48.9%–54.0%) and the specific-

ity was 55% (95% confidence interval 53.7%–55.6%). The sensitivity and specificity of this case definition did not show significant variation by age, sex, and year of reporting (Table 1). However, the positive predictive value was significantly higher for persons ≥15 years of age (71% for persons 15–24 years of age compared with 1% for persons 5–9 years of age), for men (21% compared with 5% for women), and during the 1995–1998 epidemic period (20% compared with 1% in January 1999–July 2003).

Enhanced Surveillance, 1999–2008

During 1999–2008, a total of 13,952 clinically suspected rubella cases were reported, and the number of cases per year (1,000–2,000) remained stable (Figure). Oral fluid was tested for 11,709 (84%) case-patients; 143 (1.0%) positive results were confirmed, and 11,566 (99%) of the results were discarded (Table 2) This finding is equivalent to an annual investigation and discard rate of 2,208 clinically suspected rubella cases per 100,000 population. Over the 10-year period, the proportion of confirmed cases for which oral fluid was tested increased from 39% (49/127) in 1999 to 49% (16/33) in 2008. The annual number of cases confirmed positive by oral fluid test remained <30 (Figure). The proportion of cases with a confirmatory test result has decreased since 1999 and remained <2%, except for a temporary increase in 2007 to 2.8%. During the 10-year period, 263 additional cases were confirmed by serum testing, resulting in 406 confirmed cases compared with 13,952 clinically diagnosed cases. Because laboratories in England and Wales report only cases that were confirmed positive, the number of additional cases tested and results discarded after serum testing alone is not known.

The age and sex distribution of case-patients reported from each surveillance source differed markedly (Table 2). The proportion of reported cases for which oral fluid was tested was higher for children <15 years of age (10,763/12,588 [86%]) than for persons ≥15 years (717/1,242 [58%]).

However, cases in children <15 years of age were significantly less likely to be confirmed positive by oral fluid testing than were cases in persons ≥15 years of age (54/10,763 [0.5%] vs. 89/717 [12%]; p = 0.0001). Adults represented the largest proportion of all serum-confirmed case-patients during the 10-year period. However, a higher proportion of cases for which oral fluid was tested were in children <15 years of age than cases confirmed by serum testing (54/89 [61%] vs. 52/209 [25%], respectively; p = 0.0001).

Reports of rubella showed an approximately equal sex distribution among persons <15 years of age but showed a slight preponderance of female patients among adults (Table 2). The proportion of reported cases for which oral fluid was tested did not differ by sex (6,084/7,211 [84%] male patients and 5,521/6,644 [83%] female patients). The proportion of cases confirmed positive by oral fluid testing was similar for boys and girls <15 years of age but significantly higher for men than for women (63/265 [24%] vs. 26/442 [5.9%]; p = 0.0001). Cases confirmed by serum testing showed an equal sex distribution, whereas cases confirmed by oral fluid testing showed a predominance of male patients (125/253 [49%] and 92/141 [65%] male patients among serum-confirmed and oral fluid–confirmed cases, respectively; p = 0.002).

Data sources also differed with respect to vaccination status. The proportion of reported cases that could be confirmed as rubella was almost 7× higher for unvaccinated than for vaccinated persons (128/6,543 [2.0%] and 15/5,122 [0.3%], respectively; p<0.0002). However, the absolute difference varied with age. Although a small but significant difference occurred between unvaccinated and vaccinated children <5 years of age (40/5,515 [0.7%] and 5/3,007 [0.2%], respectively; p = 0.0007), the difference was much larger in persons 15–24 years of age (46/150 [31%] and 2/171 [1.2%], respectively; p<0.0001).

Regional distribution varied by data source (Table 3).

Table 1. Accuracy of World Health Organization–modified clinical case definition for rubella, England and Wales, 1999–2008*

Characteristic	Sensitivity		Specificity		Positive predictive value	
	No. positive/ no. tested	% (95% CI)	No. positive/ no. tested	% (95% CI)	No. positive/ no. tested	% (95% CI)
Age, y						
<1	22/37	59.5 (43.6–75.3)	1,467/2,804	52.3 (50.5–54.2)	22/1,359	1.6 (1.0–2.3)
1–4	34/75	45.3 (34.1–56.6)	2,303/4,522	50.9 (49.5–52.4)	34/2,253	1.5 (1.0–2.0)
5–9	7/11	63.6 (35.2–92.1)	1,006/1,640	61.3 (59.0–63.7)	7/641	1.1 (0.3–1.9)
10–14	10/32	31.3 (15.2–47.3)	298/426	70.0 (65.6–74.3)	10/138	7.2 (2.9–11.6)
15–24	433/871	49.7 (46.4–53.0)	281/462	60.8 (56.4–65.3)	433/614	70.5 (66.9–74.1)
≥25	256/455	56.3 (51.7–60.8)	257/439	58.5 (53.9–63.2)	256/438	58.4 (53.8–63.1)
Sex						
M	649/1,278	50.8 (48.0–53.5)	2,877/5,326	54.0 (52.7–55.4)	649/3,098	20.9 (19.5–22.4)
F	112/202	55.4 (48.6–62.3)	2,744/4,954	55.3 (54.0–56.8)	112/2,322	4.8 (3.95–5.7)
Year of report						
1995 Jan–1998 Dec	743/1,435	51.8 (49.2–54.4)	3,630/6,677	54.4 (53.2–55.6)	743/3,790	19.6 (18.3–20.9)
1999 Jan–2003 Jul	18/46	39.1 (25.0–53.2)	1,983/3,616	54.8 (53.2–56.5)	18/1,651	1.1 (0.6–1.6)

*CI, confidence interval.
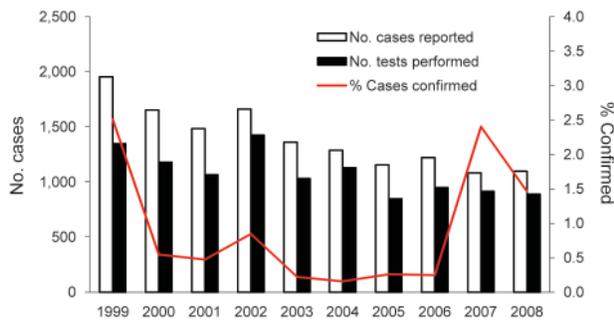
Figure. Number of rubella cases reported, number of oral (crevicular) fluid tests performed, and proportion of reported cases confirmed by oral fluid testing, England and Wales, 1999–2008.

The annual incidence based on reported cases was highest in the North East region and lowest in Wales, whereas the annual incidence based on laboratory reports of serum confirmation was highest in London. Incidence based on oral fluid test results also differed, even after adjustment for the proportion of reported cases tested. The estimated overall incidence of confirmed rubella increased by 54% (from 0.50 cases/1 million population to 0.77 cases/1 million population) when data for oral fluid testing were included. Oral fluid data also changed the ranking of regions; the Eastern region overtook London in reporting the highest overall incidence. Although the West Midlands and Yorkshire and Humberside regions reported the lowest incidence on the basis of serum testing alone, after oral fluid testing was included, the East Midlands region reported the lowest overall incidence.

Discussion

Before vaccination was introduced, epidemics of rubella occurred regularly and caused mild rash illness, predominantly in children. In 1970, introduction of a selective vaccination policy in the United Kingdom aimed to reduce the risk for infection in early pregnancy and the risk for fetal death and CRS. Despite the success of the selective policy, MMR was adopted into the routine childhood vaccination schedule to eliminate circulating rubella and to further reduce the risk for CRS.

Since 1988, a clinical diagnosis of rubella has been reportable by registered medical practitioners in England and Wales under the statutory Notification of Infectious Diseases; there is no case definition. When an infection is commonly occurring in an area, the positive predictive value of a clinical diagnosis may be sufficient for accurate surveillance (24). However, because rubella has become less common, an increasing proportion of reported cases are likely to be caused by other infections that have similar clinical manifestations. The rash of rubella may be temporary and can resemble the rash caused by other viruses. For example, infection with parvovirus B19, human herpesvirus 6 (roseola infantum), and human herpesvirus 7 all involve rash and fever and may be misdiagnosed as rubella (25,26).

We have confirmed the low sensitivity and specificity of the clinical case definition and that this definition is not affected by age, sex, and period of reporting. Despite some missing clinical information and the absence of information about arthralgia, this finding suggests that the WHO clinical case definition is not sufficiently accurate for surveillance in the postvaccine era. This finding also emphasizes the need to have laboratory confirmation of all clinically diagnosed cases to accurately monitor rubella elimination (27).

Over the 10-year period of elimination, only ≈1 of 100 persons reported with clinically diagnosed rubella and who underwent oral fluid testing had confirmed cases. In addition, reported cases differed from laboratory-confirmed cases with respect to patient age, sex, vaccination status, and geographic distribution. We have therefore shown that surveillance based only on clinical reports would substantially overestimate the true incidence of rubella, particularly in children, and therefore give a misleading epidemiologic picture. Furthermore, we have shown that testing of oral fluid is acceptable in the United Kingdom and can be used to augment routine serologic diagnosis. Approximately one third of confirmed rubella cases were diagnosed by testing of oral fluid, which improved ascertainment of confirmed infections in children and men. In addition, by using

Table 2. Reported and confirmed rubella cases in enhanced surveillance program, England and Wales, 1999–2008*

Age, y	Total no. reports				No. cases confirmed by oral (crevicular) fluid testing				No. additional cases confirmed by serum testing			
	M	F	UNK	Total no. (%)	M	F	UNK	Total no. (%)	M	F	UNK	Total no. (%)
<1	1,823	1,674	23	3,520 (25.0)	10	10	1	21 (15.0)	4	2	1	7 (2.7)
1–4	3,406	2,882	37	6,325 (45.0)	14	9	1	24 (17.0)	19	10	1	30 (11.0)
5–9	1,083	1,005	14	2,102 (15.0)	2	3	0	5 (3.5)	2	2	0	4 (1.5)
10–14	339	298	4	641 (4.6)	3	1	0	4 (0.3)	7	3	1	11 (4.2)
≥15	506	729	7	1,242 (8.9)	63	26	0	89 (62.0)	93	109	7	209 (79.0)
UNK	54	56	12	122	0	0	0	0	0	2	0	2
Total no. (%)	7,211 (52.0)	6,644 (48.0)	97	13,952	92 (64.0)	49 (34.0)	2	143	125 (48.0)	128 (48.0)	10	263

*UNK, unknown.

Table 3. Regional variation in rubella reports by oral (crevicular) fluid testing and confirmed cases from oral fluid and serum, England and Wales, 1999–2008

Region	No. reported cases	Incidence of reported cases*	No. oral fluid tests (% total reports)	No. cases confirmed by oral fluid testing	No. cases confirmed by serum testing	Annual incidence of confirmed cases*		
						Confirmed by oral fluid testing†	Confirmed by serum testing	Total
East Midlands	1,336	319	1,065 (80)	3	9	0.09	0.21	0.29
Eastern	1,212	224	1,232 (102)‡	44	32	0.80	0.59	1.41
London	1,653	226	1,452 (88)	32	67	0.67	0.92	1.35
North East	840	331	574 (68)	0	12	0.00	0.47	0.47
North West	1,662	245	1,505 (91)	12	13	0.25	0.19	0.37
South East	2,363	295	2,350 (99)	18	48	0.34	0.60	0.82
South West	1,109	224	857 (77)	13	40	0.31	0.81	1.07
West Midlands	1,235	234	821 (66)	12	10	0.33	0.19	0.42
Wales	639	220	518 (81)	1	10	0.02	0.34	0.38
Yorkshire and Humberside	1,903	382	1,260 (66)	7	22	0.20	0.44	0.58
Not specified			75	1	0			
Total	13,952	266	11,709 (84)	143	263	0.31	0.50	0.77

*Incidence per 1 million population. Annual incidence calculated by using 2001 census population figures.
†Adjusted for proportion of cases tested.
‡Several oral fluid tests were conducted for cases that were not formally reported during university outbreaks in 3 regions in 1999, including the South West, Eastern, and North West regions (17).

PCR, we obtained genotype information on 12 samples that would have otherwise not been available.

Currently, there is only 1 commercial assay for testing rubella IgM in oral fluid, and this assay does not have an In Vitro Diagnostics license, thus limiting its use in some countries. However, in many regions, WHO is evaluating this assay as a tool for surveillance of infection (28; K. Brown, pers. comm.) Although the UK system relies upon a well-organized postal service, alternative approaches for delivering and receiving specimens directly from the patient's home or family practice may be required in other countries considering the use of oral fluid.

In addition to antibody testing, oral fluid can be used for rubella RNA detection in samples obtained early during infection (13) and for genotyping and molecular epidemiologic studies (14,15). Tests for detecting rubella-specific IgM and RNA in oral fluid samples are also suitable for confirming a diagnosis of CRS (11). The same system is also being used to monitor measles and mumps incidence and to inform MMR vaccine policy in England and Wales (18,19,29,30).

Oral fluid testing can also be used to evaluate the completeness of rubella surveillance. Since 1999, the number of reports of rubella and oral fluid tests performed annually has remained constant, and the proportion of cases tested has remained high. When combined with a low rate of confirmation, including cases diagnosed as clinical rubella, in a country with free universal access to high-quality primary-care physicians, this finding suggests that surveillance of confirmed rubella is nearly complete in England and Wales. Despite this suggestion, annual incidence of confirmed rubella remains <1 per 1 million population, which is the goal for elimination (4).

In recently published surveillance guidelines, WHO has recommended IgM detection, which can be performed with serum and oral fluid (31). These guidelines also describe performance indicators to assess the quality of national surveillance systems in the elimination phase. These indicators include a laboratory investigation rate (proportion of clinically suspected cases with adequate specimens for IgM testing) >80% and a detection rate for the number of clinically suspected rubella cases investigated and discarded by laboratory testing $\geq$2/100,000 population/year. Data from the enhanced surveillance program show that for ≈84% of reported cases, oral fluid was tested, and results for 2,208 clinically suspected rubella cases per 100,000 population were investigated and discarded. This high discard rate would be feasible only with noninvasive testing and contributes to the high quality of the UK enhanced surveillance program. Information about the low rate of rubella supplements surveillance that confirms that CRS incidence in the United Kingdom was 0.14/100,000 live-born infants in 2007 (32), which was far below the WHO elimination goal of 1/100,000 live-born infants (4).

We confirmed that a clinical case definition alone is not sufficiently specific for surveillance of rubella in the elimination era and that laboratory confirmation by testing serum samples is biased and incomplete. Since 1999, a substantial proportion of confirmed cases of rubella identified through the enhanced surveillance scheme have occurred in unvaccinated men. With the availability of oral fluid testing, a high number and high proportion of suspected cases have been tested. However, numbers of confirmed rubella cases in children remain low, which is consistent with high levels of vaccine coverage and low levels of susceptibility in this younger age group (33). The enhanced oral fluid

surveillance system has proven valuable for accurately assessing progress toward achieving the WHO goal of eliminating circulating rubella and CRS from the population of England and Wales.

Acknowledgment

Dr Manikkavasagan is a research fellow in the Immunisation, Hepatitis and Blood Safety Department at the Health Protection Agency Centre for Infections, London. Her research interests include surveillance and vaccine-preventable diseases, particularly measles, varicella, and hepatitis B.

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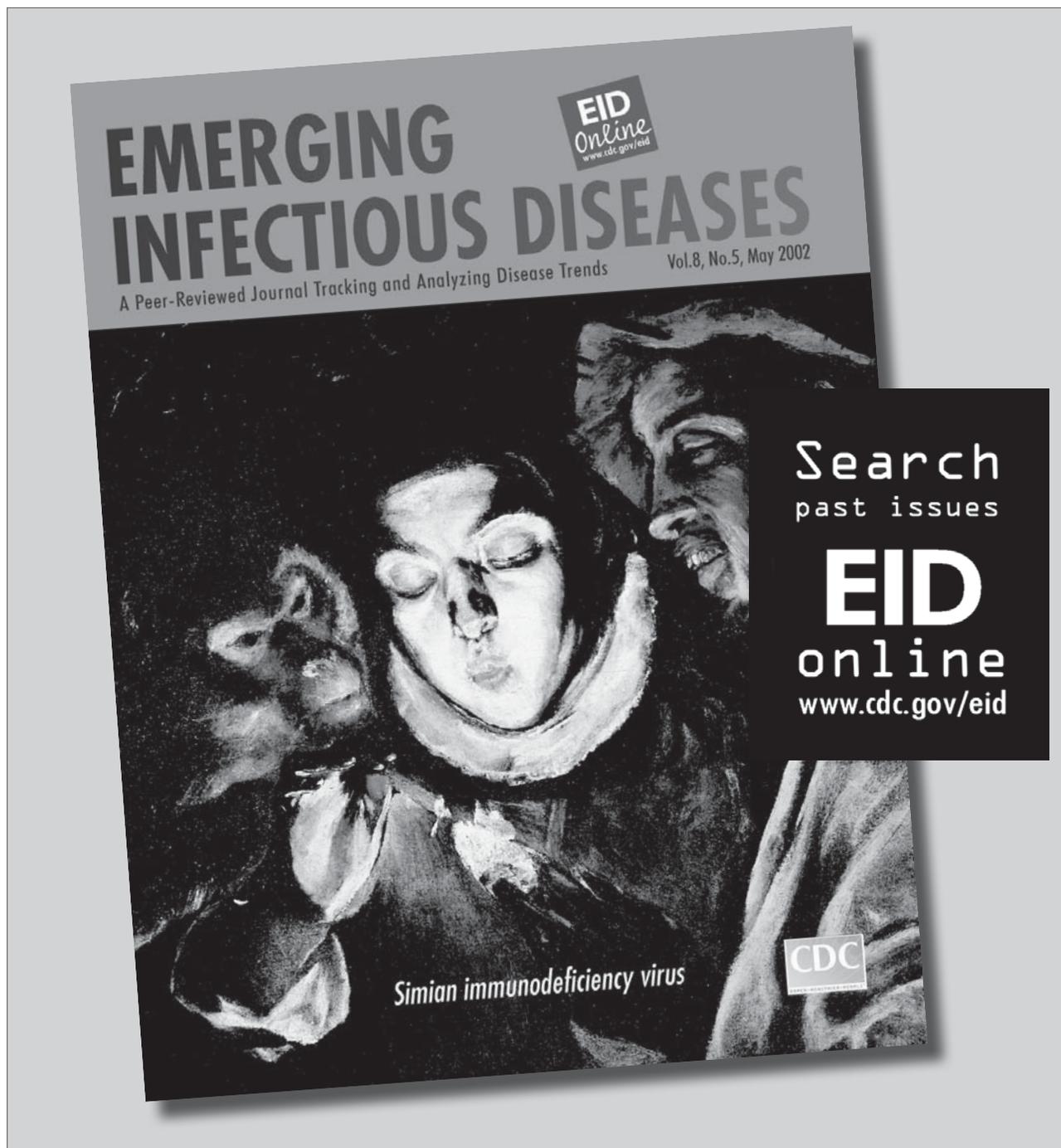
Human Monkeypox Outbreak Caused by Novel Virus Belonging to Congo Basin Clade, Sudan, 2005

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To determine the outbreak source of monkeypox virus (MPXV) infections in Unity State, Sudan, in November 2005, we conducted a retrospective investigation. MPXV was identified in a sub-Sahelian savannah environment. Three case notification categories were used: suspected, probable, and confirmed. Molecular, virologic, and serologic assays were used to test blood specimens, vesicular swabs, and crust specimens obtained from symptomatic and recovering persons. Ten laboratory-confirmed cases and 9 probable cases of MPXV were reported during September–December 2005; no deaths occurred. Human-to-human transmission up to 5 generations was described. Our investigation could not fully determine the source of the outbreak. Preliminary data indicate that the MPXV strain isolated during this outbreak was a novel virus belonging to the Congo Basin clade. Our results indicate that MPXV should be considered endemic to the wetland areas of Unity State. This finding will enhance understanding of the ecologic niche for this virus.

Monkeypox virus (MPXV) is an orthopoxvirus and the causative agent for human monkeypox, a viral disease with clinical signs in humans similar to those seen in past smallpox patients (*1,2*). Human monkeypox is regularly reported in remote villages of central Africa near tropical rainforests where persons may have contact with infected animals (*3–7*). In 2003, human monkeypox was identified in the Western Hemisphere (*8*) after importation of rodents from Ghana.

In early November 2005, the medical team at the Médecins Sans Frontières France (MSF-F) hospital in Bentiu, Unity State, Sudan, reported several suspected case-patients with generalized vesicopustular rash that resembled rash caused by MPXV. Biological samples were collected and sent to the World Health Organization (WHO) Collaborating Center for Smallpox and other Poxvirus Infections, Centers for Disease Control and Prevention (CDC), Atlanta, USA, where MPXV infection was confirmed (*9*). Because human monkeypox had been reported in a dry savannah area in Africa, an investigation team conducted a retrospective analysis of the outbreak. A description of virologic studies indicating that MPXV isolated from the Sudan outbreak is a novel virus in the Congo Basin clade will be detailed in a subsequent report. Here, we report the results of a retrospective investigation that was conducted in January 2006.

Materials and Methods

Bentiu, capital of Unity State, is situated in southern Sudan ≈750 km southwest of Khartoum, on the southern banks of the Bahr al-Ghazal River (9°14′N, 29°50′E). Unity State is uniformly flat with clay soil and its forests are scattered. During the dry season (November–April) its landscape exhibits characteristics of typical sub-Sahelian savannah; tall grass and inundated swamps predominate during the rainy season of May–October (*10,11*).

Unity State was affected greatly during the 21-year civil war in Sudan that largely destroyed the social fabric of its inhabitants (the Nuer), who were seminomadic per-

sons (*10*). Massive displacement of the indigenous population was reported in early 2000; returning refugees and displaced persons from Khartoum, Kenya, and Ethiopia occurred later in 2005. In November 2005, the underlying health infrastructure of Unity State, comprising a network of clinics and healthcare centers and a referral system, was still undergoing construction.

Initial Report of the Outbreak

On October 27, 2005, the MSF-F hospital team in Bentiu examined an 8-month-old child who had a generalized vesiculopustular rash that was clinically consistent with an orthopoxvirus-associated disease. The child originated from the village of Nuria. He became ill on October 15, 2005, with sudden onset of fever, cough, inflammation of nasal mucous membranes, and enlarged cervical lymph nodes. On October, 16, 2005, a papular rash appeared on the infant's head and within his mouth. Within 24 hours the rash had spread to cover first his extremities and later his trunk; lesions were also seen on his palms and soles. On October, 29, 2005, a similar disease developed in the child's mother with abrupt onset of fever and a papular rash, which developed pustular characteristics over the next 5 days. From October 27 through November 18, a preliminary investigation by MSF-F hospital staff identified an additional 18 similar cases in different villages. Crust and blood samples from the mother were sent to CDC in Atlanta, where MPXV infection was confirmed by PCR and virus isolation (*9*). Blood specimens, vesicular swabs, and crust specimens obtained from the 8-month-old child were sent to Institut Pasteur in Paris, where MPXV diagnosis was also confirmed.

Epidemiologic Surveillance and Investigation of Cases

During January 15, 2006–January 30, 2006, an investigation team, comprising members of the Unity State Ministry of Health (MoH), Sudan Federal MoH, MSF-F, and WHO conducted a retrospective analysis of the MPXV outbreak in Bentiu to identify its source. Three case-notification categories were developed: suspected, probable, and confirmed. A suspected case-patient was defined as any person from the outbreak zone who sought treatment during September 2005–January 2006 for fever (>37.5°C) and vesicular crusty rash. A probable case was defined as any person from the outbreak zone, evaluated by a clinician, who sought treatment during September 2005–January 2006 for fever (>37.5°C), had a vesicular-pustular rash similar to that shown in a WHO reference photograph, and had an epidemiologic link to a confirmed case. If laboratory samples were obtained during or after the illness, then the previous notification categories were reclassified as laboratory-confirmed cases or not a case. Results from a laboratory-confirmed case were considered positive either

by ELISA that showed immunoglobulin (Ig) M against orthopoxvirus, PCR amplification of MPXV DNA, or MPXV isolation. Probable cases in which test results were positive by ELISA for IgG and negative for IgM were considered laboratory confirmed if serum samples were collected >56 days after onset of rash and the patient did not have a smallpox vaccination scar or was too young to have been vaccinated against smallpox (*7*). Cases in which test results were negative by PCR and by ELISA for IgG and IgM were classified as not a case.

Suspected case-patients were identified through analysis of medical records from healthcare centers and through passive and active surveillance. Case-patients were passively identified by staff at healthcare and hospital facilities that served as sentinel surveillance sites. Active case finding was organized by the investigation team, who traveled from village to village in areas where suspected cases had been reported. Epidemiologic and clinical information for each case-patient were collected by using data obtained from patient, family, and key informant interviews and from focal group discussions. Data were also obtained by comprehensive review of available patient medical files from the Bentiu MSF-F hospital and other local healthcare centers.

Data for each patient were compiled by using a standardized MPXV investigation form designed to collect information on the identity of the patient, clinical signs, treatment administered during course of disease, potential modes of infection, and laboratory findings. Parameters established by WHO for assessment of smallpox cases (*12*) were used to determine rash severity (i.e., benign, 5–25 lesions; moderate, 26–100 lesions; grave, 101–250 lesions; and extremely grave, >250 lesions). To obtain information on potential modes of infection, investigators sought to determine the range of patient activities during the 2 weeks before illness onset, including visits to healthcare centers or traditional healers, attendance at funeral ceremonies, and any contacts that the patient may have had with other suspected human case-patients or with wild animals.

Laboratory Analysis and Clinical Specimens

After informed consent was obtained, blood specimens were collected from acute-phase and recovering suspected or probable case-patients; vesicular swabs and crust specimens were collected only from acute-phase case-patients. During October 2005–January 2006, samples (in some cases multiple samples from individual case-patients) were collected from 21 persons: 19 blood samples, 8 vesicular fluid samples, and 7 crust samples. All samples were shipped for analysis to CDC in Atlanta or to Institut Pasteur in Paris.

Molecular, virologic, and serologic assays were used for diagnosis of MPXV. PCR-based molecular assays were

performed by using DNA prepared from lesions, swabs, smears, and EDTA–whole blood specimens; in some cases whole blood and dry blood preserved on filter paper were used if no other samples were available. Specimens positive by PCR for MPXV DNA were those that yielded positive results in ≥2 independent PCR tests (specific for different loci), including 1 that discriminates MPXV-specific DNA signatures from those of other orthopoxviruses. Assays designed to detect generic-level and species-specific DNA signatures and virus culture procedures have been described (*13*). Lesion specimens that did not yield DNA signatures consistent with MPXV were subsequently examined for varicella virus (*14*).

Serologic testing alone was used to define disease status for suspected and probable case-patients who had no active lesions at the time of specimen collection. ELISAs were used for detection of orthopoxvirus-specific IgG or IgM from patient serum samples (*15*). Elevated levels of orthopoxvirus-reactive IgG in serum can indicate either MPXV infection or a previous smallpox vaccination (vaccinia virus) (*7*). Elevated IgM titer (7–56) after onset of rash in persons who had compatible clinical and epidemiologic characteristics was considered confirmation of monkeypox.

Results

During September 20, 2005–January 31, 2006, a total of 49 cases meeting confirmed, probable, or suspected status definitions were identified in Unity State, Sudan (10 confirmed, 9 probable, and 30 suspected). Among 30 suspected case-patients reported by surveillance, 18 could not be investigated because of logistic issues and 12 were reclassified as non–case-patients after investigation. Among the 12 non–case-patients, 2 who were 50 and 40 years of age were positive for IgG that was attributed to remote vaccination. Other causes of vesicular rash were found among the 12 non–case-patients: cutaneous anthrax (1), fungus in HIV patients (2), *Staphylococcus* sp. (2), and chickenpox (5).

For epidemiologic analysis, a human monkeypox case was defined as any probable or confirmed case identified during the investigation. All 19 monkeypox case-patients were Nuers, and all recovered from illness (case-fatality rate [CFR] 0%).

Of the 10 laboratory-confirmed case-patients, 3 were PCR positive, 3 were IgM and IgG positive, and 4 were IgG positive and were born after the cessation of the routine smallpox vaccination in 1975 (these 4 case-patients were 5, 11, 13, and 30 years of age, respectively). Tissue from 3 case-patients who were positive for MPXV DNA PCR yielded live virus. The hemagglutinin gene (942 bp) of Sudan viruses was identical to that of the MPXV Congo Basin strain MPXV2003_DRC and MPXV1979_Zaire and

had 6 nt changes compared with that of MPXV West Africa strains MPXV2003_US and MPXV_WalterReed267. The full genome sequence comparative analysis is ongoing, but preliminary results show that the MPXV strain isolated during this outbreak has a novel genomic structural variation related to the Congo Basin MPXV clade. All 3 PCR-positive case-patients were from the same village of Nuria; belonged to the same chain of transmission; and had blood specimens, vesicular swabs, and crust specimens obtained 5–13 days after onset of symptoms. Samples were obtained from the 3 confirmed case-patients who were IgM and IgG positive 25–70 days after onset of symptoms. From the 4 confirmed case-patients who were IgG positive, 2 had equivocal tests for IgM and samples were obtained 36 and 39 days after onset of symptoms; 2 were negative for IgM and samples were obtained 50 and 78 days after onset of symptoms.

Of 9 probable case-patients, 2 case-patients agreed to the interview and clinical investigation but declined to provide clinical specimens. The 7 remaining probable case-patients were not available for interviews during the January investigation.

The 19 monkeypox case-patients were reported from 5 villages (Figure 1): 2 in Bentiu, 3 in Modin, 5 in Nuria, 5 in Rubkona, and 4 in Wang Kay. All of these villages are located along the herbaceous wetlands along the Bahr-el Ghazal River. The alleged index case-patient, identified retrospectively, became ill on September 20, 2006, in Wang Kay village. The last cases were reported in Rubkona village, with onset of laboratory-confirmed illness occurring on December 15, 2005. The epidemic curve of the outbreak (Figure 2) has a monophasic distribution showing that the peak occurred during October 31, 2005–November 6, 2005. All case-patients were <32 years of age (range 8 months to 32 years), and most (15/19, 79%) were <20 years of age. Ten (52%) of the 19 case-patients were women.

Description of Different Chains of Transmission

Epidemiologic evidence supported 4 different chains of transmission. Person-to-person transmission was documented in 3 chains. Fourteen case-patients reported contact with a suspected monkeypox case-patient before onset of symptoms; 1 case-patient was probably exposed to infected material during his hospitalization at the MSF-F hospital; and 6 case-patients did not report any known likely mode of infection. Among the 6 case-patients who did not report any known mode of infection, 3 case-patients reported that the rash began appearing around a preexisting wound (1 on the neck, 1 on the foot, and 1 on the arm), which could have been from the bite of an infected animal. None of the monkeypox case-patients reported contact with wild animals.

Figure 3 shows 3 transmission chains hypothesized to have occurred during the outbreak. The case-patient who
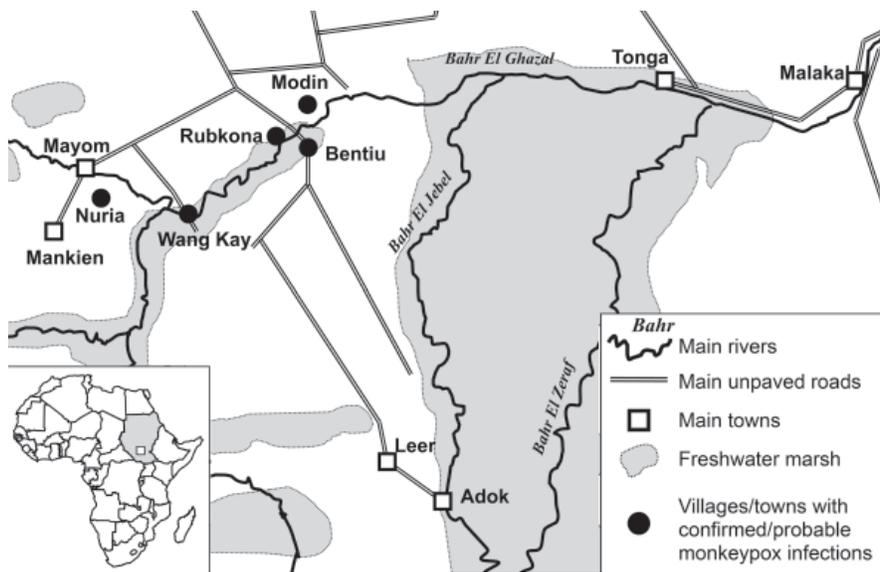
Figure 1. Geographic distribution of cases of human monkeypox virus infection in Unity State, Sudan, 2005. Inset shows location of Sudan (gray shading) and area of consideration within Unity State (white box).

we believe may have initiated the transmission chain in Unity State was an 18-year-old man from Wang Kay village. He was unavailable at the time of the investigation for follow-up, and his potential exposure to MPXV was not determined. Local authorities reported that no one was infected before him and that he had had a particularly severe form of the disease; the recovery period lasted several weeks. He may have transmitted the virus to a traditional healer and tooth extractor (case-patient 2) who had treated him and who lived in the same village.

One independent focus of infection without secondary transmission was reported in Bentiu village (1 confirmed case, IgG and IgM positive). This case-patient was a 19-year-old woman who became ill on November 3 and was admitted to the MSF-F hospital on November 10 because of fever and generalized pustular lesions over her entire body. She could not be directly linked to the other chain of transmission, and her mode of infection could not be determined.

Clinical Characteristics of Case-Patients

Of the 19 case-patients, 8 were admitted to the MSF-F Bentiu hospital. The average interval between date of fever onset and date of hospital admission was 8.5 days (median 8.5 days, minimum 4 days, maximum 14 days). The disease progression for 12 patients for whom information was available was similar: a febrile prodrome, cervical lymphadenopathy, throat and joint pain, and later skin eruptions. The median number of days from onset of fever to onset of rash was 4 days (range 1–6 days). Among the 19 case-patients, the most commonly reported signs were fever (16 case-patients), lymphadenopathy (15 case-patients), and maculopapular rash (19 case-patients). Details of the frequency of individual symptoms among the monkeypox

case-patients are reported in Figure 4.

Among 11 patients for whom we were able to obtain accurate information on number and location of lesions, 8 had lesions distributed on the entire body and 3 had lesions on the face, arms, and legs. Among the 8 patients who had lesions on the entire body, we obtained information on the number of lesions for 6 of them (3 were moderate, 26–100 lesions; 2 were grave, 101–250 lesions; and 1 extremely grave, >250 lesions). The average and median of case intervals (i.e., the number of days between symptom onsets) was 14 and could be evaluated for 7 couples (case–secondary case).

Discussion

Smallpox transmission was interrupted in Sudan in 1962, and smallpox did not return until after its reimportation during 1966–1968. The disease spread throughout
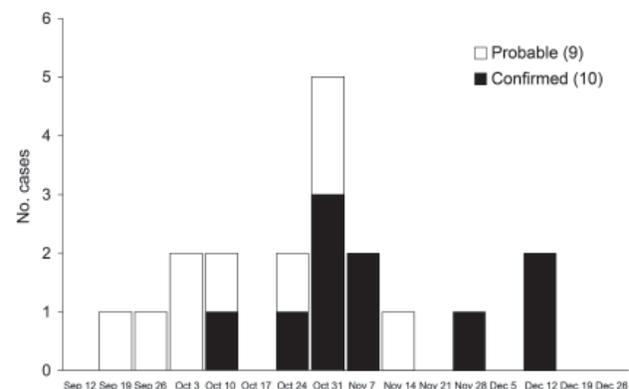
Figure 2. Date of symptom onset for 19 confirmed and probable cases of human monkeypox virus infection in Unity State, Sudan, September 2005–December 2005. Onset date estimated for 5 cases.

the country, prompting the establishment of a surveillance and containment program that successfully stopped variola transmission; the last cases were reported in Sudan in December 1972. Mass vaccinations against smallpox continued until 1975, and an international commission certified that Sudan was free of smallpox in November 1978 (*12*). After 33 years of epidemiologic silence, the events reported here from 2005 represent the sole reported outbreak of an orthopoxvirus-associated disease in Sudan in the post-smallpox era.

Despite difficulties during this investigation, we confirmed the presence of human monkeypox disease in Unity State, Sudan. Per our case definition, 10 confirmed and 9 probable cases were reported during September–December 2005. Considering the high number of suspected cases that we were unable to investigate due to logistic constraints and some missing links in several potential transmission chains, we believe the scope of the outbreak is probably underestimated.

Characteristics of the epidemiologic curve are largely compatible with a point source of infection, reinforcing our hypothesis that the traditional healer and dentist (case-patient 2) may have spread the disease to different groups of patients. Young persons were possibly infected while being treated for childhood illnesses, and young adults may have been infected during ritual teeth extraction. No deaths occurred, and evidence of human-to-human transmission for ≤5 generations was described. We found 4 chains of transmission, 3 of which were associated with the activities of a traditional healer and tooth extractor. Clinical symptoms of case-patients in Sudan was similar to previous descriptions in case-patients from central and West Africa. We found that 6 (75%) of 8 case-patients reported ulcers in the mouth, which is consistent with the frequency of oral lesions reported in unvaccinated patients in the Democratic Republic of the Congo, 1981–1986 (*2*).

In Sudan, as in much of sub-Saharan Africa, extracting incisors (and sometimes canine milk teeth) occurs just after eruption of permanent dentition. This practice is associated with achieving adulthood, beauty, and tribal identity and is necessary for emitting specific linguistic sounds and consuming softer food textures (*16,17*). In addition, it is a common belief that canine milk teeth adversely affect the health of infants, causing diarrhea, vomiting, and fever. These teeth are often removed by a traditional healer when symptoms develop in the child. Because these practices are avenues for disease transmission, an educational campaign aimed at reducing the prevalence of this practice should be implemented. Culturally sensitive education could help implement change for safer practices and discourage this harmful ritual practice.

The low CFR observed during this outbreak could not be attributed to treatment, as most case-patients were ad-
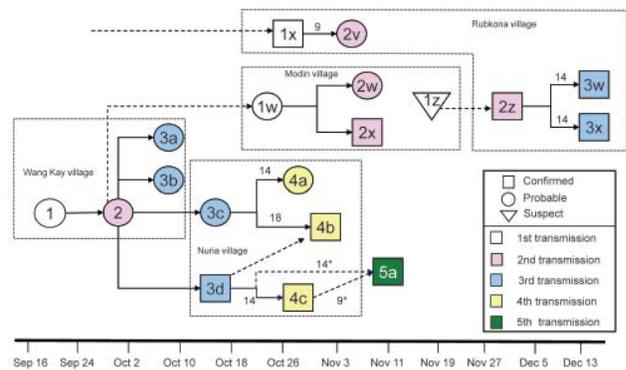
Figure 3. Pattern of virus transmission hypothesized to have occurred during outbreak of human monkeypox in Unity State, Sudan, 2005. Cases are arranged according to date of illness onset in the patient. Solid arrows indicate probable lines of person-to-person transmission; dashed arrows depict undetermined transmission events (e.g., case-patient exposed to persons with monkeypox-like symptoms in same village where no formal link could be established). Numbers near arrows refer to the number of days between case onsets (case intervals); numbers with asterisks (case-patient 5a) refer to interval between possible date of exposure and symptom onset. Dashed boxes enclose case-patients who were living in the same village. Case-patients 1x, 2v, 2w, 2x, 3d, 4c, and 5a were hospitalized in the Médecins Sans Frontières France hospital in Bentiu, Unity State. Three additional cases were not related to these chains of transmission.

mitted to the MSF-F hospital very late in the course of disease. Virulence differences between MPXV isolates from West Africa and the Congo Basin have been shown to be caused by genetic differences (*18,19*). The low CFR observed in Sudan could be linked to the MPXV circulating in Unity State and its genetic difference with West Africa and Congo Basin isolates. In this regard, our preliminary results showed that the MPXV strain isolated during this outbreak is a novel virus. Alternatively, the low CFR could be related to the transmission of the virus or dose of the virus exposure, and the relatively older ages of affected persons.

This outbreak was reported in an area outside its traditional ecology (tropical rainforests), but rodents that had been incriminated in past human infections in other African countries (e.g., Gambian rat [*Cricetomys gambianus*] or sun squirrels [*Heliosciurius* spp.]) are present in Unity State. This fact suggests that the virus may be endemic to this novel ecologic setting and could have been circulating undetected in nature for a long time. Nonetheless, we did not establish the source of animal-to-human exposure for MPXV.

Regarding the origin of this outbreak, one hypothesis is that the virus may be endemic to the wetland areas of Unity State. This hypothesis is supported by the discovery of a novel MPXV, the low CFR reported in monkeypox case-
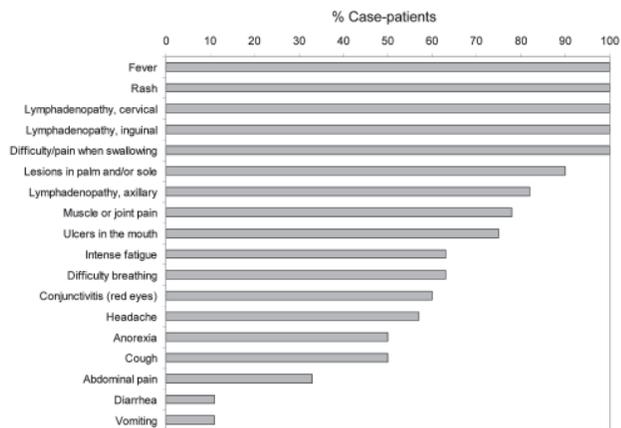
Figure 4. Frequency of individual symptoms reported among human monkeypox virus case-patients in Unity State, Sudan, 2005. Symptoms are arranged from highest to lowest percentage. Note that denominators may vary because confirmed responses were not available from all case-patients.

patients, and the identification of several chains of transmission that had no link between them, implying several introductions into the human population. The suggestion about monkeypox endemicity in Unity State is also supported by several descriptions from villagers; since 2001 similar cases that may be consistent with monkeypox have been recorded in Unity State after severe flooding as was seen during the fall of 2005. Indeed, flooding in Unity Sate during August and September 2005 may have decreased the home range of terrestrial mammals and facilitated the possible contact between potential animal reservoirs and humans. However, several suspected monkeypox cases we investigated were later found to have alternative, laboratory-confirmed diagnoses, thus making anecdotal accounts of possible past human monkeypox outbreaks impossible to confirm as orthopoxvirus infections.

An alternative hypothesis regarding the source of MPXV in Sudan is that the virus was introduced by importation from a neighboring region endemic for MPXV, either by movement of infected animals or through human migration. Still, our investigation did not establish any direct link to either the Democratic Republic of Congo or any of the countries in West Africa where monkeypox is endemic.

Additional investigation, notably ecologic studies, will be needed to demonstrate the endemicity of the virus in Sudan and to discover its natural reservoir host. These studies may have important consequences in the research of the ecologic niche of MPXV (20).

After the January 2006 investigation, the MoH in Unity State and MSF-F started a hospital-based surveillance system that enabled detection of 3 case-patients who had suspected pustular rash illnesses. Varicella virus infection

was confirmed for all. Unfortunately, surveillance was discontinued in April 2006 because of lack of resources; since then no suspected cases have been reported. To confirm that monkeypox is endemic to Unity State and possibly all of southern Sudan, additional ecologic studies are needed, as well as long-term surveillance for rash and pustular disease, combined with laboratory confirmation of suspected cases.

Acknowledgments

Dr Formenty is a field epidemiologist working at the World Health Organization. His research focuses on public health interventions (surveillance of infectious diseases and outbreak response), particularly viral hemorrhagic fevers and other emerging zoonotic diseases.

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Therapeutic Drug Monitoring for Slow Response to Tuberculosis Treatment in a State Control Program, Virginia, USA

Scott K. Heysell, Jane L. Moore, Suzanne J. Keller, and Eric R. Houpt

Therapeutic drug monitoring may be useful in tuberculosis management, but programmatic implementation is understudied. We performed a retrospective cohort study to determine prevalence of lower than expected levels of isoniazid, rifampin, ethambutol, and pyrazinamide measured at time of estimated peak serum concentration. Patients were tested for serum concentration at 2 hours after medication administration. When patients were tested, 22 had concentrations lower than expected range for rifampin, 23 of 39 patients had low levels of isoniazid, and 8 of 26 patients had low levels of ethambutol; all 20 patients tested for pyrazinamide were within expected range. Over 26 months, 42 patients met criteria for slow response. Diabetes was associated with slow response (p<0.001), and persons with diabetes were more likely than persons without diabetes to have low rifampin levels (p = 0.03). Dosage adjustment of rifampin was more likely to elevate serum concentration to the target range than adjustment of isoniazid given in daily doses (p = 0.01).

W orldwide, tuberculosis (TB) remains the leading cause of death from a curable infectious disease; ≈1.4 million deaths occurred in 2008 alone (*1*). Death is a consequence of delayed diagnosis and ineffective or incomplete treatment because cure rates exceed 95% with appropriate therapy (*2*). Slow response to therapy can lead to prolonged infectiousness, extended treatment duration, acquired drug resistance, or recurrence of TB after treatment. The reasons for slow response are diverse, but measurement of serum anti-TB drug levels, or therapeutic drug monitoring (TDM), is a potentially useful tool for uncovering the causes of slow response (*3,4*). Low serum levels can be a consequence of malabsorption, inaccurate dosing, altered metabolism, or drug–drug interactions (*4*), but in most instances low serum levels can be readily corrected with dose adjustment.

TDM is currently recommended in TB treatment guidelines as optional (*5*), and few large TB control programs have access to routine TDM. Although published reports describe patients for whom slow response was attributable to low drug levels, questions remain about how best to implement TDM on a programmatic scale (*6,7*). Definitions of slow response vary, and recommendations for which medications to prioritize for TDM are lacking. Furthermore, for general populations receiving TB therapy, TDM is unlikely to be of benefit, given the infrequency of treatment failure or TB recurrence (*8*). Although it is well known that certain patients, such as those infected with HIV and thus prone to malabsorption, are at higher risk for low drug levels (*9–12*), studies of TDM that included patients responding well to anti-TB medications found lower than expected drug levels of isoniazid and rifampin in many patients with adequate clinical response (*13,14*). Therefore, identification of patients at risk for slow response is critical within a TB control program. In addition, TDM performed earlier in the time course of slow response may also affect other major programmatic outcomes, such as treatment duration.

In the state of Virginia it is mandatory for providers to report all cases of TB to the Virginia Department of Health. Each case is assigned to a nurse case manager, who oversees and monitors the progress of each patient until treatment is completed. Directly observed therapy is administered by the nurse case manager or a trained outreach worker. After 4 weeks of therapy, patients are screened by the nurse case manager. Medical consultation for patients with ongoing symptoms is provided by the state TB clinicians in an effort

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to identify slow response earlier and to prevent acquired drug resistance. Clinicians define slow response in a patient as after ≥30 days from the start of treatment the patient has ≥2 of the following findings: sputum smear positive for acid-fast bacilli; no improvement in TB-specific symptoms, including fever, cough, weight loss, and/or night sweats; and no improvement in chest radiograph lesions previously identified as consistent with TB. Routine TDM among patients who met criteria for slow response was instituted by March 2007.

We performed a retrospective cohort study among patients slow to respond to pulmonary TB treatment in the state of Virginia to determine the prevalence of lower than expected levels of isoniazid, rifampin, ethambutol, and pyrazinamide measured at the time of estimated peak serum concentration (C_{max}). Secondary aims included investigation of risk factors for levels below the expected range, evaluation of the mean change and likelihood of achieving a level within the expected range after dose adjustment, and comparison of outcomes between persons with slow responses with those with low and expected levels. The study was approved by the institutional review boards for human subjects research at the University of Virginia and the Virginia Department of Health.

Methods

Patients

Patients were identified for inclusion in the study by using routine TB surveillance data recorded in the Virginia TB Registry. All patients who were ≥18 years of age, had confirmed *Mycobacterium tuberculosis* cultures, and started TB therapy in the state of Virginia during March 1, 2007–May 1, 2009, were eligible. We included patients who had been treated for pulmonary TB or pulmonary TB and extrapulmonary TB and who began a regimen of isoniazid, rifampin, ethambutol, and pyrazinamide. All *M. tuberculosis* specimens were sent to the state TB laboratory, where drug-susceptibility testing was performed after secondary culture of the isolate by using the automated Bactec MGIT 960 system (Becton Dickinson, Sparks, MD, USA). Patients were excluded if their original isolate was later found to be resistant to ≥1 first-line medication. Patients were also excluded if they had TDM performed for reasons other than slow response.

Surveillance data were retrieved from the state TB registry and included demographics (age, sex, race/ethnicity, country of origin, and homelessness), TB history (prior episodes of TB, sputum smear and culture status of current TB episode, and chest radiograph abnormalities), coexisting conditions (diabetes, HIV infection, intravenous drug use, and excessive alcohol use), and treatment

outcomes (completion of TB treatment, duration of completed TB treatment, relapse of TB following treatment completion, acquisition of drug resistance in a previously susceptible TB strain, and death from any cause during TB treatment). Information about medication-related adverse events following anti-TB drug dose increase was obtained from personal communication with the state TB medical consultants.

Therapeutic Drug Monitoring

The standard procedure for TDM was for patients to be given their daily dose of TB medications in the morning while fasting and then observed for 2 hours, during which they were restricted from eating or drinking. At 2 hours after medication administration, venous blood was collected and serum was separated before transport on dry ice to the regional referral laboratory. The drug levels from blood collected 2 hours after medication administration (C_{2hr}) were used as the estimated peak maximum serum concentration (C_{max}) as per standard practice and were determined by using high-performance liquid chromatography (for isoniazid and rifampin) or gas chromatography with mass spectrometry (for ethambutol and pyrazinamide). Expected C_{2hr} ranges were provided and were consistent with published norms (5). C_{2hr} levels were also recorded for patients with initial low levels in whom follow-up TDM was performed after dose adjustment.

Data Analysis

Demographic and clinical characteristics were compared with the χ^2 statistic or, for nonparametric data, the Mann-Whitney U test. For the determination of risk factors for C_{2hr} levels below the expected range, values were dichotomized into normal if the value was within or above the expected range, or low if the value fell below the expected range. Bivariate and multivariate logistic regression analyses were used to determine risk factors for either a low isoniazid or a low rifampin level. The multivariate model included any variable with p<0.1 in bivariate analysis and relevant demographic characteristics. Paired Student *t* tests were used to report the mean change in C_{2hr} levels following dose adjustment. Medications dosed ≥5× per week were considered daily dosed. Biweekly dosing was used for some patients for isoniazid and rifampin, with the isoniazid biweekly dose at 3× the usual daily dose. The rifampin dose was unchanged regardless of dosing frequency. The log-rank test was used to compare treatment duration and for patients who had not completed therapy at the time of analysis; data were right censored for survival analysis. All tests of significance were 2 sided. Data were analyzed with SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

During the study, 350 patients were treated with an initial regimen of isoniazid, rifampin, ethambutol, and pyrazinamide for pulmonary TB; of these patients, 45 (13%) met criteria for slow response. Thirty-seven patients were excluded from the study (34 with normal response and 3 with slow response) after drug-susceptibility testing showed resistance to $\geq$1 medication of the treatment regimen. An additional 2 patients were excluded because TDM was performed for reasons other than slow response. Thus, 311 patients were included in the study, of whom 42 (14%) met criteria for slow response (Table 1). At the time of TDM among patients meeting criteria for slow response, all had persistent TB-related symptoms. Of the 23 patients with initial smear-positive sputum specimens, 17 (74%) had specimens that remained smear positive.

The mean (SD) age for patients in the study was 46 years (20 years), and 204 (65%) were men (Table 1). The most common ethnicity was Asian (102 [33%]) and 228 (73%) were foreign born. Among all patients, 291 (93%) had no history of TB. Most patients tested had a positive tuberculin skin test (TST) result, although 85 (27%) did not have a TST reading recorded. There were 287 patients with a sputum smear recorded at the time of diagnosis; of these smears, 193 (62%) were positive for acid-fast bacilli. Ninety-five percent (295) of patients had a chest radiograph with findings suggestive of TB, of which 122 (39%) were cavitary. There was no significant difference in the proportion of patients with a positive TST result, a positive sputum smear, or a chest radiograph showing cavitation among the 42 with a slow response and the remaining patients with adequate response. Among patients meeting criteria for slow response, none were HIV infected and none reported using illicit drugs (either intravenous or nonintravenous). The only significant predictor of slow response was diabetes (unadjusted odds ratio [OR] 6.5, 95% confidence interval [CI] 3.2–13.5, p<0.001; adjusted OR [aOR] 6.3, 95% CI 2.8–14.0, p<0.001).

Initial C_{2hr} Levels

All 42 patients who were slow to respond were monitored for rifampin, and 22 (52%) had a C_{2hr} level below the expected range; 1 (2%) had a high level (Figure 1). For daily or biweekly dosed rifampin, the median C_{2hr} level was 7.4 µg/mL (interquartile range [IQR] 2.5–11.4 µg/mL, expected range 8–24 µg/mL) (Table 2). Thirty-nine patients were monitored for isoniazid; 23 (59%) had levels below the expected range. For daily dosed isoniazid, the median C_{2hr} was 1.90 µg/mL (IQR 1.1–3.5 µg/mL, expected range 3–6 µg/mL), and for biweekly dosing, 9.8 µg/mL (IQR 2.8–11.2 µg/mL, expected range 9–18 µg/mL). Among the 39 patients who were tested for isoniazid and rifampin levels, 13 (33%) had levels below the expected

range for both medications. Twenty-six patients were monitored for ethambutol; 8 (31%) had levels below the expected range. The median C_{2hr} level for ethambutol was 2.5 µg/mL (IQR 1.7–3.2 µg/mL, expected range 2–6 µg/mL). Twenty patients were monitored for pyrazinamide, all had levels within the expected range; median C_{2hr} level was 28.1 µg/mL (IQR 26.5–33.2 µg/mL, expected range 20–50 µg/mL).

Risk Factors for Low Isoniazid or Rifampin Levels

Analyses of risk factors for low levels of isoniazid or low levels of rifampin were performed, but small sample size precluded meaningful analysis of risk factors for low ethambutol levels. Patients with diabetes were at significantly increased risk of having a low rifampin level (OR 5.8, 95% CI 1.4–23.1, p = 0.01; aOR 5.7, 95% CI 1.2–25.7, p = 0.03) (Table 3). Patients who received isoniazid biweekly were less likely to have low isoniazid levels than those who received isoniazid daily, but this association was not statistically significant in multivariate analysis (OR 0.21, 95% CI 0.05–0.91, p = 0.04; aOR 0.47, 95% CI 0.09–2.5, p = 0.37) (Table 3).

Follow-up C_{2hr} Levels after Dose Adjustment

Eighteen patients with rifampin levels below the expected range had follow-up TDM after dose adjustment. Levels for all patients increased from the initial to the follow-up level with a mean (SD) change of 11.0 µg/mL (9.7 µg/mL; p<0.001); 16 (89%) had levels in the expected range after the first dose adjustment (Figure 2). Fourteen patients had follow-up TDM for daily-dosed isoniazid levels below the expected range; monitoring detected increased levels in 12 patients, with a mean (SD) change of 3.4 µg/mL (2.9 µg/mL; p = 0.001); 4 (29%) patients had levels in the expected range. Four patients had follow-up TDM for biweekly-dosed isoniazid levels below the expected range, and all had increased levels with a mean (SD) change of 11.8 µg/mL (6.1 µg/mL; p = 0.03); 3 (75%) patients had levels in the expected range.

Rifampin levels below the expected range were significantly more likely to be corrected to within the expected range following the first dose adjustment than were daily-dosed isoniazid levels below the expected range (p = 0.01). There was no significant difference in the likelihood of correction to the expected range between daily and biweekly dosed isoniazid. No follow-up levels of ethambutol or pyrazinamide were reported. There were no reported medication-related adverse events following dose increase.

Treatment Outcomes

Complete outcomes were available for 32 (76%) patients; 10 patients continued receiving treatment. Twenty-seven patients successfully completed treatment, 3 patients

Table 1. Baseline characteristics of adults treated for drug-susceptible pulmonary TB in a state control program, Virginia, USA, March 1, 2007–May 1, 2009*

Characteristic	All patients, N = 311, no. (%)	Slow response, n = 42, no. (%)	Normal response, n = 269, no. (%)	Bivariate OR (95% CI); p value
Age, y				
18–39	151 (49)	16 (38)	135 (50)	Referent
40–64	90 (29)	13 (31)	77 (29)	1.4 (0.65–3.10); p = 0.38
≥65	70 (22)	13 (31)	57 (21)	1.9 (0.87–4.30); p = 0.11
Sex				
M	204 (65)	29 (69)	175 (65)	Referent
F	107 (35)	13 (31)	94 (35)	0.84 (0.42–1.70); p = 0.61
Race/ethnicity				
Asian	102 (33)	19 (45)	83 (31)	Referent
Hispanic	82 (26)	11 (26)	71 (26)	0.67 (0.30–1.50); p = 0.34
Black	86 (28)	8 (19)	78 (29)	0.45 (0.19–1.10); p = 0.07
White	41 (13)	4 (10)	37 (14)	0.47 (0.15–1.50); p = 0.20
Native American	0	0	0	
Foreign born				
No	83 (27)	9 (21)	74 (28)	Referent
Yes	228 (73)	33 (79)	195 (72)	1.4 (0.64–3.00); p = 0.41
Homeless				
No	301 (97)	41 (98)	260 (97)	Referent
Yes	10 (3)	1 (2)	9 (3)	0.71 (0.09–5.70); p = 0.74
Illicit drug use				
No	298 (96)	42 (100)	256 (95)	Referent
Non-injection use	7 (2)	0	7 (3)	p>0.99
Injection use	6 (2)	0	6 (2)	p>0.99
Alcohol abuse				
No	276 (89)	38 (91)	238 (89)	Referent
Yes	35 (11)	4 (9)	31 (11)	0.81 (0.27–2.40); p = 0.70
HIV status				
Negative	266 (86)	37 (88)	229 (85)	Referent
Positive	11 (3)	0	11 (4)	p>0.99
Unknown	34 (11)	5 (12)	29 (11)	1.1 (0.39–2.90); p = 0.90
Diabetes				
No	270 (87)	25 (60)	245 (91)	Referent
Yes	41 (13)	17 (40)	24 (9)	6.9 (3.3–14.6); p<0.001†
Prior TB history				
No	291 (93)	38 (91)	253 (94)	Referent
Yes	18 (6)	3 (7)	15 (5)	1.3 (0.37–4.80); p = 0.66
Unknown	2 (1)	1 (2)	1 (1)	6.6 (0.41–108.70); p = 0.18
Tuberculin skin test result				
Negative	47 (15)	5 (12)	42 (16)	Referent
Positive	179 (58)	23 (55)	156 (58)	1.2 (0.44–3.50); p = 0.68
Unavailable	85 (27)	14 (33)	71 (26)	1.7 (0.56–4.90); p = 0.36
Sputum smear				
Negative	94 (30)	9 (21)	85 (31)	Referent
Positive	193 (62)	30 (72)	163 (61)	1.7 (0.79–3.80); p = 0.17
Not done	24 (8)	3 (7)	21 (8)	1.3 (0.34–5.40); p = 0.67
Chest radiograph				
No TB findings	16 (5)	2 (5)	14 (5)	Referent
Noncavitary	173 (56)	19 (45)	154 (57)	0.86 (0.18–4.10); p = 0.85
Cavitary	122 (39)	21 (50)	101 (38)	1.5 (0.31–6.80); p = 0.64
Disease site				
Pulmonary	212 (68)	32 (76)	180 (67)	Referent
Pulmonary/extrapulmonary	99 (32)	10 (24)	89 (33)	0.63 (0.30–1.30); p = 0.23

*Tuberculin skin test values recorded in surveillance database as positive based on guidelines from the American Thoracic Society and Centers for Disease Control and Prevention (15). TB, tuberculosis; OR, odds ratio; CI, confidence interval.
†Adjusted odds ratio 6.3 (95% CI 2.8–14.0); p<0.001.
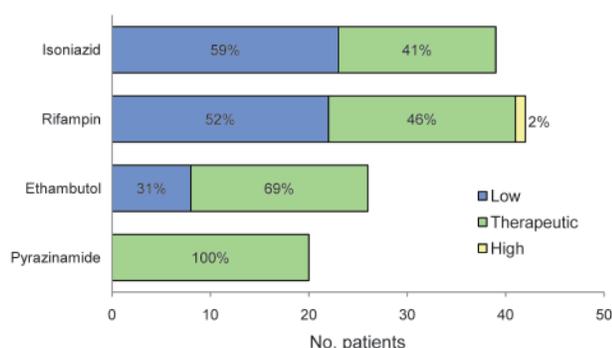
Figure 1. Results of serum concentration 2 hours after medication administration levels (C_{2hr}) of first-line antituberculosis medications among patients with a slow response to tuberculosis therapy. Frequencies are reported for low, within target, and high C_{2hr} levels corresponding to levels below, within, or above the expected range for each medication.

died, and 2 patients moved out of the state where follow-up was incomplete. Median time to completion of therapy among all 27 patients was 45 weeks (IQR 40–51 weeks). Among the 14 patients with initial rifampin levels below the expected range who completed treatment, the median duration was 40 weeks (IQR 38–48 weeks) compared with a median duration of 47 weeks (IQR 44–55 weeks) for the 13 patients with initial rifampin levels within the expected range (log-rank p = 0.17).

There were no reports of relapse of infection over a median of 14.5 months (IQR 7–25 months) from the conclusion of treatment. No patient had documented acquisition of medication resistance in follow-up TB cultures while on treatment. All 3 deaths occurred shortly after TDM was performed: 1 patient had isoniazid, rifampin, ethambutol, and pyrazinamide levels within expected ranges, 1 patient had isoniazid, rifampin, and ethambutol levels within expected ranges, and 1 patient had isoniazid, rifampin, and ethambutol levels below the expected ranges.

Discussion

The major finding of this study is that among patients being treated for pulmonary TB in Virginia, most patients that met criteria for slow response to therapy were found to have C_{2hr} levels of rifampin and isoniazid below the expected range; many patients also had low levels of ethambutol. Given the high frequency of patients who were slow to respond to both key first-line medications, isoniazid and rifampin, and the well-tolerated subsequent increase in levels documented after dose adjustment, TDM appears to be a useful strategy for identifying a remediable cause of slow response at a programmatic level. Furthermore, the median duration of therapy for patients with rifampin levels below the expected range was nearly 2 months shorter than that for patients with normal rifampin levels. Although it is not specifically known if identification and correction of lower than expected levels brought about a rapid improvement in TB clinical signs and symptoms, the comparatively shorter course represents a substantial cost savings when considering personnel involved in monitoring and medication administration, as well as diagnostic tests averted in the work-up of otherwise unexplained slow response.

We found that ≈90% of patients with lower than expected rifampin levels who were subsequently tested after the first dose adjustment achieved target levels. Dose-titration studies of rifampin confirm a continuously increasing response of early bactericidal activity by measurement of sputum colony counts with corresponding increase in rifampin dose (16,17). Rifampin has been tolerated at doses as high as 1,200 mg in small studies, and larger trials are ongoing to study high-dose rifampin in an effort to shorten therapeutic duration (18,19). Given increasing evidence that rifampin may be underdosed for many patients regardless of TB outcome (20), the findings of this study suggest that rifampin is a prime medication to prioritize for early TDM for patients for whom TB therapy is failing.

Diabetes was significantly associated with slow response in our study population, and, among persons with a slow response with diabetes, C_{2hr} levels of rifampin were significantly more likely to be below the expected range. Patients with diabetes are at greater risk for incident TB (21,22) and are more likely to have poor TB treatment outcomes (23,24), which may partially be explained by inadequate pharmacotherapy. A growing body of evidence has

Table 2. Comparison of median serum concentration at 2 hours after medication administration as estimate of peak serum concentration levels and expected range, therapeutic drug monitoring, Virginia, USA, March 1, 2007–May 1, 2009*

Medication	Median serum concentration, µg/mL (IQR)	Expected serum concentration range, µg/mL
Isoniazid		
Daily	1.9 (1.1–3.5)	3–6
Biweekly	9.8 (2.8–11.2)	9–18
Rifampin daily and/or biweekly	7.4 (2.5–11.4)	8–24
Ethambutol†	2.5 (1.7–3.2)	2–6
Pyrazinamide†	28.1 (26.5–33.2)	20–50

*IQR, interquartile range.
†All patients with therapeutic drug monitoring levels obtained for ethambutol and pyrazinamide were taking weight-based daily doses of these medications.

Table 3. Risk factors for INH or RIF serum concentration levels below the expected range 2 hours after medication administration among persons with slow responses, therapeutic drug monitoring, Virginia, USA, March 1, 2007–May 1, 2009*

Characteristic	Normal INH, n = 16, no. (%)	Low INH, n = 23, no. (%)	Bivariate risk ratio (95% CI); p value	Normal RIF, n = 20, no. (%)	Low RIF, n = 22, no. (%)	Bivariate risk ratio (95% CI); p value
Age, y						
18–39	4 (25)	8 (35)	Referent	5 (25)	10 (46)	Referent
40–64	7 (44)	8 (35)	0.57 (0.12–2.80); p = 0.49	8 (40)	7 (32)	0.44 (0.10–1.90); p = 0.27
≥65	5 (31)	7 (30)	0.70 (0.13–3.70); p = 0.67	7 (35)	5 (22)	0.36 (0.07–1.70); p = 0.20
Sex						
M	11 (69)	15 (65)	Referent	13 (65)	15 (68)	Referent
F	5 (31)	8 (35)	1.2 (0.30–4.60); p = 0.82	7 (35)	7 (32)	0.87 (0.24–3.10); p = 0.81
Race/Ethnicity						
White	1 (6)	3 (13)	1.9 (0.16–22.30); p = 0.61	3 (15)	1(5)	0.37 (0.3–4.2); p = 0.42
Asian	7 (44)	11 (48)	Referent	10 (50)	9 (41)	Referent
Hispanic/Latino	6 (38)	4 (17)	0.42 (0.09–2.10); p = 0.43	3 (15)	8 (36)	3.0 (0.60–14.70); p = 0.18
Black	2 (12)	5 (22)	1.6 (0.24–10.60); p = 0.63	4(20)	4 (18)	1.1 (0.21–5.80); p = 0.90
Foreign-born						
No	3 (19)	6 (26)	Referent	6 (30)	3 (14)	Referent
Yes	13 (81)	17 (74)	0.65 (0.14–3.10); p = 0.59	14 (70)	19 (86)	2.7 (0.58–12.80); p = 0.21
Diabetes						
No	10 (63)	13 (57)	Referent	16 (80)	9 (41)	Referent
Yes	6 (37)	10 (43)	1.3 (0.35–4.70); p = 0.71	4 (20)	13 (59)	5.8 (1.4–23.1); p = 0.01†
Alcohol abuse						
No	15 (94)	22 (96)	Referent	18 (90)	20 (91)	Referent
Yes	1 (6)	1 (4)	0.69 (0.40–11.70); p = 0.79	2 (10)	2 (9)	0.90 (0.12–7.10); p = 0.92
Dose interval						
Daily	8 (50)	19 (83)	Referent	11 (65)	16 (73)	Referent
Biweekly	8 (50)	4 (17)	0.21 (0.05–0.90); p = 0.04‡	6 (35)	6 (27)	0.88 (0.23–3.30); p = 0.85

*INH, isoniazid; RIF, rifampin; CI, confidence interval. Low is defined as below expected range; normal is defined as within or above expected range.
†Adjusted odds ratio 5.7 (1.2–25.7); p = 0.03.
‡Adjusted odds ratio 0.47 (0.09–2.50); p = 0.37.

demonstrated reduced rifampin exposure in patients with TB and diabetes, which may be in part related to impaired absorption (25,26). Although rifampin absorption may not be blunted by delayed gastric emptying (27), hyperglycemia can decrease gastric hydrochloric acid secretion, which results in a higher gastric pH and reduced rifampin absorption (25). Markers of glycemic control were not available for analysis in this study, but it may be of further use to risk stratify patients with diabetes based on disease severity. It is suspected, though not tested, that drug–drug interactions were also playing a role in the observed lower levels of rifampin in patients with diabetes from our study population. Given preexisting knowledge of the association between diabetes and poor treatment outcome, there may have been a bias on behalf of the TB control staff to characterize a patient with diabetes as a person with a slow response based
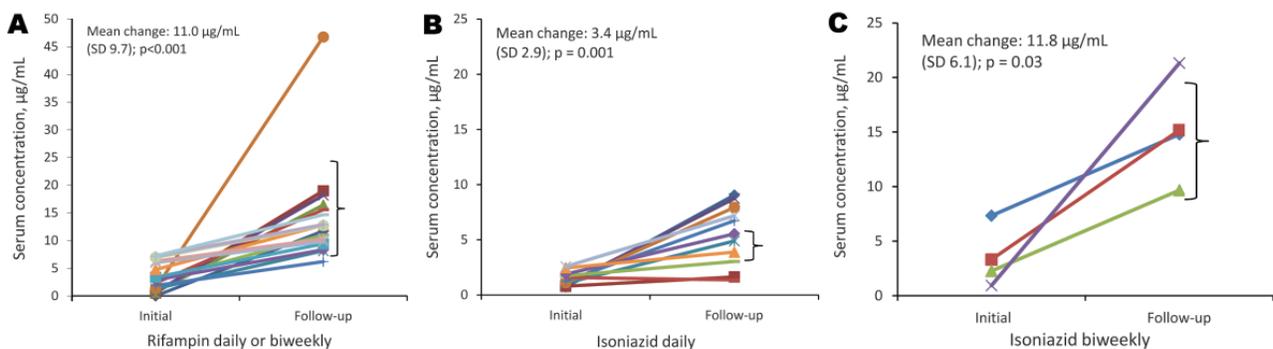
Figure 2. Results in patients with initial serum concentrations 2 h after medication below the expected range with follow-up levels after dose adjustment for rifampin daily or biweekly (A), isoniazid daily (B), and isoniazid biweekly (C). The median initial and follow-up doses of rifampin daily or biweekly were 600 mg and 900 mg, respectively; for isoniazid daily, 300 mg and 450 mg, respectively; and for isoniazid biweekly, 900 mg and 1,200 mg, respectively. Brackets represent expected ranges for each dose of medication.

on symptom persistence. Nevertheless, our findings raise the possibility of TB programs studying the benefit of routine TDM for rifampin among all patients with diabetes at the start of TB therapy.

Routine TDM among persons with slow responses at a relatively early point in the treatment course reflects policy change within Virginia's TB control program. Given the high prevalence of low levels of key medications and the observed ease of correction after dose adjustment, we recommend that similar TB programs investigate the applicability of TDM within their own settings. Further generalization must be cautiously considered, however, because relevant factors that may adversely affect pharmacokinetics, such as the patient's weight at the time of TDM, concurrent medication use, chronic kidney disease, or cirrhosis, were not available in these surveillance data for comparison (*28–30*). Other limitations to the study must be taken into account. Blood was collected at 2 hours after medication administration to estimate C_{max}. A second blood collection at 6 hours can additionally distinguish patients whose absorption may be delayed secondary to poor gastric emptying (*4*); however, the frequency of delayed absorption has been rare in other cohorts for which 2 and 6 hour measurements were performed (*10*).

Additionally, given that the prevalence of lower than expected drug levels was not known for patients with adequate response to anti-TB therapy, the overall contribution of pharmacotherapy to the cause of slow response in this cohort cannot be fully assessed. Surveillance data did not permit comparison of culture positivity in patients who met criteria for slow response at the time of TDM matched to patients with adequate response for whom TDM was not performed. Use of TDM as early as 4 weeks, as was performed in this study, may have selected for patients that might otherwise have improved after 8 weeks of therapy regardless of other interventions. Lastly, further study may find, given that the cost of TDM ($\approx$\$80 US per individual drug) may be substantial for some TB control programs, that it is more economical to start therapy with increased drug dosages for patients at higher risk for slow response.

In summary, routine TDM among patients meeting criteria for slow response to TB therapy in Virginia identified most of those tested to have C_{2hr} levels of rifampin and isoniazid below the expected range; many patients also had low levels of ethambutol. Most patients with repeat TDM following dose adjustment to rifampin and isoniazid had levels within the expected range, suggesting a clinically actionable result. Given the comparative ease of correcting low rifampin levels and the shorter duration of therapy in those with a correctable rifampin level, this medication is particularly appealing to target for programmatic intervention. Further prospective studies should evaluate the benefit of routine TDM for rifampin early in the treatment course among patients with diabetes or of higher initial doses of rifampin among all groups at risk for slow response.

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Risk Factors for Pandemic (H1N1) 2009 Virus Seroconversion among Hospital Staff, Singapore

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We describe incidence and risk factors for pandemic (H1N1) 2009 virus infection in healthcare personnel during the June–September 2009 epidemic in Singapore. Personnel contributed 3 serologic samples during June–October 2009, with seroconversion defined as a $\geq$4-fold increase in hemagglutination inhibition titers to pandemic (H1N1) 2009. Of 531 participants, 35 showed evidence of seroconversion. Seroconversion rates were highest in nurses (28/290) and lowest in allied health staff (2/116). Significant risk factors on multivariate analysis were being a nurse (adjusted odds ratio [aOR] 4.5, 95% confidence interval [CI] 1.0–19.6) and working in pandemic (H1N1) 2009 isolation wards (aOR 4.5, 95% CI 1.3–15.6). Contact with pandemic (H1N1) 2009–infected colleagues (aOR 2.5, 95% CI 0.9–6.6) and larger household size (aOR 1.2, 95% CI 1.0–1.4) were of borderline significance. Our study suggests that seroconversion was associated with occupational and nonoccupational risk factors.

During the 2003 epidemic of severe acute respiratory syndrome (SARS), large nosocomial outbreaks of SARS occurred in several hospitals in Singapore (*1,2*). Since then, concerns have been raised about how emerging infections, in particular respiratory infections, could result in transmission from patients to healthcare workers and vice versa, given the high frequency and intensity of healthcare worker contacts in the hospital environment (*3*). For pandemic influenza, additional concerns exist that even mild disease might result in staff absenteeism and, subsequently, would reduce staff strength at a time of increased demand for health services (*4*).

In April 2009, a novel influenza A virus, now referred to as pandemic (H1N1) 2009 virus, emerged in the United States and Mexico and rapidly spread worldwide (*5–7*). Published reports on pandemic (H1N1) 2009 in healthcare workers have attributed transmission to a mixture of healthcare and nonhealthcare exposures (*8–10*), with varying compliance to infection control measures implicated in some transmission events (*9,11*). Early data from the United States suggest that healthcare workers were not overrepresented among case reports of pandemic (H1N1) 2009 compared with cases in the general population (*9*), but the risk for infection for healthcare workers, and between different subgroups of healthcare workers, remains unclear (*9*).

During the initial epidemic wave of pandemic (H1N1) 2009 in Singapore, June–September 2009, we conducted a prospective seroepidemiologic cohort study among healthcare workers in Tan Tock Seng Hospital (TTSH), Singapore, by using serial blood specimens to determine antibody levels against pandemic (H1N1) 2009 as a marker of serologic infection. We describe the incidence of serologic evidence of infection and associated occupational and nonoccupational risk factors for infection in this cohort of healthcare workers.

Methods

Study Setting

TTSH is an acute-care hospital in Singapore with 1,100 beds and $\approx$6,000 healthcare workers; it has a desig-

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nated center, the Communicable Disease Centre, for management of outbreaks of emerging infections. Following the activation of Singapore's pandemic response plan by the Ministry of Health on April 25, 2009, TTSH became the designated screening center and isolation facility for all adult case-patients with pandemic (H1N1) 2009, although the first case-patient with the infection in Singapore did not receive a diagnosis and was not admitted to the hospital until May 26, 2009 (*12*). Intensive surveillance and testing of staff who had acute respiratory illness (ARI) symptoms confirmed the first case of pandemic (H1N1) 2009 in a TTSH staff member 4 weeks later, on June 22, 2009, several days after sustained community transmission had occurred in Singapore (*13,14*).

Study Design

This study was part of a larger seroepidemiologic investigation involving 3 other cohorts in Singapore: community-dwelling adults, military personnel, and staff and residents of 2 long-term care facilities (*15*). In TTSH healthcare workers (as well as in the community-dwelling adults and military personnel), up to 3 serial serum samples were taken from each person. The samples included 1) a baseline sample collected during June 22–July 7, 2009, before widespread local transmission of pandemic (H1N1) 2009; 2) an intraepidemic follow-up sample, collected during August 19–September 3, 2009, ≈4 weeks after pandemic (H1N1) 2009 epidemic activity had peaked in Singapore; and 3) a postepidemic follow-up sample, collected during September 29–October 15, 2009, >4 weeks after epidemic activity subsided in late August 2009 *(14)*.

In addition, we used standardized self-administered questionnaires to obtain baseline demographic information, seasonal influenza vaccination status, and household composition data at the time of baseline sample collection. Symptoms and possible exposures in the intervening periods between samples were elicited through follow-up questionnaires administered at the time the intraepidemic and postepidemic samples were taken. Symptom reviews covered episodes of ARI, defined as a new onset illness with any respiratory symptoms (rhinorrhea, nasal congestion, sore throat, or cough), with febrile respiratory illness (FRI) being an ARI episode with self-reported fever or a body temperature (where available) ≥37.5°C. Information on symptomatic episodes was augmented through sickness absenteeism rates and staff medical records for details such as dates of illness and tests to confirm pandemic (H1N1) 2009 infection. Exposure data covered nonoccupational exposures such as travel out of Singapore and episodes of ARI and FRI in household members, as well as occupational exposures such as care of patients with confirmed pandemic (H1N1) 2009 infection and contact with sick colleagues who have subsequently confirmed pandemic

(H1N1) 2009. Healthcare workers were also asked, when appropriate, how often they used either a surgical mask or N95 respirator during patient care and to estimate their average daily number of visitor and patient contacts. The number of colleagues in the same work area was used as a proxy indicator of the number of staff-to-staff contacts.

Recruitment of Study Participants

For the purposes of our study, we defined healthcare workers as any full-time staff personnel employed by TTSH, regardless of the nature of their work. We used internal hospital email systems and word-of-mouth referrals to invite all personnel ≥21 years of age to participate. In addition, mobile teams were sent to appropriate hospital locations such as wards, outpatient clinics, and other major work areas, such as operating theater, radiology, laboratory medicine, pharmacy, physiotherapy, and occupational therapy departments. Included in these were 3 postulated high-exposure settings: the designated isolation wards for patients with pandemic (H1N1) 2009, the emergency department through which patients with pandemic (H1N1) 2009 were being admitted, and the medical intensive care and high-dependency units where patients with the most severe pandemic (H1N1) 2009 infections were treated. Healthcare workers with ARI episodes that occurred within the 2 weeks before baseline samples were obtained were excluded, given that enrollment stopped 2 weeks after the first TTSH staff member received a diagnosis of pandemic (H1N1) 2009. Written informed consent was obtained for all participants. The study was approved by the ethics review boards of the National Healthcare Group.

Laboratory Methods and Computation of Geometric Mean Titer

Samples were tested by hemagglutination inhibition (HI) assays following standard protocols at the World Health Organization Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia (*16*). Serum samples were pretreated with receptor-destroying enzyme II (Deka Seiken Co. Ltd., Tokyo, Japan), 1:4 (vol/vol), at 37°C for 16 h before enzyme inactivation by the addition of an equal volume of 1.6% trisodium citrate (Ajax Chemicals, Melbourne, Victoria, Australia) and incubation at 56°C for 30 min. A/California/7/2009 A(H1N1) pandemic virus was purified on a sucrose gradient, concentrated, and inactivated with β-propiolactone, to create an influenza zonal pool preparation. Twenty-five microliters (4 hemagglutination units) of influenza zonal pool A/California/7/2009 virus were incubated at room temperature with an equal volume of receptor-destroying enzyme II–treated serum samples, with different wells for serum titrated in 2-fold dilutions from 1:10 to 1:1,280 in phosphate-buffered saline. After incubation of serum for 1 h,

25 µL of 1% (vol/vol) turkey erythrocytes was added to each well. HI was read after 30 min, with titers expressed as the reciprocal of the highest dilution of serum in which hemagglutination was prevented. For computing geometric mean titers (GMTs), we assigned titers <10 a value of 5, and titers ≥1,280 a value of 1,280. These values were then log transformed before we computed means and associated 95% confidence intervals (CIs). GMTs were then obtained by back transformation (17).

The HI assay was assessed on paired serum samples from 56 case-patients with pandemic (H1N1) 2009 confirmed by reverse transcription–PCR. The assay had a sensitivity of 80% when seroconversion was defined as a ≥4-fold increase in antibody titers between the first and second blood specimens (15).

Sample Size Calculations and Outcomes of Interest

We targeted a final sample size of at least 500, which would have given a power of 90% to detect (with a 2-sided $p<0.05$) seroconversion rates that were 10% higher for the healthcare workers cohort than the concurrently taken community sample, which was assumed would have seroconversion rates of 25% (on the basis of the 1957 pandemic) (18). The target sample size would also have given a power of >70% to detect a ≥2× risk of seroconversion in a healthcare worker subgroup of ≈100 than in the rest of the healthcare worker population, assuming overall seroconversion risk in healthcare workers exceeded 10%.

The primary outcome of interest was seroconversion, which was defined as a ≥4-fold increase in antibody titers between any successive pair of blood specimens. We performed univariate and multivariate logistic regression with demographic information, seasonal influenza vaccine status, titers in the baseline sample, occupational and nonoccupational related exposures to assess their contribution to seroconversion, with results presented as odds ratios (ORs) with asymptotic Wald 95% CI and 2-sided p values. Multivariate analysis involved backward stepwise logistic regression with all variables significant at $p<0.10$; only variables which improved model fit at $p<0.10$ were included in the final model. Where appropriate, 95% CIs were also presented, along with χ^2 and Student unpaired t test results for differences between proportions and means, respectively. All statistical analyses were performed by using STATA 10.0 (StataCorp, College Station, TX, USA).

Results

We enrolled a total of 558 healthcare workers into the study, of which 96% (537/558) had ≥1 follow-up blood sample; 6 participants were excluded because of missing follow-up review questionnaires, leaving 531 persons for analysis. Of these, 35 (6.6%) seroconverted. Table 1 compares selected characteristics of seroconverters and nonseroconverters. Seroconverters were sampled earlier than nonseroconverters (49% vs. 38% in the first week of enrollment), and 86% of seroconverters had both follow-up samples compared with 81% of nonseroconverters, but these differences were not significant (p = 0.20 and p = 0.73, respectively). Seroconverters were slightly more likely to have received seasonal influenza vaccine than were nonseroconverters (97% vs. 91%), but this difference was not significant. There were no also significant differences between seroconverters and nonseroconverters by age or gender. HI titers in baseline samples from nonseroconverters were higher than in baseline samples from seroconverters (GMT 7.8 vs. 5.9; p = 0.02). Among seroconverters, 63% and 51% reported having an ARI and FRI episode, respectively, and only 15% and 8% of nonseroconverters reported having an ARI and FRI episode, respectively ($p<0.01$ for both).

Table 1. Selected characteristics of healthcare workers by seroconversion status for pandemic (H1N1) 2009, Singapore, 2009*			
Characteristic	No. (%) seroconverters, n = 35	No. (%) nonseroconverters, n = 496	p value
Baseline sample timing			0.20†
Jun 22–26	17 (49)	187 (38)	
Jun 28–Jul 7	18 (51)	309 (62)	
Follow-up samples taken			0.73†
Intraepidemic only	3 (9)	65 (13)	
Postepidemic only	2 (6)	31 (6)	
Intraepidemic and postepidemic	30 (86)	400 (81)	
Female	30 (86)	411 (83)	0.66†
Seasonal influenza vaccination	34 (97)	449 (91)	0.19†
ARI episode‡	22 (63)	75 (15)	<0.01†
FRI episode‡	18 (51)	41 (8)	<0.01†
Age, y, mean (95% CI)	35 (31–39)	34 (33–35)	0.76§
GMT for baseline sample (95% CI)	5.9 (5.3–6.5)	7.8 (7.3–8.3)	0.02§

*ARI, acute respiratory illness; FRI, febrile respiratory illness; CI, confidence interval; GMT, geometric mean titer.
†χ^2 test comparing seroconverters and nonseroconverters.
‡Healthcare workers who seroconverted are considered to have had an ARI or FRI episode if the date of onset preseded the date when seroconversion was detected.
§Student t test comparing seroconverters and nonseroconverters.

Most of our participants were nurses (290/531, 55%; Table 2); allied health staff, which included mostly participants from paramedical professions such as pharmacists, laboratory medicine technicians, physiotherapists and occupational therapists, formed the second largest group (116/531, 22%); ancillary and support staff, which included mainly hospital attendants and patient service associates, formed the next largest group (69/531, 13%); and administrative support staff (35/531, 7%) and doctors (21/531, 4%) made up the rest. Seroconversion rates were highest in nurses (28/290, 10%) and lowest in allied health staff (2/116, 2%). To facilitate interpretation, allied health staff were designated the reference group for computing ORs; only nurses had a significantly higher odds of infection compared with allied health staff (OR 6.1, 95% CI 1.4–26.0; p = 0.02). Compared with those working in non–patient care areas, participants whose primary work area was an inpatient ward had higher odds for seroconversion (OR 1.4, 95% CI 0.5–3.5; p = 0.54), while those in other patient care settings had lower odds for seroconversion (OR 0.5, 95% CI 0.2–1.5; p = 0.21), but neither result was significant. Significantly higher odds for seroconversion

were also observed for participants whose primary work area was in pandemic (H1N1) 2009 isolation wards (OR 4.8, 95% CI 1.5–15.6; p<0.01).

Participants who had contact with patients who had pandemic (H1N1) 2009 had marginally but not significantly increased odds of seroconversion (OR 1.8, 95% CI 0.9–3.7; p = 0.10). Those who reported having contact with a sick colleague(s) whose illness was subsequently diagnosed as pandemic (H1N1) 2009 had significantly increased odds of seroconversion (OR 2.9, 95% CI 1.2–6.9; p = 0.02).

Results of the univariate analysis for nonoccupational exposures are presented in the Figure. Healthcare workers from larger households had increased odds of seroconversion (OR 1.2 per additional household member, 95% CI 1.0–1.4; p = 0.04), but no discernible association was seen between seroconversion and having another healthcare worker in the same household or reporting another household member with ARI or FRI symptoms during the study. However, having a child or adolescent in the household increased the odds of seroconversion. In particular, significantly higher ORs were observed if the healthcare workers

Table 2. Univariate analysis of occupational risk factors for pandemic (H1N1) 2009 for 531 healthcare workers, Singapore, 2009*				
Risk factor	No. participants	No. (%) seroconverted	Crude OR (95% CI)	p value
Occupational subgroup				
Allied health	116	2 (2)	Referent	
Nurses	290	28 (10)	6.1 (1.4–26.0)	0.02
Ancillary and support	69	2 (3)	1.7 (0.2–12.4)	0.60
Administration	35	2 (6)	3.5 (0.5–25.5)	0.22
Doctors	21	1 (5)	2.9 (0.2–32.9)	0.40
Direct patient contact				
No	71	4 (6)	Referent	
Yes	460	31 (7)	1.2 (0.4–3.5)	0.73
Primary work area				
Nonpatient care areas	83	6 (7)	Referent	
Inpatient wards	210	20 (10)	1.4 (0.5–3.5)	0.54
Other patient care settings†	238	9 (4)	0.5 (0.2–1.5)	0.21
Work in high exposure settings				
Pandemic (H1N1) 2009 isolation wards	514	31 (6)	Referent	
No	514	31 (6)	Referent	
Yes	17	4 (24)	4.8 (1.5–15.6)	<0.01
Emergency department	507	33 (7)	Referent	
No	507	33 (7)	Referent	
Yes	24	2 (8)	1.3 (0.3–5.8)	0.73
Medical ICU/HDU				
No	514	33 (6)	Referent	
Yes	17	2 (12)	1.9 (0.4–8.9)	0.39
Contact with patient who had pandemic (H1N1) 2009				
No	409	23 (6)	Referent	
Yes	122	12 (10)	1.8 (0.9–3.8)	0.10
Contact with sick colleague(s) who had pandemic (H1N1) 2009				
No	484	28 (6)	Referent	
Yes	47	7 (15)	2.9 (1.2–6.9)	0.02

*OR, odds ratio; CI, confidence interval; ICU, intensive care unit, HDU, high-dependency unit.
†Includes allied health and medical staff from departments with both inpatient and outpatient coverage and staff from all other noninpatient departments involved in patient care.
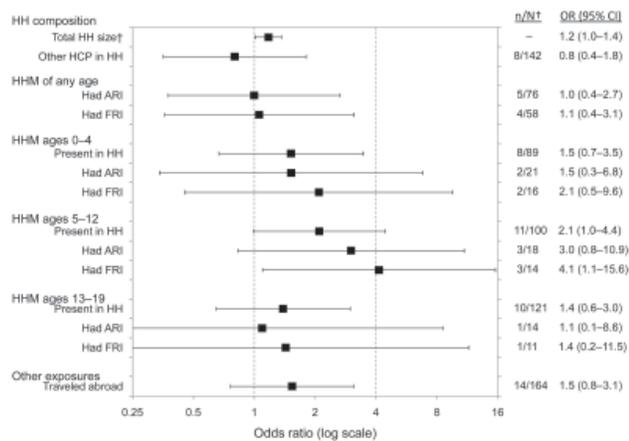
Figure. Univariate analysis for nonoccupational exposures to pandemic (H1N1) 2009 among healthcare workers, Singapore. Error bars indicate 95% confidence intervals (CIs) for odds ratios (ORs). †n/N, no. of seroconverters/no. in strata. HH, household; HCP, healthcare provider; HHM, household member; ARI, acute respiratory illness; FRI, febrile respiratory illness.

reported a child 5–12 years of age in the household (OR 2.1, 95% CI 1.0–4.4; p = 0.05), with the ORs being even higher if a child 5–12 years of age in the household had FRI symptoms (OR 4.1, 95% CI 1.1–15.6; p = 0.04).

The variables included in the final multivariate analysis are shown in Table 3. Being a nurse remained significantly associated with increased odds of infection (OR 4.5, 95% CI 1.0–19.6; p = 0.05), as did having pandemic (H1N1) 2009 isolation wards as a primary work area (OR 4.5, 95% CI 1.3–15.6; p = 0.02). Contact with colleagues with pandemic (H1N1) 2009 (OR 2.5, 95% CI 0.9–6.6; p = 0.06) and coming from a larger household (OR 1.2 per additional household member, 95% CI 1.0–1.4; p = 0.06) were of borderline significance. Having higher HI titers in the baseline serum sample was protective (OR 0.5 per unit of increase, 95% CI 0.3–1.0; p = 0.05).

In Table 4, allied health participants are compared with nurses, the groups with the lowest and highest seroconversion rates respectively; the latter was further stratified by whether they worked in inpatient wards (ward based vs. non–ward based). The proportion who seroconverted was slightly, but not significantly, higher in ward-based nurses than in non–ward-based nurses (11% vs. 8%; p = 0.53). Ward-based nurses were from significantly larger households than the other 2 groups (p<0.01 vs. allied health, p = 0.01 vs. non–ward-based nurses). No significant difference was found in the proportion who reported using face masks all or almost all of the time in patient care, but ward-based nurses were significantly more likely to have had seasonal influenza vaccine than were allied health workers (p<0.01). Key differences were found in the mean number of contacts and occupational related factors. Non–ward-based nurses mostly worked in large areas, including operating theaters, the emergency department, and outpatient clinics, and hence had significantly higher numbers of contacts than either ward-based nurses or allied health workers (p<0.01 on all measures). On the other hand, ward-based nurses were significantly more likely to be in contact with patients with confirmed pandemic (H1N1) 2009 (p<0.01 vs. non–ward-based and allied health), and allied health staff were significantly less likely to be in contact with a sick colleague who had pandemic (H1N1) 2009 (p<0.01 vs. either nursing group).

Discussion

In this study, we used paired serum samples to assess infection rates and risk factors for infection in healthcare personnel during an influenza pandemic in an acute care hospital in Singapore. We observed surprisingly lower seroconversion rates in healthcare personnel than in the rest of the community, as was emphasized in another publication (15), and found that a mixture of occupational and nonoccupational exposures were associated with risk for infection.

Table 3. Multivariate analysis of risk factors associated with seroconversion for pandemic (H1N1) 2009 in 531 healthcare workers, Singapore, 2009*

Risk factor	Adjusted OR (95% CI)	p value
Occupational subgroup		
Allied health	Referent	
Nurses	4.5 (1.0–19.6)	0.05
Ancilllary and support	1.5 (0.2–11.1)	0.69
Administration	3.6 (0.3–42.8)	0.31
Doctors	3.8 (0.5–28.7)	0.19
Pandemic (H1N1) 2009 isolation wards vs. all others	4.5 (1.3–15.6)	0.02
Contact with colleague(s) who had pandemic (H1N1) 2009 vs. none	2.5 (0.9–6.6)	0.06
Household size (per additional household member)	1.2 (1.0–1.4)	0.06
HI titer in baseline sample (per unit of increase)†	0.5 (0.3–1.0)	0.05

*OR, odds ratio; CI, confidence interval; HI, hemagglutination inhibition.
†For every unit increase in baseline (sample A) titer, where the integer values of 0–8 denote titers <0, 10, 20, 40, 80, 160, 320, 640, and ≥1,280, respectively.

Table 4. Comparison of risk factors among allied health staff, ward-based nurses, and non–ward-based nurses for exposures to pandemic (H1N1) 2009, mask use, and work-related contacts, Singapore, 2009*

Risk factor	1: Allied health staff, n = 116	2: Non–ward-based nurses, n = 103	3: Ward-based nurses, n = 187	p values†		
				2 vs. 1	3 vs. 1	3 vs. 2
Seroconverted in study period, %	2 (0–6)	8 (4–15)	11 (7–16)	0.05	<0.01	0.53
Mean age, y	32 (30–33)	34 (32–36)	32 (31–34)	0.14	0.81	0.21
Mean household size	4.8 (4.4–5.1)	4.8 (4.4–5.2)	5.5 (5.1–5.8)	0.96	<0.01	0.01
Household members with FRI in the following age groups, %						
0– 4 y	3 (1–7)	3 (1–8)	2 (1–5)	1.00	1.00	0.70
5–12 y	2 (0–6)	4 (2–10)	1 (0–4)	0.42	0.64	0.19
13–19 y	1 (0–5)	3 (1–8)	2 (1–5)	0.34	0.65	0.70
Masks for patient care all or almost all the time, %	64 (53–74)	71 (59–80)	69 (61–77)	0.48	0.54	0.87
Valid responses‡	76	69	130			
Received seasonal influenza vaccine, %	84 (77–90)	91 (84–95)	96 (92–98)	0.15	<0.01	0.19
Geometric mean no. colleagues in work area	27 (24–31)	48 (39–59)	23 (20–25)	<0.01	0.05	<0.01
Valid responses‡	101	89	147			
Geometric mean no. patient contacts per day	15 (12–18)	37 (26–52)	19 (17–22)	<0.01	0.03	<0.01
Valid responses‡	73	59	127			
Geometric mean no. visitor contacts per day	12 (9–15)	28 (21–38)	15 (13–18)	<0.01	0.04	<0.01
Valid responses‡	75	58	133			
Occupational-related exposures, %						
Direct patient contact	84 (77–90)	91 (84–95)	99 (97–100)	0.15	<0.01	<0.01
Contact with patients who had pandemic (H1N1) 2009	14 (9–21)	19 (13–28)	41 (34–48)	0.28	<0.01	<0.01
Contact with colleague(s) who had pandemic (H1N1) 2009	2 (0–6)	15 (9–23)	14 (10–20)	<0.01	<0.01	0.86

*Values in parentheses are 95% confidence intervals. FRI, febrile respiratory illness.
†p values by Fisher exact test for proportions and unpaired Student *t* test for means.
‡Based on participants who answered this questionnaire item; all other analyses are based on no. participants in that occupational subgroup.

When the study was planned, we had expected the healthcare workers cohort to have a higher seroincidence than a group of community-dwelling adults, given previous reports of pandemic and nonpandemic influenza outbreaks in hospitals (*19,20*), our own experience with SARS (*1*), and recent work showing the intensity of work-related contacts in the healthcare setting (*3*). Instead, we found that only 7% of our healthcare workers seroconverted, compared with 13% of participants in the community cohort (*15*). Our study corroborates case-reporting data in the United States, which suggest that healthcare workers did not have a higher incidence of infection than the general community, without being subject to biases that might arise from underreporting or differential case ascertainment (*9*).

Although definitively attributing the low infection rates in healthcare workers to improved infection control practices is difficult without the appropriate control groups, much evidence supports the efficacy of the common bundle of measures used in hospitals to reduce spread of respiratory viruses (*21*). Notably, there was a high level of preparedness and widespread implementation of airborne and respiratory droplet precautions and other pandemic (H1N1) 2009 infection control practices in healthcare institutions in the United States, Singapore, and elsewhere (*11,13,22–24*).

However, our study also suggests that the risk to healthcare staff should not be underestimated. We found

some occupational factors associated with seroconversion. The higher seroconversion rates in nurses posted to designated pandemic (H1N1) 2009 isolation wards should be interpreted with some caution in view of the small number of seroconversion events (2 of those infected had symptoms and 2 did not) and participants (n = 17) from these wards. Since masks (either surgical or N95 masks) were widely used in all clinical areas around the hospital, this group essentially had the same level of protection as other staff while being far more intensely exposed to pandemic (H1N1) 2009. The higher risk for seroconversion for nurses on multivariate analysis also deserves notice. Nurses had higher seasonal influenza vaccination rates and were more compliant than other occupational subgroups in following preventive measures such as mask use. However, they also were more likely to be exposed to patients as well as to have colleagues with confirmed pandemic (H1N1) 2009; the latter factor was significantly associated with seroconversion by univariate analysis (and of borderline significance on multivariate analysis), and staff-to-staff transmission was also implicated in TTSH and elsewhere (*10,13*). Non–ward-based nurses also had higher contact rates than the other main occupational subgroup (allied health staff), a factor that we could not account for in multivariate analysis (as questions on contact rates were not answered by all participants). We also could not account for the nature of patient contacts, which might be more prolonged and

intense in nurses (*3*). We suggest that our finding of the higher seroconversion risk in nurses is the result of residual confounding by the sum of these factors, many of which are an integral part of the nursing profession.

Lastly, our study suggests that nonoccupational exposures should not be forgotten as a potential source of healthcare worker infections. Other studies based on case investigations have also attributed some infections to community sources, and in our study, we found that having a child of primary school age was a risk factor on univariate analysis, particularly if that child had an FRI during the study period, although the direction of transmission in the latter could not be ascertained. Studies on nonpandemic influenza have found that index cases from pediatric age groups were more likely to generate secondary cases (*25,26*), although the same was not observed with pandemic (H1N1) 2009 (*27*). The effect of having children in the household was superseded in multivariate analysis by overall household size, which was unsurprising since households with children also tended to be larger. In any case, the significance of such nonoccupational exposures should be taken into account in any hospital-level pandemic preparedness plan.

We do acknowledge several limitations in our study. First, our findings are based on data from healthcare workers from just 1 hospital. Moreover, the unexpectedly low seroconversion rates in our cohort reduced the power of the study to investigate exposures more weakly associated with the outcome. We were also unable to assess the usefulness of personal protective equipment due to the lack of appropriate control groups. The resolution of exposure data from what was a self-administered questionnaire survey was also lower than insights that may be gained from detailed case investigations or exposure diaries that have been used in the healthcare setting (*3,11*). Finally, the lack of randomization also leaves scope for bias in our results.

An effective vaccine for pandemic (H1N1) 2009 has now been introduced, and this will likely reduce intrahospital risk of infection from this particular strain of influenza until significant genetic drift occurs, provided healthcare institutions can overcome the challenges to achieving high vaccine coverage rates in healthcare personnel (*28,29*). Seasonal influenza vaccination rates may be atypically high in TTSH because of its designated status as a first-line screening and referral center; 1 study on healthcare workers from 2 other hospitals in Singapore found that only 39% of participants were vaccinated (*30*). Although the low incidence of healthcare workers infections provides some suggestion that measures in place during the pandemic were effective, our findings suggest that some occupation-related risk factors remain. Nurses, particularly those working in pandemic (H1N1) 2009 isolation wards, were disproportionately affected, possibly because their higher levels of protective behaviors inadequately compensated for their increased oc-

cupational risk. This situation should be recognized when planning for future pandemics.

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Effectiveness of Personal Protective Equipment and Oseltamivir Prophylaxis during Avian Influenza A (H7N7) Epidemic, the Netherlands, 2003

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We analyzed the effectiveness of personal protective equipment and oseltamivir use during the 2003 avian influenza A (H7N7) epidemic in the Netherlands by linking databases containing information about farm visits, human infections, and use of oseltamivir and personal protective equipment. Using a stringent case definition, based on self-reported conjunctivitis combined with a positive hemagglutination-inhibition assay, we found that prophylactic treatment with oseltamivir significantly reduced the risk for infection per farm visit from 0.145 (95% confidence interval [CI] 0.078–0.233) to 0.031 (95% CI 0.008–0.073). The protective effect was ≈79% (95% CI 40%–97%). These results are comparable with the reported effect of prophylactic treatment with oseltamivir on human seasonal influenza. No significant protective effect was found for use of respirators or safety glasses, possibly because of limitations of the data.

Avian influenza A viruses are considered a threat to public health because they may result in new human influenza A strains. Thus, knowledge about preventing human infections with avian influenza viruses is essential. In 2003, a devastating epidemic caused by an avian influenza virus of subtype H7N7 occurred among the poultry sector of the Netherlands (*1*). During this epidemic, an unexpectedly high number of persons reported illness that appeared to be associated with subtype H7N7 infection after they were exposed to infected poultry (*2*); 1 veterinarian died of acute respiratory distress syndrome (*3*). Previous reports have documented transmission between poultry, between

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humans, and from poultry to humans (*1,4–8*). In this report, we extend earlier work by analyzing the effect of personal protective measures on poultry-to-human transmission. Specifically, we investigated the effects of use of respirators and safety glasses and the prophylactic use of oseltamivir on the risk for infection during depopulation of infected farms. Our quantitative estimates of the effect of personal protective measures can guide efforts to prevent human infections with avian influenza.

Materials and Methods

Data

Immediately after the epidemic, the National Institute of Public Health and the Environment of the Netherlands sent a questionnaire to 1,747 persons, of whom 872 (49.9%) responded. Response was lowest among persons who actively handled the culling of poultry (*9*). Of the 872 persons who responded, 450 could be linked to a farm-visits database kept during the epidemic that contained information about who had visited which farm on which date for what reason. Of these 450 persons, 194 had been actively involved in hands-on culling during the depopulation; activities included catching live poultry and picking up dead poultry. Because this group had the highest exposure (*4*), it was used to analyze the effect of personal preventive measures.

The questionnaire asked for information about symptoms of infection of the eyes, from which a self-reported conjunctivitis result was derived as an outcome measure for subtype H7N7 infection. The presence of ≥2 of the following eye symptoms was classified as conjunctivitis: redness, tearing, itchiness, pain, burning, purulence, or sensitivity to light. Blood samples were collected from survey respondents 3 weeks after possible exposure and tested by

a hemagglutination-inhibition (HI) assay for antibodies by using a modified cutoff based on validation studies of persons known to be infected and of nonexposed controls (*10*). The serologic result was available in addition to the self-reported conjunctivitis information.

We used a case definition that combined both outcomes, i.e., a person needed to self-report conjunctivitis and have a positive HI assay result. For sensitivity analysis, both the conjunctivitis and the serologic result were used in separate case definitions.

The survey also contained information about prophylaxis with a neuraminidase inhibitor (oseltamivir [Tamiflu; Roche, Basel, Switzerland]) and the use of personal protective equipment (PPE [safety glasses and respirator]). Beginning on March 14, prophylaxis with oseltamivir (75 mg daily) was prescribed to persons in contact with potentially infected poultry (2 weeks after the first infection was diagnosed and 10 days after culling began). Continuation of oseltamivir treatment was recommended until 2 days after possible exposure. The subtype H7N7 strain that circulated was susceptible to oseltamivir (*2*). To determine oseltamivir use, persons were asked the following questions: 1) Were you prescribed oseltamivir by a medical doctor? (yes/no). 2) If oseltamivir was prescribed, when was it prescribed and when did you stop using it? 3) How often did you fail to take a capsule? Persons who were not prescribed oseltamivir were classified as "did not use." On the basis of the period of use stated in the survey, persons who were prescribed oseltamivir were classified per visit as "used" or "did not use." Within the used category, persons who missed ≤3 capsules were classified as consistent users; if more capsules were missed, they were classified as inconsistent users.

PPE (respirator and safety glasses) were provided during the entire epidemic to persons involved in the depopulation. The Dutch Food and Consumer Product Safety Authority provided the PPE and supervised its use. For each visit, workers received new PPE. They were instructed how to use the PPE but received no extensive training. For both respirators and safety glasses, workers were asked the following questions: 1) Did you use respirators/safety glasses (yes/no)? 2) How often did you use respirators/safety glasses (always, almost always, sometimes, almost never, never)? 3) How often did you not use respirators/safety glasses? From responses to these questions, we classified persons into 3 categories: used, sometimes used, and did not use. Persons who had either always or almost always used respirators or safety glasses and who stated that they had not missed using them more than twice were placed in the "used" category. Persons who had not used them were classified as did not use, and all remaining persons with answers were classified as sometimes used. For safety glasses and respirators, information was available only about their

use during the whole epidemic and not per visit. We therefore assumed that a person's use of a respirator and safety glasses did not change during the epidemic. The respirators provided were type FFP2 (US equivalent N95, Figure 1), which protected both nose and mouth and were all the same size. Safety glasses covered only the front of the eyes and were open above, below, and on both sides of the eyes (Figure 1). They were effective against splashes but not against dust. No information was available about the overall health of the workers.

Statistical Analysis

The probability of becoming infected during a visit was calculated from the number of visits each person had made and whether each person was infected (according to the case definitions). The analysis comprised only visits to farms with infected poultry. During each visit, a person could either escape infection or become infected. The probability of each sequence of events could be added, from which the probability of infection per visit could be estimated by maximum likelihood (online Technical Appendix, www.cdc.gov/eid/

Figure 1. Poultry worker wearing respirator and safety glasses, the Netherlands, 2003.

Table 1. Number of infections and use of oseltamivir prophylaxis during avian influenza A (H7N7) epidemic, on the basis of combined conjunctivitis and serology-based case definition, the Netherlands, 2003*

	No. persons		No. visits	
Oseltamivir use	Infected	Total	By infected persons	Total†
Not prescribed	4	16	6	26
Prescribed but not used during visit	9	34	17	64
Prescribed and used consistently	4	41	6	85
Prescribed and used inconsistently	2	18	7	39
Prescribed and consistency of use unknown	2	7	2	18
Prescribed but period of use unknown	2	29	5	64

*Data resulted from a survey conducted among poultry workers after the epidemic.
†Because visits were classified into groups, persons may be in 2 categories (prescribed/not used and prescribed/used).

Table 2. Risk for infection with avian influenza A (H7N7) with different levels of oseltamivir use, on the basis of combined conjunctivitis and serology-based case definition, the Netherlands, 2003*

Oseltamivir use	Risk† (95% CI)
Not used	0.145 (0.078–0.233)
Not prescribed	0.161 (0.052–0.336)
Prescribed but not used during visit	0.138 (0.061–0.246)
Used	0.031 (0.008–0.073)
Prescribed and used consistently	0.015 (0.0008–0.0630)
Prescribed and used inconsistently	0.049 (0.004–0.157)
Prescribed and consistency of use unknown	0.068 (0.004–0.258)
Unknown	0.019 (0.001–0.080)
Prescribed but period of use unknown	0.020 (0.001–0.080)

*CI, confidence interval.
†Probability of infection per visit.

content/16/10/1562-Techapp.pdf). The dependent variable in this analysis was the case definition, i.e., whether a person was infected. The probability of infection was calculated according to the categorical level of personal protection used, e.g., use of oseltamivir or not, as independent variables. We calculated confidence intervals (CIs) using profile likelihood methods (*11*). Statistical testing was performed by using likelihood ratio tests (*11*).

For oseltamivir, risk for infection was calculated per group and then aggregated over the groups of persons who had and had not used oseltamivir (Tables 1, 2). Use of respirators, safety glasses, and oseltamivir were classified into the earlier described groups. We then calculated the risk for infection for each combination of groups, e.g., always used respirators/sometimes used safety glasses (Tables 3, 4). Although onset date of infection was unknown, the likelihood function took into account that a person could

become infected only once and that infection could occur at any farm visit.

Results

The 194 persons analyzed together had made 458 active culling visits. A conjunctivitis result was available for 193 persons (36 positive), a serologic result for 131 persons (81 positive), and a combined conjunctivitis/serology result for 130 persons (19 positive). Mean age of the study population was 40 years (range 18–64 years). Most (110) persons were veterinarians. Ninety percent of the study population was male. Persons made an average of 2.4 (range 1–9) active culling visits.

Oseltamivir

Oseltamivir had been prescribed for 159 persons; 35 stated that oseltamivir had not been prescribed. Forty-five

Table 3. Number of infections and use of respirator and safety glasses during avian influenza A (H7N7) epidemic, on the basis of combined conjunctivitis and serology-based case definition, the Netherlands, 2003*

		No. persons		No. visits	
Respirator used	Safety glasses used	Infected	Total	By infected persons	Total
Always	Always	1	19	3	44
	Sometimes	5	19	13	53
	Not used	4	27	8	45
Sometimes	Always	–	–	–	–
	Sometimes	3	17	11	58
	Not used	2	22	3	45
Not used	Not used	3	4	4	8
Unknown	Unknown	1	22	1	43

*Data resulted from a survey conducted among poultry workers after the epidemic. –, no data in this group.

Table 4. Risk for infection with avian influenza A (H7N7) with different levels of respirator and safety glasses use, on the basis of combined conjunctivitis and serology-based case definition, the Netherlands, 2003*

Respirator used	Safety glasses used	Risk† (95% CI)
Always	Always	0.023 (0.001–0.099)
	Sometimes	0.079 (0.018–0.188)
	Not used	0.093 (0.029–0.204)
Sometimes	Always	–
	Sometimes	0.056 (0.014–0.14)
	Not used	0.045 (0.007–0.133)
Not used	Not used	0.408 (0.119–0.755)
Unknown	Unknown	0.023 (0.001–0.099)

*CI, confidence interval; –, no data in this group.
†Probability of infection per visit.

persons for whom oseltamivir had been prescribed did not use it during some of their visits. For the group of 130 (combined case definition), oseltamivir was prescribed for 114 and not prescribed for 16; for 34 persons, oseltamivir was prescribed but not used.

The estimated risk for infection per visit without use of oseltamivir was 0.145 (95% CI 0.078–0.233). This risk dropped significantly to 0.031 (95% CI 0.008–0.073; p = 0.005) per visit when oseltamivir was used (Table 2; Figure 2). When calculated over the infection probabilities, oseltamivir use had a protective effect of 79% (95% CI 40%–97%; relative risk [RR] 0.21, 95% CI 0.03–0.60). The risk for infection seemed to increase when oseltamivir was used inconsistently, but this risk did not reach significance.

Results with case definitions that used either the serologic results or self-reported conjunctivitis differed slightly. With the serologic result, the estimated risk for infection without oseltamivir use was 0.513 (95% CI 0.370–0.656). This risk dropped to 0.274 (95% CI 0.157–

0.418; p = 0.01) with oseltamivir use (Tables 5, 6), which resulted in a protective effect of 46% (95% CI 19%–68%; RR 0.54, 95% CI 0.32–0.81). With self-reported conjunctivitis used as case definition, the estimated risk for infection without oseltamivir use was 0.115 (95% CI 0.064–0.180). This risk dropped to 0.073 (95% CI 0.032–0.135; p = 0.08) with oseltamivir use (Tables 5, 7), which resulted in a protective effect of 53% (95% CI 2%–82%; RR 0.47, 95% CI 0.18–0.98).

We could not analyze interactions between use of oseltamivir and PPE because the resulting groups would have had too few visits. In the group that always used respirators and protective glasses and the group that sometimes used respirators and protective glasses, oseltamivir use was relatively equal (63%–74%). In the group that sometimes used respirators and did not use safety glasses, oseltamivir was used in 38% of the visits. In the group that did not use safety glasses or respirators, oseltamivir was used in 25% of the visits. These findings indicate no strong correlation.

Respirators and Safety Glasses

Persons generally were more inclined to use respirators than safety glasses (Tables 3, 8). Persons who always used safety glasses also used a respirator. Only a small number of persons stated they had used no respirator and no safety glasses.

We gauged the effect of using safety glasses by comparing the risk for infection in persons with different safety glasses use within the group who always used respirators (Table 4). Within this group, risk for infection decreased with use of safety glasses; however, this trend was not significant. We gauged the effect of respirator use by comparing the risk for infection in persons with equal levels of safety glasses use but different respirator use (Table 4). Risk for infection was lower in persons who sometimes used a respirator than in persons who always used a respirator (within the groups that sometimes used or did not use safety glasses). Risk was higher for persons who had not used respirators than for those who had sometimes or always used them (within the group that had not used safety glasses). However, the group
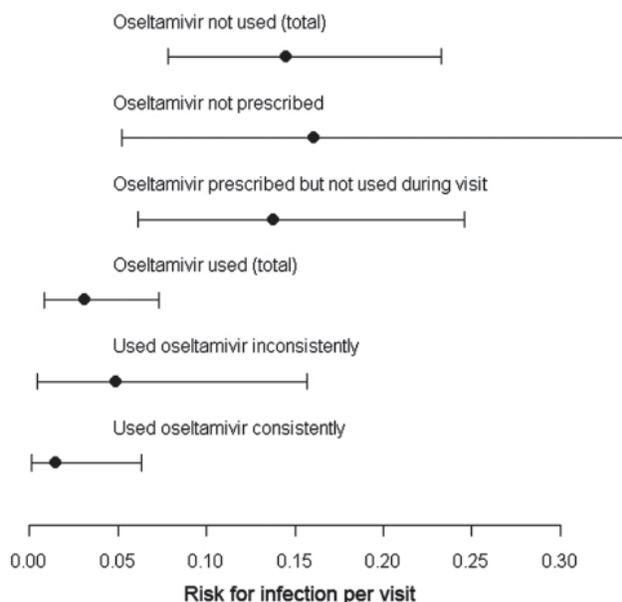

Figure 2. Point estimates and 95% confidence intervals of the risk for infection per visit in relation to oseltamivir use for the combined serology/conjunctivitis case definition, the Netherlands, 2003.

Table 5. Comparison of conjunctivitis and serologic result case definitions for number of infections and prophylactic oseltamivir use during avian influenza A (H7N7) epidemic, the Netherlands, 2003*

Oseltamivir use	Conjunctivitis		Serologic result	
	Persons†	Visits‡	Persons	Visits
Not prescribed	6/35	9/63	12/16	22/26
Prescribed but not used during visit	12/45	25/80	26/34	48/64
Prescribed and used consistently	7/54	9/113	24/41	54/85
Prescribed and used inconsistently	5/26	13/65	11/18	24/39
Prescribed and consistency of use unknown	4/9	8/23	4/7	11/18
Prescribed but period of use unknown	9/47	15/109	17/30	40/68

*Data resulted from a survey conducted among poultry workers after the epidemic.
†No. persons infected/total no. persons.
‡No. visits by infected persons/total no. visits. Because visits were classified into groups, persons may be in 2 categories (prescribed not used and prescribed used).

that had used neither respirators nor safety glasses was small. In both comparisons, the protective effect of respirators was not statistically significant. In the sensitivity analysis, results for which conjunctivitis and the serologic result were used as case definitions were similar to results with the combined case definition (Table 9).

Discussion

Quantifications of the effect of prophylactic use of oseltamivir on the risk for infection with avian influenza A (H7N7) virus can be used to guide efforts to reduce human infections with avian influenza. The risk for infection per visit was remarkably high among persons who did not use prophylactic oseltamivir (0.145, 95% CI 0.078–0.233). Although significantly lower, the risk for infection per visit for persons who did use oseltamivir prophylactically was still considerable (0.031, 95% CI 0.008–0.073).

Given the lack of research on the prophylactic effect of oseltamivir use on avian influenza in humans, we compared our result with studies on human seasonal influenza. Our estimated protective effect of oseltamivir use (79%) compares with that reported for laboratory-confirmed symptomatic human seasonal influenza, which ranges from 68% to 90% (12–17). The protective effect estimated with the case definition based on the serologic result only (46%) is close to the range of 49%–68% found with laboratory-confirmed human influenza (12–14). This finding suggests that the specificity of the serologic outcome measure is high, even though in our

study it has a relatively low cutoff (10). The standard for use of HI assays in serologic studies is a cutoff of 40. This cutoff was lowered on the basis of evidence from an epidemiologic study in which no serologic responses were found in any of the 89 known infected persons by using the standard criteria, but a high proportion had low-level antibody reactivity with high specificity, which has triggered some debate about the validity of this serologic approach. However, finding a significantly lower risk for persons that used oseltamivir and had antibody reactivity cannot be explained by nonspecific reactivity. Therefore, we conclude that, for some reason, subtype H7N7 infections do not provoke a strong immune response, possibly related to the ocular tropism. Since then, similar findings have been reported (18,19). Finally, the protective effect when conjunctivitis is used as case definition was 53% in our study, compared with 29% for human influenza based on influenza-like illness (14), which suggests a reasonable specificity of conjunctivitis as indicator for subtype H7N7 infection.

Considering the effect of oseltamivir in reducing the risk for infection, prophylactic treatment of all persons involved in depopulating farms may seem wise. However, oseltamivir is also the fallback drug used for treating patients with severe influenza, and resistance against oseltamivir has increased in seasonal influenza viruses (20). A decision regarding prophylactic use of oseltamivir in a future epidemic would need to account for the risk for infection, risk for resistance, and severity of the infection.

Table 6. Risk for infection with avian influenza A (H7N7) with different levels of oseltamivir use, on the basis of serologic result as case definition, the Netherlands, 2003*

Oseltamivir use	Risk† (95% CI)
Not used	0.513 (0.370–0.656)
Not prescribed	0.653 (0.398–0.870)
Prescribed but not used during visit	0.446 (0.277–0.623)
Used	0.275 (0.182–0.381)
Prescribed and used consistently	0.294 (0.178–0.432)
Prescribed and used inconsistently	0.281 (0.117–0.493)
Prescribed and consistency of use unknown	0.181 (0.016–0.502)
Unknown	0.274 (0.157–0.418)
Prescribed but period of use unknown	0.274 (0.157–0.419)

*CI, confidence interval.
†Probability of infection per visit.

Table 7. Risk for infection with avian influenza A (H7N7) with different levels of oseltamivir use, on the basis of conjunctivitis as case definition, the Netherlands, 2003*

Oseltamivir use	Risk† (95% CI)
Not used	0.115 (0.064–0.180)
Not prescribed	0.098 (0.040–0.189)
Prescribed but not used during visit	0.127 (0.055–0.227)
Used	0.054 (0.026–0.097)
Prescribed and used consistently	0.041 (0.012–0.091)
Prescribed and used inconsistently	0.052 (0.010–0.135)
Prescribed and consistency of use unknown	0.149 (0.029–0.360)
Unknown	0.073 (0.032–0.135)
Prescribed but period of use unknown	0.073 (0.032–0.135)

*CI, confidence interval.
†Probability of infection per visit.

Although our results provided clear evidence that prophylactic use of oseltamivir is effective in reducing risk for infection, the results were less clear with regard to PPE. In fact, although use of safety glasses appeared to reduce the risk for subtype H7N7 infection, this effect was not significant. Considering that the main symptom of subtype H7N7 is conjunctivitis, safety glasses may protect the eyes to some extent against influenza infection. For respirators, we also did not find a clear protective effect. Persons who always wore respirators may have done work that exposed them more. For both respirators and safety glasses, people received a limited amount of training that perhaps led to in-

effective use of PPE and unsafe removal of contaminated PPE. Respirators were available in only 1 size and thus may not have fit well. The safety glasses were effective against splashes but were open on all sides and were not effective against dust. Possibly the PPE used were not appropriate for the high-exposure work. The number of available visits per group was low, data were also of limited temporal resolution (because PPE use per visit was not available), and potential for a recall bias also existed. Because of these limitations, we cannot conclusively determine from this study that respirators and safety glasses do not provide a protective effect. Findings of an experimental situation (21,22) indicated that

Table 8. Comparison of conjunctivitis and serologic result case definitions for number of infections and use of respirators and safety glasses during avian influenza A (H7N7) epidemic, the Netherlands, 2003*

Use of respirator	Use of safety glasses	Conjunctivitis		Serology	
		Persons†	Visits‡	Persons	Visits
Always	Always	3/28	5/60	11/19	30/44
	Sometimes	7/25	16/76	12/19	38/53
	Not used	7/38	14/65	19/27	32/45
Sometimes	Always	–	–	–	–
	Sometimes	4/25	13/84	10/18	40/62
	Not used	5/28	12/62	10/22	19/45
Not used	Not used	4/14	6/34	4/4	8/8
Unknown	Unknown	6/35	13/72	15/22	32/43

*Data resulted from a survey conducted among poultry workers after the epidemic. Totals of respirator and safety glasses use can be derived by adding up the separate groups. –, no data in this group.
†No. infected persons/total no. persons.
‡No. visits by infected persons/total no. visits.

Table 9. Comparison of conjunctivitis and serologic result case definitions for risk for infection with avian influenza A (H7N7) with different level of respirator and safety glass use, the Netherlands, 2003*

Use of respirator	Use of safety glasses	Risk† with conjunctivitis used as case definition (95% CI)	Risk† with serology used as case definition (95% CI)
Always	Always	0.051 (0.012–0.127)	0.338 (0.181–0.534)
	Sometimes	0.063 (0.017–0.144)	0.274 (0.125–0.471)
	Not used	0.115 (0.050–0.210)	0.494 (0.320–0.672)
Sometimes	Always	–	–
	Sometimes	0.050 (0.015–0.114)	0.222 (0.108–0.380)
	Not used	0.082 (0.027–0.173)	0.233 (0.118–0.383)
Not used	Not used	0.121 (0.039–0.261)	1 (0.463–1)
Unknown	Unknown	0.088 (0.035–0.171)	0.48 (0.296–0.676)

*CI, confidence interval; –, no data because no persons in this group sometimes used a respirator and always used safety glasses.
†Probability of infection per visit.

respirators are likely to modify exposure and thus risk for infection. In Norfolk, United Kingdom, in 2006, incomplete use of PPE (safety glasses and respirator) was associated with conjunctivitis and influenza-like illness in an outbreak of avian influenza A (H7N3) (*23*).

In our study, prophylactic use of oseltamivir greatly reduced risk for infection with avian influenza A (H7N7). However, even with oseltamivir use, risk for infection remains considerable. Oseltamivir use should be part of an integrated approach to reduce human exposure, together with the use and appropriate training of PPE.

Dr te Beest works as postdoctoral fellow on the modeling of avian influenza at both Utrecht University and the National Institute of Public Health and the Environment. His research interests include mathematical modeling of the transmission dynamics of infectious diseases and quantifying the effect of interventions.

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Bloodstream Infections among HIV-Infected Outpatients, Southeast Asia

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Bloodstream infections (BSIs) are a major cause of illness in HIV-infected persons. To evaluate prevalence of and risk factors for BSIs in 2,009 HIV-infected outpatients in Cambodia, Thailand, and Vietnam, we performed a single Myco/F Lytic blood culture. Fifty-eight (2.9%) had a clinically significant BSI (i.e., a blood culture positive for an organism known to be a pathogen). *Mycobacterium tuberculosis* accounted for 31 (54%) of all BSIs, followed by fungi (13 [22%]) and bacteria (9 [16%]). Of patients for whom data were recorded about antiretroviral therapy, 0 of 119 who

had received antiretroviral therapy for >14 days had a BSI, compared with 3% of 1,801 patients who had not. In multivariate analysis, factors consistently associated with BSI were fever, low CD4+ T-lymphocyte count, abnormalities on chest radiograph, and signs or symptoms of abdominal illness. For HIV-infected outpatients with these risk factors, clinicians should place their highest priority on diagnosing tuberculosis.

Bloodstream infections (BSIs) are a major cause of illness in HIV-infected persons. A series of studies, most of which were conducted in sub-Saharan Africa during the 1990s, demonstrated a high prevalence of BSIs (ranging from 10% to 63%) among hospitalized HIV-infected persons who had fever (1–17). In studies that measured clinical outcomes, the in-hospital death rate for patients with a BSI was high (19%–47%). A variety of pathogens cause BSIs in febrile, hospitalized persons with HIV, most notably non-Typhi *Salmonella* spp. (6%–15%) and *Mycobacterium tuberculosis* (2%–19%). BSI with *M. tuberculosis* appears to be particularly lethal, causing death during hospitalization in up to 47% of patients (9). Although untreated BSIs are believed to lead rapidly to severe illness, sepsis, and death, patients with BSIs may be able to be identified before they are ill enough to require hospitalization, potentially improving clinical outcomes. Despite the large number of studies that have evaluated BSIs in HIV-infected persons, all previous studies have focused on patients seeking care at hospitals because of fever and did not evaluate infections among outpatients with or without fever.

Although overall transmission rates have declined and antiretroviral therapy (ART) has become more widely available, HIV infection remains a major public health problem in Southeast Asia (18). Previous studies of BSI in Southeast Asia enrolled only inpatients, and only 1 evaluated a predominantly HIV-infected population (1,19–21). In this study, we prospectively enrolled patients from multiple HIV testing and treatment clinics in Cambodia, Thailand, and Vietnam to assess BSI prevalence, etiology, and risk factors in outpatients with HIV.

Methods

Enrollment and Specimen Collection

From September 2006 through July 2008, HIV-infected persons were enrolled consecutively from community outpatient facilities that perform HIV counseling, testing, and clinical care: 4 clinics in Cambodia (2 in Băntéay Méan Chĕăy Province, 1 in Bătdâmbâng Province, 1 in Phnom Penh); 1 in Bangkok, Thailand; and 3 in Ho Chi Minh City, Vietnam. At each facility, HIV-infected persons who came to the clinic during the enrollment period were screened for eligibility and, if eligible, were offered enrollment. The enrolled group comprised both persons newly diagnosed with HIV and persons previously diagnosed with HIV, some of whom were receiving ART. Patients were asked to participate in the study regardless of the presence or absence of symptoms or prior suspicion of clinical illness. Patients were eligible for the study if they had documented HIV infection and were >6 years of age. Because the study was designed primarily to evaluate different strategies for diagnosing tuberculosis (TB) in HIV-infected persons, patients were excluded if they had undergone TB screening with chest radiograph or sputum smears in the previous 3 months and if they had taken medications with anti-TB activity within the past month (22).

After providing written informed consent, patients underwent a standardized interview and physical examination, chest radiograph, and blood testing for complete blood cell count and CD4+ T lymphocytes. Patients were asked about a broad range of symptoms and exposures during the past 4 weeks. Trained phlebotomists obtained 5 mL of blood and directly injected it into Myco/F Lytic bottles (Becton Dickinson, Franklin Lakes, NJ, USA). Bottles were kept at room temperature and shielded from light, then transferred to a centralized laboratory, where they were placed into an automated blood culture instrument (BACTEC 9050/9120/9240 system; Becton Dickinson).

The human subjects review committees at Centers for Disease Control and Prevention (Atlanta, GA, USA) approved the study. Collaborating institutions in each country also approved it.

Specimen Processing

A detailed description of processing and testing of the nonsputum specimens has been published (22). Myco/F Lytic bottles were incubated for 42 days in a BACTEC 9050/9120/9240 instrument. Cultures flagged as positive by the instruments were removed, acid-fast bacilli (AFB) smears were carried out, and specimens were subcultured onto blood agar plates. AFB-positive cultures were subcultured onto 2 Lowenstein-Jensen media slants. AFB-negative Myco/F Lytic cultures that had growth on blood agar plates underwent bacterial or fungal identification. Blood cultures <42 days old with no organisms found on smear and blood agar plates were returned to the instrument. All Myco/F Lytic cultures were removed after 42 days, visually inspected for growth, subcultured onto 2 Lowenstein-Jensen slants, incubated for an additional 3 weeks, and then discarded. Cultures positive for *M. tuberculosis* were identified by using the niacin production and nitrate reduction tests. Nontuberculous mycobacteria (NTM) were speciated by using high-performance liquid chromatography or the Genotype Mycobacterium CM/AS assay (Hain Lifescience, Nehren, Germany) (23).

Data Analysis

Blood cultures were classified as negative, positive for a likely contaminant, or positive for a clinically significant pathogen. Because only 1 blood specimen for culture was drawn from each patient, the identity of the organism was used as the only criteria for determining whether the blood culture result was due to contamination or a clinically significant pathogen (24,25).

We calculated proportions and medians to describe patient characteristics and calculated bivariate odds ratios (ORs) and 95% confidence intervals (CIs) to analyze factors associated with clinically significant BSI. To calculate adjusted ORs for clinically significant BSI, factors significant in bivariate analysis at p<0.05 and factors hypothesized a priori to be associated with BSI were entered in a multiple logistic regression model, and a final model was chosen through stepwise automated variable selection. We assessed all factors for colinearity; for colinear factors, we retained the factor that had largest OR and that we judged to be most clinically meaningful. For the bivariate analysis, we analyzed only observations with complete (non-missing) data; for the multivariate analysis, we created a separate missing values stratum for 3 variables (CD4 cell count, hemoglobin level, leukocyte count) to maximize the number of observations in the final model. Analyses were conducted by the same approach to evaluate independent clinical predictors of mycobacterial, bacterial, and fungal BSI. All analyses were conducted in SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Enrollment

Of 2,115 patients evaluated, 2,013 (95.2%) were eligible for the study; of eligible patients, 2,009 were enrolled. Reasons for ineligibility included current or recent TB treatment (87 patients), use of medications with anti-TB activity in the past month (5), recent TB screening (3), age <7 years (2), and other or missing reasons for noneligibility (5).

Patient Characteristics

Almost half (945 [47.0%]) of the patients enrolled were from Cambodia. Median age was 31 years (interquartile range [IQR] 27–38); 1,019 (50.7%) patients were male. A clinically significant BSI was found in 58 (2.9%) patients and a contaminant in 131 (6.5%). *M. tuberculosis* caused 31 (54%) BSIs; 13 (22%) BSIs were caused by fungi, 9 (16%) by bacteria, and 5 (9%) by NTM (Table 1).

Risk Factors for BSI

In bivariate analysis, a large number of symptoms, signs, chest radiography findings, and laboratory studies were associated with BSI (online Appendix Table, www.

cdc.gov/EID/content/16/10/1569-appT.htm). BSI was associated with recent diagnosis of HIV infection, which we defined as receipt of an HIV diagnosis within the 14 days before enrollment, to account for delays between initial diagnosis and visit to an HIV clinic (OR 1.80, 95% CI 1.06–3.05). The 1 sign or symptom most strongly associated with BSI was temperature >38°C, which was documented in 25 (43%) patients with BSI compared with 5% of those without BSI (OR 13.58, 95% CI 7.78–23.68). CD4 cell count was strongly associated with BSI. The median CD4 count for patients with a BSI was 15 cells/mm³ (IQR 8–50), compared with 261 cells/mm³ (IQR 102–405) for those without a BSI (p<0.01). Of patients with BSI, 50 (86%) had CD4 count <100 cells/mm³, including 44 (76%) with a CD4 count <50 cells/mm³. Only 5 (9%) patients with BSI had CD4 count ≥200 cells/mm³. Of enrolled patients, 83 (4%) had a temperature >38°C and a CD4 count <100 cells/mm³. Of these, 21 (25%) had a BSI, including 14 caused by mycobacteria (13 *M. tuberculosis* and 1 NTM) and 7 caused by fungi. The characteristic that was most protective was receipt of ART. Of patients for whom data were recorded about receipt of ART, 0 of 119 who had received ART for at least 14 days had a BSI, compared with 3% of 1,801 who did not receive ART or who had started ART <14 days previously (OR 0).

In multivariate analysis, several factors were significantly associated with BSI. These factors were loss of appetite, nausea or vomiting; temperature >38°C, oral hairy leukoplakia, CD4 count <100 cells/mm³, anemia, leukocytosis, and paratracheal adenopathy or a miliary pattern on a chest radiograph (Table 2).

Risk Factors for Mycobacterial, Fungal, and Bacterial BSIs

When we restricted the multivariate analysis to different subsets of BSI, we found that several factors were independently associated with mycobacterial BSI: shaking chills, difficulty breathing, diarrhea, temperature >38°C, leukocytosis, thrombocytopenia, paratracheal adenopathy or miliary pattern on the chest radiograph, and receipt of an antibiotic drug other than co-trimoxazole (Table 2). The only independent predictors of bacterial BSI were jaundice and self-report of fever in the previous 24 hours. No factors were statistically significant in the analysis of fungal BSI.

Discussion

In this large study of HIV-infected persons in Southeast Asia, 1 in 35 outpatients had a BSI; the highest prevalence was in patients with low CD4 counts and clinical signs of infection. *M. tuberculosis* remains one of the most common causes of BSI in HIV-infected persons who live in resource-limited settings. In all analyses performed, several factors were consistently associated with BSI: self-reported

Table 1. Clinically significant organisms isolated from cultures of blood samples from 2,009 HIV-infected outpatients from Thailand, Cambodia, and Vietnam, September 2006–July 2008

Organism	No. (%), n = 58
Mycobacteria	36 (62)
Mycobacterium tuberculosis	31 (86)
M. avium-intracellulare	3 (8)
M. simiae	1 (3)
Non-tuberculous mycobacteria not identified	1 (3)
Other bacteria	9 (16)
Non-Typhi _Salmonella_ spp.	2 (22)
S. cholerasius	3 (33)
Pseudomonas spp.	1 (11)
Neisseria spp.	2 (22)
Escherichia coli	1 (11)
Fungi	13 (22)
Cryptococcus neoformans	6 (46)
Penicillium marneffei	5 (38)
Histoplasma capsulatum	1 (8)
Non-albicans _Candida_ spp.	1 (8)

fever or documented elevated temperature, low CD4 count, abnormalities on chest radiograph, and signs or symptoms of abdominal illness.

BSI correlated strongly with immunosuppression. In fact, 10% of outpatients with HIV and CD4 count <100 cells/mm^3 had a BSI, a prevalence similar to that seen in a previous study of febrile, HIV-infected inpatients in Thailand (6). No patients who received ART had a BSI, consistent with the observation from other settings that highly active ART may reduce the incidence of bacteremia

in HIV-infected persons (26–28). _M. tuberculosis_ was the most frequent pathogen isolated in our study, findings consistent with studies that have shown that undiagnosed TB disease is common in patients with newly diagnosed HIV-infection and that the invasiveness of TB increases with declining CD4 cell counts and with the absence of ART (29–31). These findings are particularly valid in countries with a high incidence of TB, such as Thailand, Cambodia, and Vietnam; in these 3 countries, the estimated incidence is >140 TB cases (all forms) per 100,000 persons (32). We found that strong predictors of _M. tuberculosis_ BSI included clinical features that suggest pulmonary TB, including difficulty breathing and adenopathy or a miliary pattern shown on a chest radiograph. In an analysis published separately, we demonstrated that the incremental yield of blood culture for detecting TB was extremely low in HIV-infected persons who have 3 sputum specimens cultured on liquid media (22). Our study, therefore, further supports the World Health Organization policy of focusing on pulmonary, rather than extrapulmonary, TB case finding and of recommending routine, regular TB screening for HIV-infected patients (33).

More than one fifth of all BSIs were attributable to fungi, but we were unable to identify any clinical characteristics independently associated with fungal BSI. Because cryptococcosis and penicilliosis are commonly associated with advanced immunosuppression and have high death rates if left untreated, further studies are needed to improve case finding for and prevention of these infections (34,35).

Table 2. Multivariate analysis of risk factors for clinically significant BSI caused by mycobacterial or other bacterial infection in HIV-infected outpatients, Thailand, Cambodia, and Vietnam, September 2006–July 2008*

Characteristic	Adjusted odds ratio (95% CI) for BSI		
	Any pathogen†	Mycobacteria‡	Bacteria§
CD4 cell count <100/mm^3	5.8 (2.5–13.7)	11.2 (3.0–41.3)	–
Female sex	0.4 (0.2–0.9)	–	–
Fever in past 24 h	–	–	4.7 (1.2–17.6)
Loss of appetite in past 24 h	2.0 (1.0–3.9)	–	–
Nausea or vomiting in past 24 h	2.5 (1.2–5.0)	–	–
Shaking chills in past 24 h	–	3.1 (1.1–9.1)	–
Difficulty breathing	–	4.1 (1.5–11.1)	–
Diarrhea in past 24 h	–	4.2 (1.6–11.3)	–
Jaundice	–	–	12.5 (1.4–112.3)
Ever injected drug	–	–	–
Temperature >38°C	3.2 (1.6–6.3)	5.7 (2.1–15.4)	–
Heart rate >100 bpm	–	–	–
Oral hairy leukoplakia	2.8 (1.4–5.7)	–	–
Hemoglobin level <12 g/dL	4.8 (2.0–11.7)	–	–
Leukocyte count >12 × 10^3/μL	3.7 (1.4–9.3)	15.3 (4.9–48.2)	–
Thrombocyte count <100,000 cells/μL	–	7.0 (1.8–27.6)	–
Paratracheal adenopathy on chest radiograph	5.0 (2.1–11.6)	11.3 (3.7–34.3)	–
Miliary pattern on chest radiograph	6.0 (1.8–19.5)	14.2 (3.4–58.6)	–
Took antimicrobial medication other than cotrimoxazole	–	5.1 (1.6–16.8)	–

*BSI, bloodstream infection; CI, confidence interval; bpm, beats per minute; –, variable was not included in the model.
†1,961 patients in final model.
‡1,944 patients in final model.
§1,961 patients in final model.

In contrast, we found that self-reported fever and the finding of jaundice on physical examination were strong predictors of bacterial infection. The reasons for an association with jaundice are unclear, but the association is consistent with our finding that most bacterial infections were of enteric origin. Symptoms of abdominal illness, such as loss of appetite and nausea or vomiting, also were associated with BSI caused by any pathogen. Our finding that non-Typhi *Salmonella* spp. infections were the most common bacterial infection in HIV patients is consistent with results of other studies and provides further evidence that efforts are needed to prevent invasive salmonellosis in HIV-infected persons, through improvements in food and water safety and the development of new vaccines (*36*).

Our study has several limitations. First, we collected only 1 blood culture per patient and used only 1 type of culture media, which potentially reduced the sensitivity for detection of bacteremia (*37*). This limitation is a likely explanation for the lack of *Streptococcus pneumoniae* detected in our study. Invasive pneumococcal disease is a common cause of bacteremia in HIV-infected patients throughout the world, but *S. pneumoniae* is challenging to isolate from blood. In addition, 12% of study patients were receiving co-trimoxazole preventive therapy, and our study was conducted among outpatients, a population less likely to have undiagnosed severe disease caused by a virulent pathogen, such as pneumococcus. Although other investigators in Southeast Asia have found similarly low pneumococcal isolation rates and have speculated that this is attributable to low incidence, at least 1 high-quality study demonstrated that the incidence of invasive pneumococcal disease in Thailand is similar to that in other regions (*19–21,38*).

A major strength of our study, however, is that, unlike all previous studies, which were conducted at single referral hospitals, our study was conducted at multiple urban and rural clinical facilities in 3 countries. Thus, our results can be broadly generalized to HIV-infected patients throughout Southeast Asia.

Mycobacterial, fungal, and bacterial BSIs remain a major health problem for HIV-infected persons in Southeast Asia. Any HIV-infected outpatients (regardless of whether they have newly diagnosed HIV, are newly seeking care, or are already receiving care) who report experiencing fever or abdominal symptoms in the previous day, have a temperature >38°C or jaundice on physical examination, or have a chest radiograph demonstrating paratracheal adenopathy or a miliary pattern, have a high likelihood of a BSI, particularly if their CD4 count is <100 cells/mm³. In such patients, blood culture, when available, should be performed immediately to facilitate diagnosis and accelerate access to treatment of BSI. Regardless of blood culture availability, clinicians should place their highest priority on early diagnosis and treatment of pulmonary TB. Ultimately, increasing use of ART most likely will have the greatest effect on reducing BSIs.

Dr Varma is a physician and epidemiologist currently serving as director of the US Centers for Disease Control and Prevention International Emerging Infections Program in Beijing, People's Republic of China. His research interests include surveillance, treatment, and control of infectious diseases in resource-limited settings.

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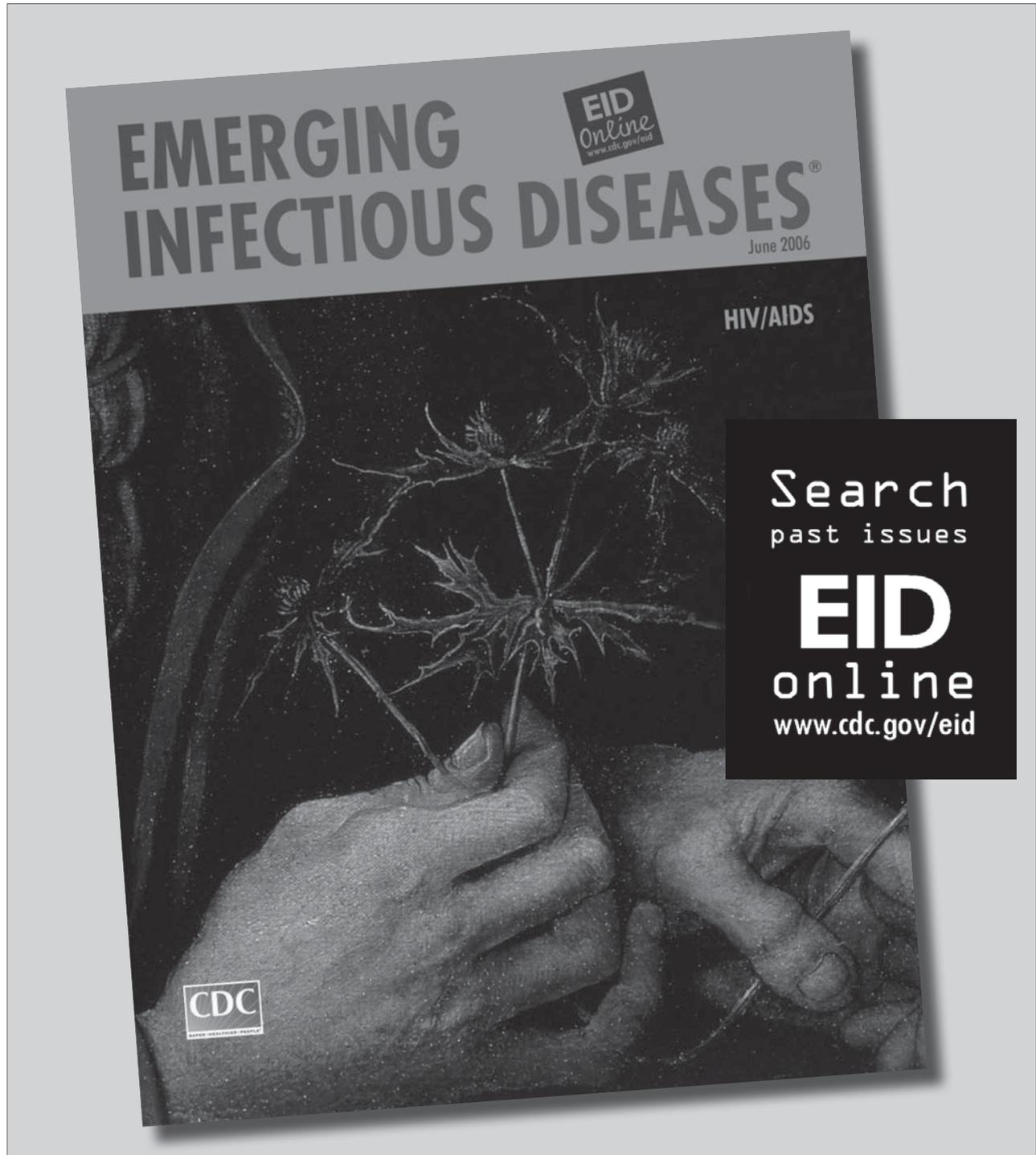
Changing Epidemiology of Pulmonary Nontuberculous Mycobacteria Infections

Rachel M. Thomson, on behalf of the NTM working group at the Queensland TB Control Centre and Queensland Mycobacterial Reference Laboratory

Nontuberculous mycobacteria (NTM) disease is a notifiable condition in Queensland, Australia. Mycobacterial isolates that require species identification are forwarded to the Queensland Mycobacterial Reference Laboratory, providing a central opportunity to capture statewide data on the epidemiology of NTM disease. We compared isolates obtained in 1999 and 2005 and used data from the Queensland notification scheme to report the clinical relevance of these isolates. The incidence of notified cases of clinically significant pulmonary disease rose from 2.2 (1999) to 3.2 (2005) per 100,000 population. The pattern of disease has changed from predominantly cavitary disease in middle-aged men who smoke to fibronodular disease in elderly women. *Mycobacterium intracellulare* is the main pathogen associated with the increase in isolates speciated in Queensland.

Worldwide, pulmonary disease caused by nontuberculous mycobacteria (NTM) appears to be increasing (*1–4*), yet accurate data to support this assumption are difficult to produce. Patients traditionally described are middle-aged men with underlying chronic lung disease, such as chronic obstructive pulmonary disease, who have upper lobe cavity formation and nodules of various sizes. An increasing number of patients have nodules, bronchiolitis, and bronchiectasis involving the middle lobe and lingula. These patients are more commonly female nonsmokers and have no preexisting lung disease (*5,6*).

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NTM disease is not a reportable condition in most countries because no evidence of human-to-human transmission exists; therefore, it is not considered a public health concern. However, the organisms are ubiquitous in the environment, and substantial evidence shows that the environmental niche for *Mycobacterium intracellulare* (the most common pulmonary pathogen) is in biofilms lining suburban water pipes. Many NTM pathogens have been isolated from drinking water (*7*). Some clinicians believe the condition should be classified as an environmental health concern, similar to that caused by *Legionella* spp.

Globally, geographic variability in environmental exposure and prevalence of NTM disease is significant (*8*). Without detailed clinical information, differentiating between contamination of specimens, colonization/infection, and disease is difficult; laboratory reports of isolates do not always reflect the true incidence of disease. To determine if disease is present, sputum specimens and often a bronchoscopic sample of a patient's lower respiratory tract must be collected. In addition, computed tomography scanning and clinical appointments with primary care providers and specialists are needed. Because this investigative process is costly to the healthcare system and the patient, accurate epidemiologic data on this condition should be of interest to public health experts.

Studies of avian versus human Mantoux testing in schoolchildren have shown that exposure to NTM organisms is common in Queensland (*9–11*). Therefore, since the introduction of TB control services, disease caused by NTM in Queensland has been notifiable. This practice has been continued primarily to avoid confounding of smear-positive cases with TB. The notification process provides a unique opportunity to study the clinical significance of isolates positive for NTM and the features such as age and sex, symptoms, underlying conditions, and radiology results of patients with disease, avoiding the inherent bias that occurs in case series that are reported by tertiary and quaternary referral centers. The incidence of clinically reported cases of pulmonary disease caused by *M. avium* complex (MAC) in Queensland has been increasing (1985, 0.63/100,000 population; 1994, 1.21/100,000; and 1999, 2.2/100,000). In 2005, the Queensland Tuberculosis Control Centre (QTBCC) revised the notification process to ensure collection of meaningful clinical data and follow-up of clinically significant NTM cases.

Methods

At the time of this study, public hospitals requesting mycobacterial cultures forwarded all specimens to the Queensland Mycobacterial Reference Laboratory (QMRL). Requests in the private sector are processed by 2 main laboratories and a few smaller laboratories. The 2 private laboratories (representing >90% of the state's private mycobacterial pathology service) identify mycobacteria in specimens and report as "atypical mycobacteria, not further specified." After culture, not all isolates are forwarded to the QMRL for species identification. However, isolates are forwarded if the specimen is smear positive (to exclude TB), was obtained from a bronchial wash or a site other than sputum, and if >1 specimen is positive for that patient or the treating clinician requests it. In 1999, a specific research project was undertaken to capture all unspeciated isolates from private laboratories and better assess the true impact of mycobacteriology in the state. This study was approved by the Research Ethics Committee of the Princess Alexandra Hospital (138/98) in accordance with the Australian National Health and Medical Research Council guidelines. Because it proved quite labor intensive for all isolate reports to be forwarded for this study, it did not continue after 1999. Reports of all positive cultures identified by QMRL are forwarded to the QTBCC, which sends questionnaires to clinicians asking for the clinical significance of all mycobacterial isolates. Clinicians are provided with guidelines (as per American Thoracic Society/Infectious Disease Society of America [ATS/IDSA]) to assist in the diagnosis of significant disease.

More detailed clinical information is requested for patients who have disease, including details on predisposing conditions, radiologic and bacteriologic test results, and treatment received. Provision of this information is voluntary. Follow-up surveys evaluate any changes in regimen brought about by side effects or intolerance to drugs or failure of the starting regimen. Bacteriology is monitored for success of treatment. An update on the patient's status is requested yearly for 3 years after treatment has stopped. All case files notified as clinically significant disease are reviewed by QTBCC clinicians to confirm concordance with ATS/IDSA criteria for disease (*12*).

Most nonpulmonary isolates grown by private laboratories are sent to the QMRL for speciation. For patients with nonpulmonary NTM isolates, the clinical significance of the isolate is sought by QTBCC; species of organism and site of infection is recorded. However, details on individual treatment regimens and outcomes are not collected.

Statistical calculations were performed by using OpenEpi: Open Source Epidemiologic Statistics for Public Health, version 2.3. (www.openepi.com). Population statistics were obtained from the Australian Bureau of Statistics (*13,14*), including population data for different age categories. The rate of positive NTM isolates is reported as number per 100,000 population, as are age-specific and gender-specific rates of pulmonary disease. Comparisons of statistics for 1999 and 2005 were made by using χ^2 2 × 2 tables in OpenEpi.

Results

A more aggressive capture of single, positive, unspeciated isolates occurred in 1999; the total number of NTM isolates was 14.8/100,000 population. Because the number of speciated pulmonary isolates in each year (1999 and 2005) should not have been affected by the increase in data collection in 1999, the increase in speciated isolates for these 2 years most likely reflects the true increase in significant isolates (9.1–13.6/100,000 population). The total number of isolates in 2005 was 15.8/100,000 population. Assuming the proportion of unspeciated isolates remained constant, and on the basis of the increase in speciated isolates, we estimated the total number of isolates in 2005 to be 22.1/100,000 population.

The difference between the 2 years was due to the higher number of pulmonary isolates as demonstrated in Table 1. Speciated pulmonary isolates rose significantly, from 5.5 to 10.2/100,000 population.

There were differences between the species of NTM isolated in 1999 and 2005 (Figures 1, 2). The species accounting for most of the change in NTM isolates were *M. intracellulare* and *M. abscessus*. More of the MAC isolates are now identified by multiplex PCR as either *M. avium* or *M. intracellulare*. During 1999–2005, the number of *M. avium* isolates increased from 35 (1/100,000) to 62 (1.55/100000) and *M. intracellulare* from 77 (2.2/100,000) to 212 (5.3/100,000), while MAC decreased from 35 to 3. *M. abscessus* isolates increased from 23 (0.065/100, 000) in 1999 to 40 (1/100,000) in 2005. *M. fortuitum* isolates increased from 41(1.17/100,000) in 1999 to 50 (1.25/100,000) in 2005.

The notified significance of pulmonary and extrapulmonary isolates is shown in Table 2. Of 488 patients with pulmonary NTM isolates, only 26.6% had significant disease overall. Percentages were slightly higher for patients with *M. intracellulare* isolates (39.4%), *M. avium* (33.3%), *M. kansasii* (52.6%), and much lower for patients infected with species traditionally thought more likely to be contaminants, such as *M. gordonae* (11.1%). Other rarer species were not thought to be associated with pulmonary disease (Table 3).
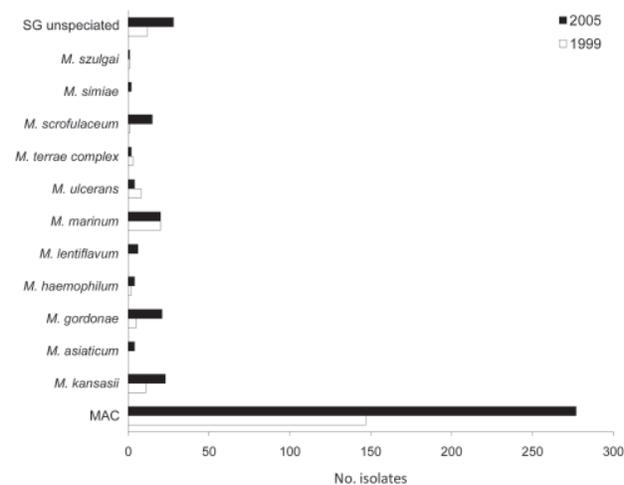
Figure 1. Changes in frequency of isolation for slow-growing (SG) nontuberculous mycobacteria (absolute numbers), Queensland, Australia, 1999 and 2005. MAC, *Mycobacterium avium* complex.

Of the isolates from nonpulmonary sites, the most common species was *M. fortuitum* (33/143), followed by *M. intracellulare* (12), *M. abscessus* (11), and *M. chelonae* (11). Overall, 68.5% of nonpulmonary isolates were felt to be associated with clinically significant NTM disease. Table 4 shows the statistical significance of these isolates according to species.

Pulmonary Isolates

Of the 111 new notifications in 2005 with significant pulmonary disease that met ATS/IDSA criteria, *M. intracellulare* (63) was the most common pathogen, accounting for 56.8% of cases. *M. avium* (17) was the next most common (15.3%), followed by *M. kansasii* (9 [8.1%]). A total of 19 cases were repeat notifications (i.e., relapse/reinfection; *M. intracellulare* (15), *M. avium* (1), *M. kansasii* (1), *M. fortuitum* (1), and *M. abscessus* (1).

The age and sex distribution of patients with significant pulmonary disease differed in 1999 and 2005. In 1999, the absolute number of men with disease outnumbered women in all age groups with the exception of the >75-year

Table 1. Comparison of numbers of nontuberculous mycobacteria isolates in 1999 and 2005, Queensland, Australia*

Isolates	No. isolates (rate/100,000 population; 95% CI)		p value
	1999	2005	
Total patient isolates	517† (14.8; 13.4–14.9)	631‡ (15.8; 14.3–16.9)	NS
Pulmonary	382 (10.9; 9.9–12.1)	488 (12.2; 11.1–13.3)	NS
Nonpulmonary	135 (3.9; 3.4–4.2)	143 (3.6; 3.1–4.0)	NS
Speciated isolates	318 (9.1; 8.2–10.2)	544 (13.6; 12.3–14.7)	<0.0000001
Pulmonary	192 (5.5; 4.5–6.6)	413 (10.2; 9.4–11.5)	<0.0000001
Nonpulmonary	119 (3.4; 3.0–3.8)	131 (3.3; 2.9–3.7)	NS

*CI, confidence interval; NS, not significant. Population of Queensland in 1999, 3.5 million; in 2005, 4 million.
†In 1999, more smear-negative, single-positive unspeciated isolates were captured than usual, inflating these numbers.
‡The calculated number of positive isolates in 2005 was 22.1/100,000 population, based on the observed increase in speciated isolates, assuming the proportion of isolates not referred for speciation remained constant.
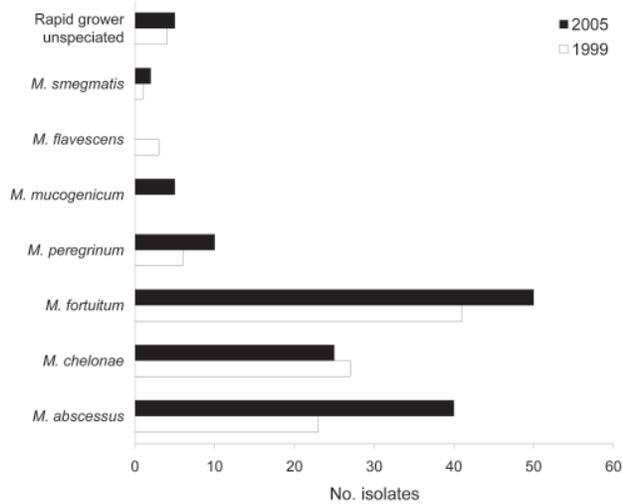
Figure 2. Changes in frequency of isolation for rapid-growing nontuberculous mycobacteria (absolute numbers), Queensland, Australia, 1999 and 2005.

age group. In 2005, women having NTM disease outnumbered men in all age groups >30 years; the difference was most marked in elderly persons. When adjusted for population within each age bracket and represented as rates per 100,000 (Figure 3), a significant increase was seen in women with disease who were 60–74 years of age (p = 0.0005), a decrease in men 30–44 years of age (p = 0.030) There was a combined overall increase in patients 60–74 years of age (p = 0.002) and a decrease in those 30–44 years of age (p = 0.013).

The radiologic pattern of disease has also changed (Figure 4). Most of the increase was in patients with noncavitary disease, from 25 (0.7/100,000) to 84 (2.1/100,000) cases (p<0.0001). A slight drop from 33 (0.9/100,000) cases of cavitary disease in 1999 to 28 (0.7/100,000) occurred in 2005 (p = 0.25).

Discussion

In 2005, NTM were isolated from 15.6 persons per 100,000 population; however, the true number of isolates from that year is estimated at 22.1/100,000, a significant increase from 1999. Little change has been recorded in the isolation of NTM from nonpulmonary sites. However, the incidence of isolation of NTM from human pulmonary specimens has increased to 12.1/100,000 population. The incidence of notified significant pulmonary disease has increased from 2.2 to 3.2/100,000 population per year. This increase is largely due to an increase in the number of elderly women with noncavitary disease.

The observed changes in speciation are interesting epidemiologic observations, but their clinical relevance is yet to be defined. *M. intracellulare* is the main pathogen associated with the increase in disease, followed by *M. abscessus,* and *M. kansasii,* all clinically significant pulmonary pathogens. We have observed a change in the age and gender ratio. NTM disease has increased in elderly female patients who have predominantly nodular bronchiectasis, but disease has decreased in middle-aged men.

The epidemiology of NTM disease is difficult to study and equally difficult to report in a meaningful way. We have reported the incidence of new cases of clinically significant disease notified by clinicians that met the 1997 ATS/ISDA criteria (*12*) for disease. The true number of cases of disease may be an underestimate because the experience and attitudes of clinicians with regard to assessing the clinical significance of isolates is varied. It is likely that other cases of significant disease were not notified. The more recent ATS/IDSA standards of 2007 (*15*) have more relaxed microbiological criteria, and it is likely more case-patients would now be considered to have disease. Because NTM disease is a chronic disease, often over many years, the prevalence of disease will be much higher and more likely will reflect the impact on the healthcare system. Even with the current notification process in Queensland, we failed to capture all single, positive, unspeciated isolates.

A rising body of literature supports the observation by clinicians that the incidence and prevalence of disease is increasing. Other studies in Australia have also shown an increase over earlier time periods (*16–18*). The most recent report is from the Northern Territory. All isolates from 1989–1997 were examined (*16*). Disease incidence increased from 2.7 to 4.7 per 100,000 population between the first and second halves of this time period. Pang re-

Table 2. Significance of nontuberculous mycobacteria isolates as notified, Queensland, Australia, 1999 and 2005*				
Nontuberculous mycobacteria isolates	1999		2005	
	No. (%) isolates	Rate/100,000 population	No. (%) isolates	Rate/100,000 population
Pulmonary				
Significant	77 (20.2)	2.2	130 (26.6)	3.25
Under evaluation	1 (0.26)	0.03	52 (10.6)	1.3
Not significant	304 (79.6)	8.7	306 (62.7)	7.65
Extrapulmonary				
Significant	106 (78.5)	2.65	98 (68.5)	2.45
Under evaluation	0	–	4 (2.8)	–
Not significant	29 (21.5)	0.83	41 (28.7)	1.03
*–, not applicable				

Table 3. Clinical significance of nontuberculous mycobacteria isolates from pulmonary sites, Queensland, Australia, 2005*

Species	Significant	Under evaluation	Not significant	Total
Slow growers				
Mycobacterium intracellulare	79 (39.7)	22 (11.1)	98 (49.2)	199
M. avium	18 (33.3)	8 (14.8)	28 (48.3)	54
MAC	–	–	3 (100)	3
M. kansasii	10 (52.6)	3 (15.8)	6 (31.6)	19
M. scrofulaceum	3 (30)	–	7 (70)	10
M. gordonae	2 (11.1)	–	16 (88.9)	18
M. lentiflavum	–	–	5 (100)	5
M. asiaticum	–	–	3 (100)	3
M. haemophilum	–	–	1 (100)	1
M. simiae	–	–	2 (100)	2
Unspeciated	–	–	24 (100)	24
Rapid growers	–	–	–	–
M. abscessus	7 (24.1)	4 (13.8)	18 (62.1)	29
M. chelonae	3 (21.4)	2 (14.3)	9 (64.3)	14
M. fortuitum	1 (5.3)	1 (5.3)	17 (89.4)	19
M. peregrinum	–	1 (33.3)	2 (66.7)	3
M. mucogenicum	–	–	2 (100)	2
M. nonchromogenicum	–	1 (50)	1 (50)	2
Unspeciated	2 (28.6)	–	5 (71.4)	7
Atypical mycobacteria NFI	5 (7.0)	10 (14.1)	56 (78.9)	71
Other	–	–	3 (100)	3
Total	130	52	306	488

*Values are given as no. isolates (% of total for species). MAC, *Mycobacterium avium* complex; –, not applicable; NFI, not further identified.

ported a 3-fold increase in *M. kansasii* disease in Western Australia during 1962–1982 and 1983–1987 (*19*). More recently Marras et al. (*3*) reported an increase in population prevalence of NTM isolates in Ontario, Canada from 2003 (9.1/100,000) to 2007 (14.1/100,000). Although adequate clinical data were not available for all patients, it is likely that the proportion of isolates responsible for disease increased in a similar manner. Skin testing by using MAC an-

tigens to assess infection or at least exposure to NTM was initially used to demonstrate the considerable geographic distribution of NTM infection (*20,21*). More recently Khan et al. demonstrated an increase in skin test reactivity to *M. intracellulare* in 1971 and 1972 (11.2%) and 1999–2000 (16.6%) among representative US population cohorts (*22*).

If sensitization is a reflection of infection with NTM, then the observed increases cannot be attributed to the in-

Table 4. Clinical significance of nontuberculous mycobacteria isolates from nonpulmonary sites, Queensland, Australia, 2005*

Species	Significant	Under evaluation	Not significant	Total
Mycobacterium intracellulare	7 (58.3)	–	5 (41.7)	12
M. avium	6 (66.7)	2 (22.2)	1 (11.1)	9
M. scrofulaceum	3 (60)	1 (20)	1 (20)	5
M. gordonae	1 (33.3)	–	2 (66.7)	3
M. abscessus	9 (81.8)	–	2 (18.2)	11
M. chelonae	8 (72.7)	–	3 (27.3)	11
M. fortuitum	23 (69.7)	3 (9.1)	7 (21.2)	33
M. peregrinum	7 (100)	–	–	7
M. asiaticum	–	–	1 (100)	1
M. haemophilum	1 (33.3)	–	2 (66.7)	3
M. lentiflavum	–	–	1 (100)	1
M. mucogenicum	–	–	1 (100)	1
M. marinum	–	–	1 (100)	1
M. smegmatis	1 (50)	–	1 (50)	2
M. szulgai	1 (100)	–	–	1
M. ulcerans	4 (100)	–	–	4
Atypical mycobacteria NFI	3 (27.3)	–	8 (72.7)	11
Slow grower unspeciated	1 (50)	–	1 (50)	2
Other	–	–	1 (100)	1
Total	97 (67.8)	6 (4.2)	40 (28)	143

*Values are given as no. isolates (% of total for species). –, not applicable; NFI, not further identified.

Figure 3. Age-adjusted rates for cases of nontuberculous mycobacteria disease among women (A), men (B), and all residents (C), Queensland, Australia, 1999 and 2005. *p = 0.0005; †p = 0.030; ‡p<0.005; §p = 0.057.

Thomson, unpub. data). During the study period, Queensland had been affected considerably by drought, and restrictions to domestic water use had been enforced. The reduction in flow within distribution systems may have led to an increased chlorine degradation time and, hence, lower disinfectant levels at point of use. Previously, *M. scrofulaceum* was the main pathogen associated with childhood lymphadenitis. A change from chloramination to chlorination of water led to a reduction in the isolation of this species. The resurgence of this species in 2005 supports the notion of increased chlorine degradation, brought about by flow reductions but potentially also by temperature increases associated with climate change.

Changes in behavior associated with water restrictions may also have been a factor in resurgence of disease. The changes in turbulence that occur with the turning on and off of taps lead to disruptions in biofilms and release of mycobacteria into the water. However, some authors have suggested that patients may inhale aerosolized mycobacteria while showering (23,24). Recently, Feazel et al. (25) found mycobacterial DNA concentrated in the biofilms of showerheads in the United States. Queensland implemented a strong public awareness campaign promoting 4-minute showers to reduce water usage, which in theory should reduce exposure to mycobacteria through this route.

Several case series have reported gender imbalances in this disease. Earlier studies have demonstrated a male predominance with cavitary disease (8,16,26). More recent studies have shown a female predominance (3,27–29) of older patients with less preexisting lung disease. The differences between these groups of studies may be attributed to sampling bias and population differences. We have been able to demonstrate that, in fact, the pattern of disease is changing, from cavitary disease in middle-

creased use of computed tomography scans, better laboratory techniques, or greater clinician awareness. Improved detection methods, such as the introduction of broth culture (MGIT BACTEC 960; Becton Dickinson, Franklin Lakes, NJ, USA) before the data collection of 1999, also cannot explain our observations. An increase in environmental exposure may have contributed to the increasing incidence of disease. The main environmental niche for *M. intracellulare* appears to be in biofilms lining drinking water pipes (7). *M. intracellulare* has been isolated from drinking water in Brisbane, as have *M. abscessus* and *M. kansasii* (R.

Figure 4. Changes in the radiologic appearance of cases of nontuberculous mycobacteria disease, Queensland, Australia, 1999 and 2005.

aged men who smoke to the nodular bronchiectatic form of disease in elderly women. Queensland government statistics suggest that the median age of the population is predicted to rise from 36.3 years in 2006 to 45 years in 2051, and the number of persons ≥65 years of age will increase 4-fold by 2051. As the vulnerable population increases, NTM disease is also likely to increase. Therefore, the impact of this disease on health budgets is worthy of further attention.

Acknowledgments

Dr Thomson is a thoracic physician at the QTBCC, The Prince Charles Hospital, and Greenslopes Private Hospital. She is also a PhD student researching environmental aspects of nontuberculous mycobacteria related to pulmonary infections.

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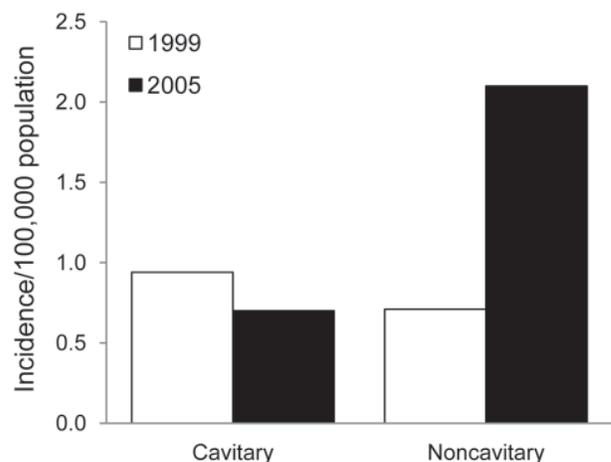14. Australian Bureau of Statistics. Australian Demographic Statistics December quarter 2005. Commonwealth of Australia; 2006 [cited 2010 Aug 12]. http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3101.0Dec%202005?OpenDocument

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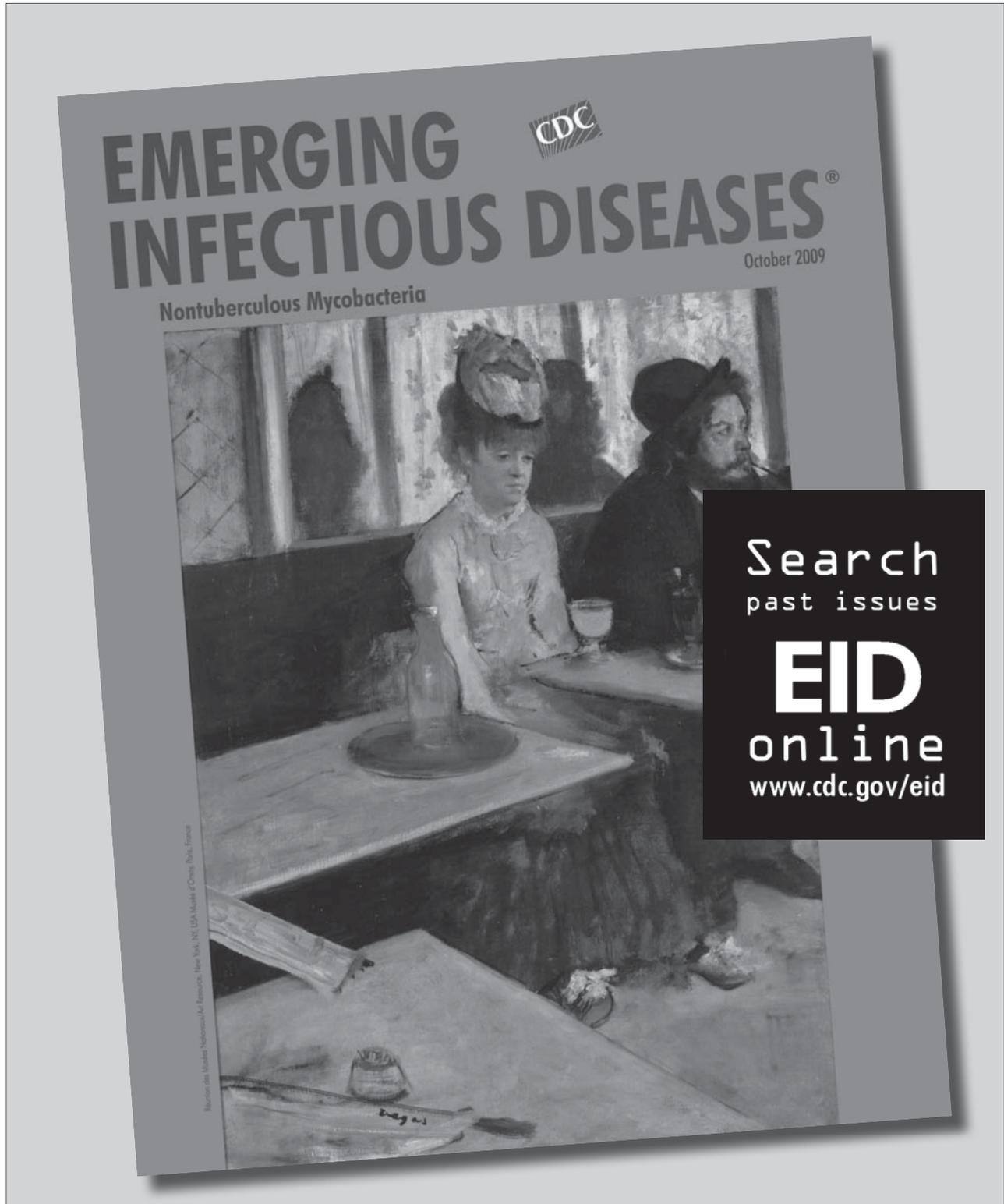
Mortality Rate Patterns for Hemorrhagic Fever with Renal Syndrome Caused by Puumala Virus

Marika Hjertqvist, Sabra L. Klein, Clas Ahlm, and Jonas Klingström

To investigate nephropathia epidemica in Sweden during 1997–2007, we determined case-fatality rates for 5,282 patients with this disease. Overall, 0.4% died of acute nephropathia epidemica ≤3 months after diagnosis. Case-fatality rates increased with age. Only women showed an increased case-fatality rate during the first year after diagnosis.

Hantaviruses cause 2 severe emerging zoonotic diseases: hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. These diseases have a case-fatality rate ≤40% depending on the specific hantavirus (1,2). Hantaviruses are primarily maintained in the environment by rodents, with humans serving as incidental hosts who are typically infected by inhalation of virus-contaminated rodent excreta (1,2). In parts of Europe, including Sweden, Puumala virus (PUUV) causes nephropathia epidemica (NE), a relatively mild form of HFRS with case-fatality rates of 0.1%–1% (1–4).

For many infectious diseases, frequency of infection is generally higher, and the clinical outcome often worse, in male patients (5–8). The reported male:female ratio for NE cases varies from 2 to 5:1 (1,3). Male-biased rates of infection have also been reported for other hantaviruses (1,9). However, in northern Sweden, seroprevalence does not differ by patient sex, which suggests the same number of persons of either sex might be infected with PUUV (10). Why males are overrepresented for diagnosed NE cases is not known.

We recently reported sex differences in cytokine responses during acute NE (11). Whether sexually dimorphic immune responses during hantavirus infection cause differences in the severity of disease between men and women has not been investigated. Furthermore, whether age is a risk factor in outcome of HFRS/HCPS is unknown. To investigate NE in Sweden during 1997–2007, we determined case-fatality rates for 5,282 patients with this disease.

The Study

NE is a reportable disease according to the Swedish Communicable Disease Act. Clinicians and microbiologic laboratories report diagnosed cases to the Swedish Institute for Infectious Disease Control. These notifications are stored in a database. In this study, we included all 5,282 NE cases reported to the Swedish Institute for Infectious Disease Control and registered in the database during January 1, 1997–December 31, 2007. OpenEpi (www.openepi.com) was used to calculate the standardized mortality ratio (SMR). Because NE is an acute viral infection, and as a comparison for the numbers of deaths during the different phases of NE, we used the number of deaths during the second year after diagnosis in the studied population.

Incidence was highest in the group 55–59 years of age for men and women (Figure 1, panel A), and the mean ± SD age at diagnosis was 49.3 ± 16.7 years for men and 50.7 ± 16.5 years for women. The overall male:female ratio for NE cases was 1.52:1. Sex hormones can play major roles in susceptibility to infectious diseases (5,7). To test whether increased incidence of PUUV infection in men was dependent on sex hormones, we compared sex ratios in NE cases between children (persons <10 years of age and presumably prepubertal) and adults (≥10 years of age). Sixty-two children were given a diagnosis of NE during 1997–2007. The male:female ratio for these children was 1.58:1, which was similar to the sex ratio for adult NE patients (1.52:1). This finding suggested that circulating sex hormones may not play a major role in the observed overrepresentation of NE in men.

Of persons with a diagnosis of NE, 21 (0.4%) patients died ≤3 months after diagnosis. The SMR was 3.5 (95% confidence interval [CI] 2.22–5.26) for all patients and 6.4 (95% CI 2.97–12.15) for female patients and 2.7 (95% CI 1.52–4.56) for male patients during the acute phase of NE.

No persons <50 years of age died during the acute phase (Figure 1, panel B). However, the case-fatality rate increased with age (Figure 1, panel B), and the case-fatality rate was 6.5% for patients ≥80 years of age, which showed age-dependent differences in mortality rates for NE. The mean ± SD age at time of death for patients with acute NE was 73.7 ± 10.4 years (range 50.9–88.5 years). Women died at a slightly, but not significantly, younger mean ± SD age (71.5 ± 12.4 years, range 50.9–85.7 years, n = 8) than men (75.1 ± 9.2 years, range 57.8–88.5 years, n = 13) (p = 0.347, by t test). Mean age at time of death for patients with NE was 10 years less than the expected

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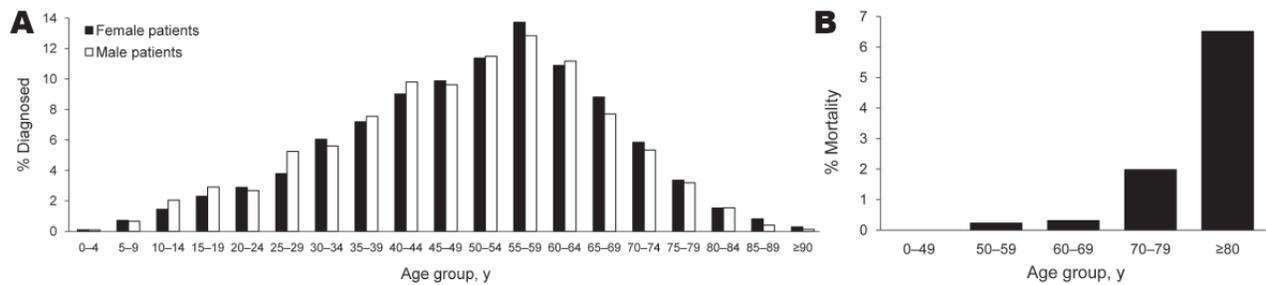
Figure 1. Incidence of acute nephropathia epidemica (NE) and case-fatality rates, Sweden, 1997–2007. A) Age distribution for male and female patients with acute NE. B) Age distribution of case-fatality rates for all patients with acute NE. The Swedish Death Register was used to identify all deceased persons with a diagnosis of NE. Numbers of deaths during different periods after diagnoses were 21 (13 male patients and 8 female patients) during the acute phase (≤3 months after diagnoses), 7 (5 male patients and 2 female patients) after the acute phase (>3 months after diagnoses) but ≤1 year later, and 24 (19 male patients and 5 female patients) during the second year after diagnoses.

life span for women and 3 years less than the expected life span for men (mean age at time of death in Sweden in 2003 was 78 years for men and 82 years for women; www.scb.se/statistik/_publikationer/BE0701_1986I03_BR_BE51ST0404.pdf).

Because no patient <50 years of age died of NE during the acute phase and the mean age at time of death was relatively high, we suggest that NE is rarely life threatening. To assess this possibility, we analyzed mortality rate patterns early after acute NE. The SMR decreased for the 9 months after acute NE in female (SMR 0.67, 95% CI 0.11–2.20) and male (SMR 0.36, 95% CI 0.13–0.79) patients (Figure 2).

We also analyzed SMRs for the first year (including the acute phase of NE [<3 months after diagnosis] and early phase [3–12 months] after NE diagnosis). For 52 persons who died ≤2 years after NE diagnosis, the case-fatality rate was only slightly higher in the first year (n = 28) than in the second year (n = 24) after diagnosis (SMR 1.17, 95% CI 0.79–1.66). A clear difference was observed between
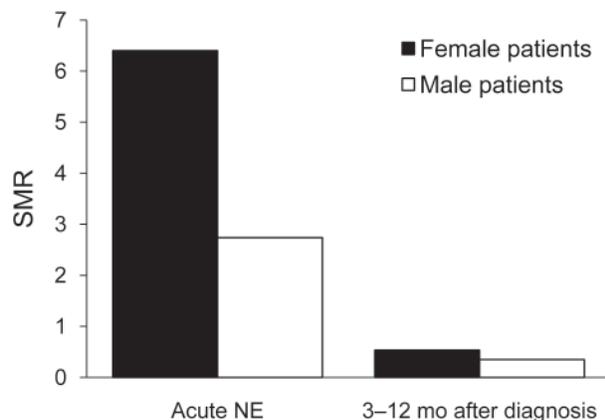
men and women. Deaths did not increase in men between the first (n = 18) and second (n = 19) years after diagnosis (SMR 0.95, 95% CI 0.58–1.49). However, we observed a 2× difference in deaths for women between the first (n = 10) and second (n = 5) years after diagnosis (SMR 2.0, 95% CI 1.02–3.57).

Conclusions

We report an age-dependent case-fatality rate for the hantavirus disease NE; most deaths occurred in older persons. There is a report of patients <50 years of age dying of NE (12), but this finding is rare. Increased case-fatality rates in older persons have been described for other infectious diseases. For example, during a typical influenza season, 90% of deaths caused by influenza occur among persons ≥65 years of age (13). Whether this finding is unique to relatively mild infection with PUUV or is a conserved feature of all hantaviruses causing HFRS/HCPS is unknown.

We previously showed that there are sex differences in cytokine responses during acute NE (11), which suggested that there might be sex differences in severity of infection. Women showed a 2× difference in number of deaths between the first year and second year after NE diagnosis. However, men showed no difference in number of deaths between the first year and second year after diagnosis. These results suggest that there are sex differences in mortality rates after infection with PUUV. Whether NE is a more lethal disease in women than men or causes increased mortality rates in men up to 2 years after diagnoses is unknown.

The finding that the case-fatality rate for NE is associated with the age and sex of patients might have practical implications on healthcare issues. It may also indicate that age and sex should be considered predictive variables in clinical studies of hantavirus infections.

Figure 2. Overall standardized mortality ratios (SMRs) for male and female patients with a diagnosis of acute nephropathia epidemica (NE) and SMRs 3–12 mo after diagnosis, Sweden, 1997–2007.

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Pandemic (H1N1) 2009 Virus on Commercial Swine Farm, Thailand

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A swine influenza outbreak occurred on a commercial pig farm in Thailand. Outbreak investigation indicated that pigs were co-infected with pandemic (H1N1) 2009 virus and seasonal influenza (H1N1) viruses. No evidence of gene reassortment or pig-to-human transmission of pandemic (H1N1) 2009 virus was found during the outbreak.

In April 2009, a novel swine origin influenza A (H1N1) virus, now referred to as pandemic (H1N1) 2009 virus, emerged in humans in Mexico and the United States and spread worldwide (*1*). In May 2009, pandemic (H1N1) 2009 was confirmed in 2 patients in Thailand who had a history of travel to Mexico. Shortly after emergence of this virus, reports of transmission from humans to pigs on pig farms were documented (*2,3*). Human-to-pig transmission of this virus was reported in Thailand on December 17, 2009 (www.dld.go.th/dcontrol/Alert/Ah1n1/H1N1%20 update22_12_2009.pdf). Pigs showed mild respiratory signs; only 1 pandemic (H1N1) 2009 virus was isolated from 80 nasal swab specimens.

Swine influenza virus (SIV) was reported in Thailand in 1981 (*4*). All 3 subtypes (H1N1, H3N2, and H1N2) of this virus are circulating in Thailand (*5*). A recent pathogenesis study demonstrated that subtype H1N1 induces typical SIV-like illness and slightly more severe gross lesions than illness induced by subtype H3N2 (*6*). Genetic data indicate that SIV (H1N1) in Thailand differs from pandemic (H1N1)

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2009 virus. SIV (H1N1) in Thailand contains surface proteins of influenza viruses from North America and Eurasia, which are also found in pandemic (H1N1) 2009 virus; SIV (H1N1) in Thailand contains internal proteins of viruses from Eurasia; and pandemic (H1N1) 2009 viruses contain swine, human, and avian virus gene segments (*5,7*).

We report an outbreak of infection with pandemic (H1N1) 2009 virus during November 2009–March 2010 on a commercial pig farm in Thailand. The outbreak presumably resulted from human-to-pig transmission because 1 of the workers on this farm had influenza-like clinical signs at the beginning of the outbreak. Infection in this worker was not confirmed because he quit his job on the farm after the start of the outbreak and could not be located.

The Study

In early November 2009, a small commercial pig farm in central Thailand reported respiratory problems in pigs (morbidity rate 50%, mortality rate 10%) in nursery pigs. The farm contained 3,235 pigs (700 sows, 35 boars, 1,000 piglets, 1,000 nursery pigs, and 500 finishing pigs). It has a conventional open-house production system in which both sides of the unit have natural air flow ventilation. The farm also has continuous nursery herd flow in which new pigs are continuously added when they are old enough. This process results in pigs of different ages being in the same unit. Sick pigs had clinical signs (fever, cough, nasal discharge, edematous eyelids, and conjunctivitis) of infection.

Nasal swabs from 20 nursery pigs (4–9 weeks of age) were submitted to Chulalongkorn University Veterinary Diagnostic Laboratory. All samples were positive for porcine circovirus type 2 and porcine reproductive and respiratory syndrome virus (these viruses are major causes of swine respiratory disease), and 2 samples were positive for influenza A virus by reverse transcription–PCR (RT-PCR) with primers for each specific pathogen (*8–10*).

Because respiratory problems in nursery pigs continued, nasal swabs specimens from 20 nursery pigs and finishing pigs, gilts (young females), and sows (10 per group) with clinical signs were submitted to the diagnostic laboratory by the end of December 2009. Two samples from nursery pigs were positive for influenza virus A (H1N1) by multiplex RT-PCR (*11*). Both samples were subjected to virus isolation in MDCK cells (*12*) and designated RA20 and RA29 (Table 1). Genome characterization identified RA20 as SIV and RA29 as pandemic (H1N1) 2009 virus (Table 2; Figure). SIV-positive nasal swabs obtained in November were then characterized. Results showed that isolates RA4 and RA9 were pandemic (H1N1) 2009 virus, which indicated that pigs on the farm were infected with this virus.

Pandemic (H1N1) 2009 investigations on the farm included clinical surveillance and sample collection from

Table 1. Influenza (H1N1) viruses studied, Thailand*

Influenza (H1N1) virus isolate	Collection date	Identification	Study designation	GenBank accession no. (gene segment 1–8)
A/sw/Thailand/CU-RA4/2009	2009 Nov 6	Pandemic (H1N1)2009	RA4	CY062305–CY062312
A/sw/Thailand/CU-RA9/2009	2009 Nov 6	Pandemic (H1N1) 2009	RA9	CY062321–CY062328
A/sw/Thailand/CU-RA20/2009	2009 Dec 26	Thai SIV	RA20	CY062281–CY062288
A/sw/Thailand/CU-RA29/2009	2009 Dec 26	Pandemic (H1N1)2009	RA29	CY062297–CY062304
A/sw/Thailand/CU-RA114/2010	2010 Jan 17	Pandemic (H1N1)2009	RA114	CY062265–CY062272
A/sw/Thailand/CU-RA204/2010	2010 Jan 17	Thai SIV	RA204	CY062289–CY062296
A/sw/Thailand/CU-RA15/2010	2010 Jan 30	Pandemic (H1N1) 2009	RA15	CY062273–CY062280
A/sw/Thailand/CU-RA75/2010	2010 Jan 30	Pandemic (H1N1) 2009	RA75	CY062313–CY062320
*SIV, swine influenza virus.				

sick and contact pigs and close monitoring of swine workers and farm pets for influenza-like illness. Nasal swab specimens were obtained from pigs on January 17, 2010, January 30, 2010, and March 9, 2010. Because initial laboratory findings indicated that the outbreak involved the nursery herd, weaned pigs were moved to a separate site on the farm to control disease in the nursery pigs. Following Food and Agriculture Organization (www.fao.org) sample collection recommendations, we obtained 20 nasal swabs from pigs with SIV-like illness. In addition, nasal swab specimens (n = 10 per group) were collected from gilts, sows, and finishing pigs to test for pandemic (H1N1) 2009 virus, although no clinical signs were observed in any pigs from these age groups. All SIV-positive samples were subjected to virus isolation (12), virus subtyping by multiplex RT-PCR (11), and whole genome sequencing of subtype H1N1 viruses (13). Of 175 samples obtained during December 26, 2009–March 9, 2010, fifteen swab specimens from nursery pigs with clinical signs were positive for influenza (H1N1) 2009 virus; 8 viruses were characterized. No other SIV subtypes were found. On March 9, ≈1 month after implementing the change in handling of

pigs, no pigs showed respiratory signs and 34 nasal swab specimens were negative for influenza virus.

Gene sequences were compared for corresponding genes of other influenza virus strains obtained from GenBank by using the MegAlign program (DNASTAR, Madison, WI, USA). Phylogenetic trees were constructed by using MEGA4 (www.megasoftware.net/) and the neighbor-joining method with 1,000 bootstrap replicates. Whole genome analysis showed that contemporary SIV (H1N1) and pandemic (H1N1) 2009 virus were concurrently circulating in the nursery herd (Table 2; Figure). On the basis of virus hemagglutinin 1 gene grouping (14), our findings show that newly isolated SIV (H1N1) from Thailand are grouped in the classical swine cluster with other SIV (H1N1) isolates (online Appendix Figure, www.cdc.gov/EID/content/16/10/1587-appF.htm). There was no evidence of gene reassortment between SIV (H1N1) and pandemic (H1N1)2009 virus during the investigation (Table 2).

To test for evidence of pandemic (H1N1) 2009 virus interspecies transmission, we obtained serum samples on January 17, 2010, from 40 pigs in 8 age groups (5/group),

Table 2. Gene origin and percent homology of SIV RNA segments compared with pandemic (H1N1) 2009 virus, Thailand*

Influenza (H1N1) virus	PB2 (1–2229)†	PA (1–2153)†	NA (1–1347)†	M (1–982)†	HA (1–1698)†	NS (1–778)†	NP (1–1443)†	PB1 (1–2153)†
Pandemic (H1N1) 2009‡	Avian TRIG		Eurasian swine		Classical swine			Human TRIG
SIV from Thailand§	Eurasian swine				Classical swine		Eurasian swine	
RA4	Avian TRIG		Eurasian swine		Classical swine			Human TRIG
RA9	Avian TRIG		Eurasian swine		Classical swine			Human TRIG
RA20¶	Eurasian swine				Classical swine		Eurasian swine	
	83.1%	85.1%	89.5%	94.2%	86.4%	90.8%	82.4%	85.1%
RA29	Avian TRIG		Eurasian swine		Classical swine			Human TRIG
RA114	Avian TRIG		Eurasian swine		Classical swine			Human TRIG
RA204¶	Eurasian swine				Classical swine		Eurasian swine	
	83.2%	85.2%	89.5%	94.2%	86.8%	90.8%	82.3%	85.1%
RA15	Avian TRIG		Eurasian swine		Classical swine			Human TRIG
RA75	Avian TRIG		Eurasian swine		Classical swine			Human TRIG

*All swine influenza virus (SIV) isolates except RA20 and RA204 have >99% homology with corresponding genes of A/Nonthaburi/102/2009. PB, polymerase B; PA, polymerase A; NA, neuraminidase; M, matrix; HA, hemagglutinin; NS, nonstructural; NP, nucleoprotein; TRIG, triple reassorted internal gene.
†Nucleotide positions compared.
‡A/Nonthaburi/102/2009 (H1N1) virus.
§A/sw/Ratchaburi/NIAH1481/2000 (H1N1) virus.
¶Percent homology of compared sequences with those of corresponding genes of A/Nonthaburi/102/2009.
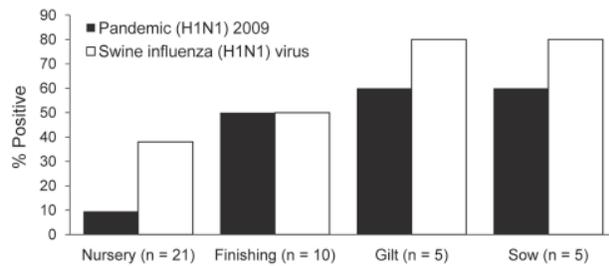
Figure. Percentage of pigs with antibodies against pandemic (H1N1) 2009 virus and swine influenza virus (H1N1) detected by hemagglutination-inhibition test, by pig type, Thailand. Serum samples were obtained from pigs of different ages in January 2010. Samples were positive when titer was $\geq$40.

15 workers, and 4 farm pets (3 dogs and 1 cat). Samples were subjected to hemagglutination-inhibition (HI) testing with SIV (H1N1) and pandemic (H1N1) 2009 virus antigens (*12*).

Control rabbit antibodies against SIV (H1N1) viruses did not cross-react with pandemic (H1N1) 2009 virus. Serologic results showed that only 2 (9.5%) of 21 test samples from the nursery group had positive HI titers for pandemic (H1N1) 2009 virus and 8 (38%) of 21 had positive HI titers for SIV (H1N1) virus. For pigs in other age groups, 11 (55%) of 20 had antibodies against pandemic (H1N1) 2009 virus and 14 (70%) of 20 had antibodies against SIV (H1N1) by HI test. No human cases of co-infection were observed. We found no evidence of pandemic (H1N1) 2009 virus interspecies transmission from pigs to humans or to farm pets.

Conclusions

Consistent with findings of previous reports (*2*,*3*), our findings demonstrate that young pigs are susceptible to infection with pandemic (H1N1) 2009 virus. Infection in pigs substantiates the hypothesis that the clinical outcome caused by infection with pandemic (H1N1) 2009 virus differs from that of infection with SIV (H1N1), which currently circulates in pigs in Thailand. Serologic results demonstrated that uninfected populations are susceptible to infection with pandemic (H1N1) 2009 virus. Results of genome analysis did not show gene reassortment between the 2 different influenza (H1N1) viruses. However, a previous report showed that reassortment of influenza virus genes occurs in pigs (*15*). Continued monitoring, characterization of SIVs, and serologic surveillance of pigs are necessary for future influenza pandemic preparedness.

Acknowledgments

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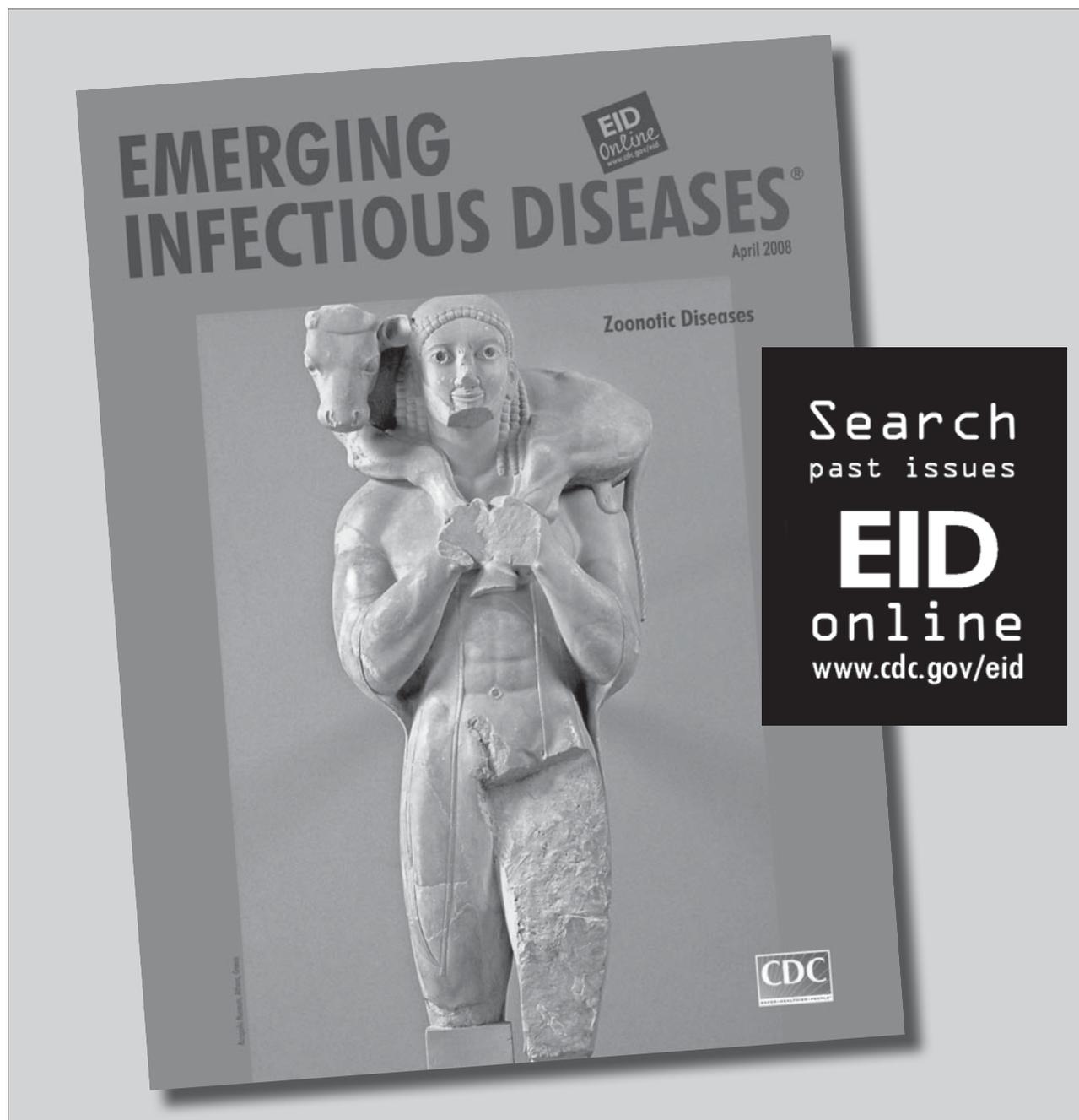
Toxoplasma gondii Oocyst–specific Antibodies and Source of Infection

Claudia A. Muñoz-Zanzi, Paulina Fry, Blaz Lesina, and Dolores Hill

Infection source can determine cost-effective public health interventions. To quantify risk of acquiring *Toxoplasma gondii* from environmental sources versus from meat, we examined serum from pregnant women in Chile. Because 43% had oocyst-specific antibodies, we conclude that contaminated meat remains the primary source of infection but that environmental sources also pose substantial risk.

Toxoplasmosis is a zoonotic disease that occurs worldwide and is caused by the protozoon *Toxoplasma gondii*. The public health relevance of toxoplasmosis relates to congenital (*1*) and postnatal infection (*2–4*). The distribution of postnatal infection is highly variable worldwide, or even within a country, probably because of environmental, socioeconomic, and cultural factors. Seroprevalence estimates range from 11% in the United States (*5*) to >70% in Brazil (*6*). Postnatal infection is caused by ingestion of undercooked meat containing tissue cysts; ingestion of water, fruits, vegetables, and shellfish contaminated with oocysts; or unintentional ingestion of cat feces or soil that contain oocysts (*7,8*). Population studies to determine specific risk factors for infection and source attribution have been based on the epidemiologic analysis of information from questionnaires administered to infected and uninfected persons; however, applicability of this method is limited. The relative roles of various potential sources are not known and probably vary from population to population. Knowledge of sources of infection within a specific type of community can provide valuable information for designing cost-effective food safety and public health interventions. Our objective, therefore, was to quantify the risk of acquiring infection from environmental sources (oocysts) compared with the risk from eating meat. We did so by detecting antibodies against a recombinant sporozoite-specific protein

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(SSP), which is found only in the oocyst (sporozoite) stage of the parasite (*9,10*).

The Study

We used 494 banked serum samples from pregnant women in Valdivia Province, southern Chile, who had participated in an unrelated study. *T. gondii* prevalence in this population was high (39%). A convenience sample was selected from among women who were at least 18 years of age at the time of their first prenatal visit at 1 of 5 public clinics, which served mostly low-income persons from urban communities. Information available was age, gestation time, residency (urban or rural), socioeconomic status, and education level. Because of the small sample size, p<0.1 was considered significant. The study protocol (0901E57685) was approved by the University of Minnesota Institutional Review Board.

Past exposure to *T. gondii* was determined qualitatively by immunoglobulin (Ig) G, detected by using a commercial enzyme immunoassay kit (*Toxoplasma* IgG EIA, Bio-Rad Laboratories, Redmond, WA, USA). According to the manufacturer, sensitivity and specificity of this assay were 83% and 93%, respectively. Approximate timing of infection for patients with IgG-positive samples was ascertained by using a commercial solid-phase enzyme immunoassay (*Toxoplasma gondii* IgG Avidity EIA; Ani Labsystems Ltd. Oy, Vantaa, Finland) to determine avidity (binding ability) index. According to manufacturer recommendations, results were interpreted as follows: avidity <15%, acute infection; 15%–30%, possible infection during the past 6 months; and >30%, infection not within the past 3 months.

A Western blot assay developed at the Animal Parasitic Diseases Laboratory of the US Department of Agriculture, with estimated sensitivity and specificity of 88% and 100%, respectively, was used to qualitatively evaluate IgG-positive serum samples for antibodies to SSP. Details of the assay protocol and validation procedures have been submitted for publication (D. Hill et al., unpub. data, www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=409642&showpars=true&fy=2009).

A total of 193 samples were positive for *T. gondii* IgG; overall seroprevalence was 39.1% (90% confidence interval [CI] 34.9%–43.5%) (Figure). Age of seropositive women was higher than that of seronegative women (p = 0.011). In addition, college-level education was protective for *T. gondii* exposure (p = 0.077) (Table). Of 180 *T. gondii* IgG–positive women with available SSP results, 64 (35.5%) had SSP antibodies. Validation of the SSP assay indicated that SSP antibodies tend to decrease over time and can become undetectable after 6–8 months (Hill et al., unpub. data); therefore, we calculated the proportion of the study population with SSP antibodies among women with evidence of recent (within the past 6 months) infec-
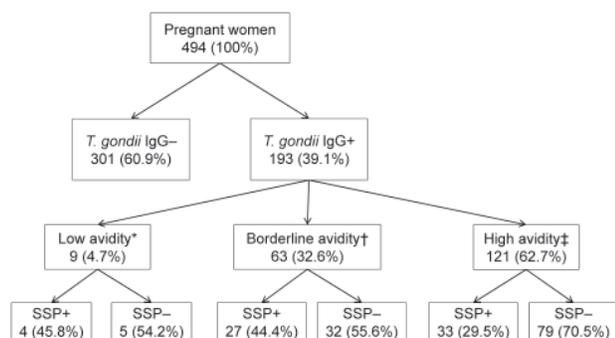
Figure. Detection of *Toxoplasma gondii* in 494 low-income pregnant women from Valdivia Province, Chile. *<15%, indicates acute infection; †15%–30%, indicates possible infection within ≤6 mo (4 samples from recently infected women were not tested for SSP antibodies); ‡>30%, excludes recent (within 3 mo) infection (9 samples from women with chronic infection were not tested for SSP antibodies). –, negative; +, positive; Ig, immunoglobulin; SSP, sporozoite-specific protein antibodies.

tion only. Of the 193 *T. gondii*–seropositive women, 72 (32.6%) had evidence of recent or acute infection (avidity <30%; Figure), although this was probably an overestimation because avidity increases slowly in certain persons. Among these 72 women, 31 (43.1%) had SSP antibodies, including 4 (45.8%) of 9 acutely infected women and 27 (44.4%) of 59 women with evidence of recent infection (Figure). Socioeconomic status was the only demographic factor significantly associated with SSP antibodies (p = 0.056). The age-adjusted odds ratio for socioeconomic status was 4.06 (90% CI 1.21–13.60), indicating that the odds of having SSP antibodies was 4× higher for women of very low (≤$3,500/year) compared with low (>$3,500/year to <$5,000/year) socioeconomic status.

Conclusions

Our finding that 43% of toxoplasmosis infections were associated with direct or indirect exposure to an occyst-contaminated environment indicates that cyst-containing meat was the major (57%) source of infection in the study population (Figure). Although this value is likely to vary by population, it is consistent with findings from a multicenter case–control study that reported, on the basis of calculation of population-attributable fractions, that 30%–63% of acute infections could be attributed to eating meat (*11*). Although data on prevalence of infection in meat-producing animals from the area are limited, in general, the high risk for human infection is consistent with the high seroprevalence reported for swine (9%) (C.A. Muñoz-Zanzi et al., unpub. data), free-range chickens (55%) (*12*), and sheep (28%) (*13*). In communities such as our study area, consumption of meat produced locally from small farms and backyard pens is common, especially for populations of low socioeconomic status. In these informal farm management systems, animals are at higher risk of acquiring *T. gondii*.

Our detection of SSP antibodies in 43% of recently infected women (Figure) implies that infection from ingestion of oocysts is an almost equally large public health problem in this population as is contaminated meat. A high level of oocyst contamination of soil and especially water (because of runoff) probably results from high (33%) prevalence of infection in cats (*14*) and high annual precipitation. Oocysts in the local environment have not been studied; however, infection of local aquatic or semiaquatic species, such as sea lions and feral mink (M. Sepulveda, unpub. data), suggests that surface water is contaminated with oocysts. Although less recognized than contact with cat feces (while handling cat litter), eating raw shellfish, which can accumulate oocysts (*7*), and drinking contaminated water are major risk factors for *T. gondii* infection (*15*). Use of a study population of pregnant women affects generalizability of findings; nevertheless, results indicate that toxoplasmosis is a major public health problem in this area of Chile. Despite knowledge of how to prevent or minimize risk for infection (e.g., cook meat thoroughly, freeze meat, wash vegetables, wash hands after handling raw meat and after gardening), the high risk for infection in this population highlights the need for improved education programs, especially for populations of low socioeconomic status.

A study limitation is the possibility of misclassifications (false-positive or false-negative results) with use of

Table. Demographics of low-income pregnant women evaluated for exposure to *Toxoplasma gondii*, Valdivia Province, Chile*				
Variable	Seronegative	Seropositive	Total	Odds ratio (p value)†
Urban residence‡	289/300 (96.3)	184/189 (97.4)	473/489 (96.7)	– (0.538)
Very low socioeconomic status§	261/300 (87.0)	167/191 (87.4)	428/491 (87.2)	– (0.888)
College/technical education¶	41/300 (13.7)	16/191 (8.4)	57/491 (11.6)	0.58 (0.077)
Age, y, median#	25 (18.0–39.0)	27 (18.0–41.0)	25 (18.0–40.2)	1.46** (0.011)
Total, no. (%)	301 (60.9)	193 (39.1)	494	

*Values are no./no. tested (%) except as indicated.
†Odds ratio and corresponding p value from univariate logistic regression analysis.
‡Reference is rural residence.
§Household income ≤$3,500/y; reference is low socioeconomic status, household income >$3,500/y to <$5,000/y.
¶Reference is no college or technical education.
#Median, 2.5th, and 97.5th percentiles (in parenthesis) are reported for age as a continuous variable.
**Odds ratio for a 28-year-old woman compared with an 18-year-old woman as an example.

the IgG, SSP, and avidity assays. Despite this limitation, the fraction of infection attributed to oocysts was consistent for all infected groups. A method for quantifying error rates, sensitivity, and specificity of the entire testing algorithm is being developed. Lastly, the potential effects of strain variability of immune response and detection of SSP antibodies remain unknown for this new tool.

The new tool reported here can be used for source attribution as well as epidemiologic analysis of self-reported data. Its use should lead to improved effectiveness of intervention programs.

Acknowledgment

Dr Muñoz-Zanzi is an assistant professor in the Division of Epidemiology, School of Public Health, University of Minnesota. Her research involves the epidemiology, diagnosis, and control of zoonotic disease pathogens of global public health relevance.

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etymologia

Toxoplasma

[tok″so-plaz′mə]

From the Greek *tóxikon* (poisoned arrow, from *tóxon* [bow]) and *plásma* (something molded). This genus of intracellular parasitic protozoa was named for its arc-like shape. Charles Nicolle and Louis Manceaux published the definitive description of the organism in 1909 after they observed the parasites in the blood, spleen, and liver of a North African rodent, *Ctenodactylus gondii*. The species *T. gondii* was formally designated at that time.

Source: Ajioka JW, Morrissette NS. A century of *Toxoplasma* research. Int J Parasitol. 2009;39:859–60; http://emedicine.medscape.com/article/229969-overview; Dorland's illustrated medical dictionary, 31st edition. Philadelphia: Saunders Elsevier; 2007.

Predicting Need for Hospitalization of Patients with Pandemic (H1N1) 2009, Chicago, Illinois, USA

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In the absence of established guidelines for hospitalization of patients with pandemic (H1N1) 2009, we studied emergency department patients to identify clinical parameters that predict need for hospitalization. Independent predictors of hospitalization include multiple high-risk medical conditions, dyspnea, and hypoxia. These findings are easily applicable, with a 79% positive predictive value for hospitalization.

Past influenza outbreaks have shown that limited health-care resources may be rapidly overwhelmed during an outbreak (*1,2*). Guidelines for hospitalization of persons with influenza would help physicians by providing a framework for the initial evaluation and management of patients with influenza. We conducted a study of patients with pandemic (H1N1) 2009 to identify predictors for hospitalization.

The Study

All patients with confirmed pandemic (H1N1) 2009 infection seen in the emergency department (ED) of Rush University Medical Center (a 613-bed teaching hospital in Chicago) from April 29, 2009, through June 22, 2009, were included in the study. Patients were stratified into 2 groups: hospitalized patients (admitted for at least 24 hours) and nonhospitalized patients (patients discharged from the ED).

Respiratory specimens from ED patients with influenza-like illness were tested by reverse transcription–PCR for respiratory viruses by using the Luminex xTAG RVP (Luminex, Austin, TX, USA), and clinical data were entered into electronic medical records. Specimens positive for nontypeable influenza A by Luminex xTAG RVP were confirmed as pandemic (H1N1) 2009 by using the Centers for Disease Control and Prevention (CDC) reverse transcription–PCR for pandemic (H1N1) 2009 (*3*). Continuous

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variables that vary with age (respiratory rate, blood pressure, hematologic counts) were regrouped as normal or abnormal by using age-specific normal ranges (*4,5*). Obesity (body mass index $\geq$30) for adults and children 2–19 years of age and for those with high-risk medical conditions was defined according to CDC guidelines (*6,7*).

The Mann-Whitney U test and Pearson χ^2 test or Fisher exact test were used to compare continuous and categorical variables, respectively. p values <0.05 were considered significant. Backwards stepwise logistic regression was performed for factors associated with hospitalization and intensive care unit (ICU) admission. Goodness-of-fit was determined with the Hosmer-Lemeshow statistic. Data were analyzed by using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The study was approved by the institutional review board of Rush University Medical Center.

A total of 189 cases that were identified by review of microbiology records were considered eligible for the study. However, only 83 patients who were examined in the ED were included in the study; the remaining 106 patients were seen at outpatient clinics and private doctors' offices. Demographic, clinical, and laboratory data of hospitalized patients (32 [39%]) were compared with data from those discharged from the ED (51 patients) (Tables 1, 2).

Most patients were African American (63%) or Hispanic (27%); 48 patients (58%) were female. The median age of hospitalized patients was 12 years (interquartile range 2–38.8 years) versus 20 years (interquartile range 9–28 years) for nonhospitalized patients (p = 0.70). Of 32 hospitalized patients, 17 (53%) were children; most (71%) of these children were <5 years of age. The most common admitting diagnoses were pneumonia (11 patients), viral syndrome (5 patients), influenza (4 patients), and asthma exacerbation (4 patients). Univariate analysis showed that being <5 years of age was significantly associated with hospitalization (38% hospitalized vs. 16% nonhospitalized, odds ratio 3.2, 95% confidence interval 1.1–9.1; p = 0.02).

Hospitalized patients were significantly more likely to report a high-risk medical condition than were nonhospitalized patients (p<0.0001). Univariate analysis showed that the following high-risk medical conditions were also significantly associated with hospitalization: history of prematurity, hemoglobinopathy, and chronic neurologic disease (p<0.05). A trend toward a higher incidence of chronic pulmonary disease was seen in hospitalized patients (41% vs. 22% of nonhospitalized patients; p = 0.06). Obesity was not found to be a significant risk factor for hospitalization (p = 0.18).

Patients with dyspnea were significantly more likely to be hospitalized (p<0.0001). Hospitalized patients had lower pulse oximeter oxygen saturation (SpO$_2$; median 95%, range 65%–100%) than nonhospitalized patients (median 99%, range 96%–100%; p<0.0001). Tachypnea and hy-

poxia ($SpO_2 \leq 92\%$) were significantly associated with hospitalization (p = 0.002 and p<0.0001, respectively). Five of 39 patients with measured creatinine had evidence of acute renal failure, which was significantly associated with hospitalization (p = 0.007). A chest radiograph showed an infiltrate in 11 of 51 patients, and all 11 patients were hospitalized (p = 0.001). Hypoxia was a strong predictor of a chest radiograph finding of infiltrate (odds ratio 50.7, 95% confidence interval 7.2–354.3; p<0.0001). Multivariate analysis showed that high-risk medical conditions (median number of high-risk conditions 2 vs. 0; p = 0.01), dyspnea (p = 0.01), and oxygen saturation (median SpO_2 95% vs. 99%; p = 0.004) were found to be significantly associated with hospitalization (online Appendix Table 1, www.cdc.gov/EID/content/16/10/1594-appT1.htm).

Sixteen of 83 (19%) study patients were admitted to an ICU. No deaths occurred. Univariate analysis showed that the following factors were significantly associated with ICU admission: greater median number of high-risk medical conditions (2 vs.1; p<0.0001), patient age <5 years (p = 0.002), chronic pulmonary disease (p = 0.01), history of prematurity (p = 0.001), congenital heart disease (p = 0.04), dyspnea (p<0.0001), tachypnea (p = 0.003), lower median oxygen saturation (SpO_2 92% vs. 98%; p <0.0001), acute renal failure (p = 0.004), and an infiltrate on chest radiograph (p<0.0001). Multivariate analysis showed that dyspnea (p = 0.01) and oxygen saturation (median SpO_2 92% vs. 98%; p = 0.02) were significantly associated with ICU admission (online Appendix Table 2, www.cdc.gov/EID/content/16/10/1594-appT2.htm).

Table 1. Demographics of nonhospitalized and hospitalized patients who had pandemic (H1N1) 2009 infection, Rush University Medical Center, Chicago, Illinois, USA, April 29–June 22, 2009*

Characteristic	Nonhospitalized patients, n = 51	Hospitalized patients, n = 32	ICU patients, n = 16	Nonhospitalized vs. hospitalized patients		ICU vs. non–ICU patients†	
				p value	OR (95% CI)	p value	OR (95% CI)
Median age, y (IQR)	20.0 (9.0–28.0)	12.0 (2.0–38.8)	2.5 (1.13–31.8)	0.70‡	–	0.16‡	–
Age <5 y, no. (%)	8 (15.7)	12 (37.5)	9 (56.3)	0.02§	3.2 (1.1–9.1)	0.002	6.6 (2.0–21.3)
Sex, M/F (% M)	23/28 (45.1)	12/20 (37.5)	5/11 (31.3)	0.50§	0.73 (0.30–1.8)	0.33§	0.56 (0.18–1.8)
Presence of high- risk conditions,¶ no. (%)	21 (41.2)	29 (90.6)	16 (100)	<0.0001§	13.8 (3.7–51.3)	<0.0001	–
No. high-conditions per patient,¶ median (range)	0 (0–2)	2 (0–4)	2 (1–3)	<0.0001‡#	–	<0.0001‡	–
Chronic pulmonary disease, no. (%)	11 (21.6)	13 (40.6)	9 (56.3)	0.06§	2.5 (0.94–6.6)	0.01	4.5 (1.4–14.0)
History of prematurity, no. (%)	0	6 (18.8)	5 (31.3)	0.002	–	0.001	30.0 (3.2–281.8)
Congenital heart disease,** no. (%)	0	2 (6.3)	2 (12.5)	0.15	–	0.04	–
Transplantation,†† no. (%)	1 (2.0)	3 (9.4)	1 (6.3)	0.29	5.2 (0.51–52.1)	1.00	1.4 (0.14–14.6)
Hemoglobinopathy, no. (%)	0	4 (12.5)	2 (12.5)	0.02	–	0.17	4.6 (0.6–35.8)
Diabetes mellitus, no. (%)	3 (5.9)	5 (15.6)	3 (18.8)	0.25	3.0 (0.66–13.4)	0.18	2.9 (0.6–13.5)
Chronic neurologic disease	2 (3.9)	7 (21.9)	4 (25.0)	0.02	6.9 (1.3–35.5)	0.07	4.1 (1.0–17.7)
Immunosuppression, no. (%)	2 (3.9)	5 (15.6)	3 (18.8)	0.10	4.5 (0.82–25.0)	0.13	3.6 (0.7–18.2)
Malignancy, no. (%)	0	3 (9.4)	1 (6.3)	0.054	–	0.48	2.2 (0.2–25.5)
Pregnancy, no. (%)‡‡	1 (4)	3 (15.0)	0	0.29	4.8 (0.46–49.6)	0.56	–

*p values by Fisher exact test except as indicated. ICU, intensive care unit; OR, odds ratio; CI, confidence interval; IQR, interquartile range; –, not applicable.
†Nonhospitalized patients + hospitalized patients not in ICU; n = 67.
‡Mann-Whitney U test.
§Pearson 2-sided χ^2 test.
¶High-risk conditions as defined by Centers for Disease Control and Prevention: <5 y or $\geq$65 y; pregnancy; immunosuppression; chronic pulmonary, cardiovascular, hepatic, hematologic, neurologic, neuromuscular, or metabolic disorders; long-term aspirin therapy in those $\leq$18 y of age.
 #Significant on multivariate analysis.
**Tetralogy of Fallot (1), patent ductus arteriosus status postmedical closure (1).
†† Renal transplant (2), liver transplant (1), heart transplant (1).
‡‡Percentage of female patients.

Conclusions

We sought to identify predictors of hospitalization in patients with confirmed pandemic (H1N1) 2009 infection. Univariate analysis showed that presence of high-risk medical conditions, age <5 years, dyspnea, and findings of tachypnea, hypoxia (SpO$_2$ $\leq$92%), chest radiograph infiltrate, and acute renal failure were significant risk factors for hospitalization. Notably, headache, rhinorrhea, sore throat, and cough were inversely associated with hospital admission. We hypothesize that treating physicians perceive these symptoms as more suggestive of upper respiratory tract disease and hence are less likely to hospitalize such patients.

Multivariate analysis showed that only a higher number of high-risk medical conditions (including age <5 years), dyspnea, and a lower median oxygen saturation level were predictive of hospitalization. We found that dyspnea and a low median oxygen saturation level were also associated with ICU admission. These findings suggest that clinicians' decision to hospitalize was not influenced by mere perception of illness severity, but rather it accurately reflected the risk for complicated or severe disease. Our findings are also

Table 2. Clinical characteristics of nonhospitalized and hospitalized patients who had pandemic (H1N1) 2009 infection, Rush University Medical Center, Chicago, Illinois, USA, April 29–June 22, 2009*

Characteristic	Nonhospitalized patients, n = 51	Hospitalized patients, n = 32	ICU patients, n = 16	Nonhospitalized vs. hospitalized patients		ICU vs. non–ICU patients†	
				p value	OR (95% CI)	p value	OR (95% CI)
Duration of ILI before evauulation, d, median (range)	2 (0–7)	3 (1–7)	3 (1–7)	0.15‡	–	0.20‡	–
Subjective fever, no. (%)	46 (90.2)	27 (84.4)	14 (87.5)	0.50	0.59 (0.16–2.2)	1.00	0.95 (0.18–5.0)
Headache, no. (%)	18 (35.3)	5 (15.6)	1 (6.3)	0.05§	0.34 (0.11–1.0)	0.03	0.14 (0.02–1.1)
Cough, no. (%)	50 (98.0)	25/31 (80.7)	14 (87.5)	0.01	0.08 (0.01–0.73)	0.62	0.57 (0.10–3.3)
Rhinorrhea, no. (%)	40 (78.4)	13/31 (41.9)	7 (43.8)	0.001§	0.20 (0.08-0.53)	0.05§	0.34 (0.11–1.0)
Sore throat, no. (%)	24 (47.1)	2/31 (6.5)	1 (6.3)	<0.0001§	0.08 (0.02–0.36)	0.02§	0.11 (0.01–0.88)
Myalgia, no. (%)	21 (41.2)	6/31 (19.4)	2 (12.5)	0.04§	0.34 (0.12–0.98)	0.053§	0.23 (0.05–1.1)
Dyspnea, no. (%)	2 (3.9)	15 (46.9)	11(68.8)	<0.0001§¶	21.6 (4.5–104.4)	<0.0001¶	22.4 (5.8–86.2)
Nausea/vomiting, no. (%)	14 (27.5)	9/31 (29.0)	4 (25.0)	0.88§	1.1 (0.40–2.9)	1.00	0.83 (0.24–2.9)
Obesity (BMI $\geq$30 or weight >95th percentile), no. (%)	12/37 (32.4)	11/22 (50.0)	4/10 (40.0)	0.18§	2.1 (0.71–6.2)	1.00	1.1 (0.26–4.2)
Tachypnea, no. (%)	9/49 (18.4)	16/31 (51.6)	10 (62.5)	0.002§	4.7 (1.7–13.0)	0.003§	5.4 (1.7–17.5)
O$_2$ saturation, % (range)	99 (96–100)	95 (65–100)	92 (65–100)	<0.0001‡¶	–	<0.0001‡¶	–
Hypoxia (SpO$_2$ $\leq$92%), no. (%)	0	10 (31.3)	9 (56.3)	<0.0001	–	<0.0001	84.9 (9.3–772.0)
Lymphopenia,# no. (%)	9/12 (75.0)	21 (65.6)	10 (62.5)	0.72	0.64 (0.14–2.80)	0.54§	0.67 (0.18–2.5)
Thrombocytopenia,# no. (%)	1/12 (8)	8 (25.0)	5 (31.3)	0.41	3.70 (0.41–33.00)	0.25	2.7 (0.6–12.2)
Acute renal failure,** no. (%)	0	5 (15.6)	4 (25.0)	0.007	–	0.004	22.0 (2.3–214.2)
Infiltrate on chest radiograph, no. (%)	0	11/29 (37.9)	11 (68.9)	0.001	–	<0.0001	–

*p values by Fisher exact test except as indicated. Values given as no./no. indicate number of patients for whom results were available (if less than total no. patients in category). ICU, intensive care unit; OR, odds ratio; CI, confidence interval; ILI, influenza-like illness; –, not applicable; BMI, body mass index; SpO$_2$, pulse oximeter oxygen saturation.
†Nonhospitalized patients + hospitalized patients not in ICU; n = 67.
‡Mann-Whitney U test.
§Pearson 2-sided χ^2 test.
¶Significant on multivariate analysis.
#Lymphopenia <1,500 lymphocytes/mm^3, thrombocytopenia <150,000 thrombocytes/mm^3.
**Assuming that patients who did not have biochemical testing did not have acute renal failure.

consistent with CDC alerts on emergency warning signs of pandemic (H1N1) 2009 influenza (8) and a recent report by Echevarría-Zuno et al. (9) from Mexico in which dyspnea, tachypnea, and cyanosis were prognostic factors for admission and death. Although the presence of any 1 underlying high-risk medical condition has been previously described as a risk factor for complication with seasonal influenza including hospitalization (10), a higher number of high-risk medical conditions is a stronger predictor of hospitalization (median number 2 vs. 0; p = 0.01).

Although excellent clinical prediction rules for hospitalization of patients with community-acquired pneumonia are available (e.g. CURB-65 or pneumonia severity index), few data exist for influenza admissions (10,11). We propose a simple clinical guide for hospitalization of patients with pandemic (H1N1) 2009 infection by using results of our multivariate analysis. The presence of any of 3 predictors—$\geq$2 high-risk medical conditions (including age <5 years), dyspnea, or hypoxia—has sensitivity, specificity, positive predictive value, and negative predictive value of 72%, 88%, 79%, and 83%, respectively, for hospitalization. Dyspnea or hypoxia was also predictive of ICU admission, with sensitivity, specificity, positive predictive value, and negative predictive value of 94%, 91%, 71%, 98%, respectively. Identification of these risk factors is widely applicable as a triage tool, especially in settings such as physician's offices where laboratory and radiologic data are not immediately available.

Dr Vasoo is a fellow in the Section of Infectious Diseases at Rush University Medical Center. His research interests include respiratory pathogens, diagnostic microbiology, and tropical infectious diseases.

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Imported Lassa Fever, Pennsylvania, USA, 2010

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We report a case of Lassa fever in a US traveler who visited rural Liberia, became ill while in country, sought medical care upon return to the United States, and subsequently had his illness laboratory confirmed. The patient recovered with supportive therapy. No secondary cases occurred.

Lassa fever is a potentially severe viral infection caused by *Lassa virus* (family *Arenaviridae*, genus *Arenavirus*), with an overall case-fatality rate of 1%–2% and a case-fatality rate of 15%–20% for hospitalized patients (*1,2*). The virus is endemic to West Africa, with the reservoir host being *Mastomys* spp. rodents (*3*). Person-to-person transmission of Lassa virus can occur through direct exposure to infected blood or secretions, and instances of nosocomial transmission have been documented (*4,5*). Primary symptoms of Lassa fever are fever, headache, nausea, diarrhea, sore throat, and myalgia; hemorrhagic signs or deafness may also occur during illness (*1,6*).

Because the incubation period ranges from a few days to >2 weeks (*1,5*) and many symptoms are nonspecific, the potential exists for human carriage of Lassa virus to areas outside those to which it is endemic, putting travel companions, close contacts, and healthcare providers at risk for secondary infection. Before 2010, five instances of imported Lassa virus were recorded in persons from West Africa to the United States. Although early instances involved sick persons who were airlifted to the United States for diagnosis and treatment (*7–9*), the 2 most recent occurrences (1989 and 2004) involved persons who were not identified as potentially infectious until healthcare was sought in the United States (*10,11*). Here we report a case in a person who became infected and sick during a trip to Liberia and sought care upon return to the United States.

The Case

A Liberian man 47 years of age living in the United States traveled to Liberia in January 2010. He arrived in Monrovia, then spent 5 days traveling throughout Nimba County in north-central Liberia, bordering Guinea and Côte d'Ivoire. He reported sleeping nightly in his rural native village in a dwelling infested with rats and recalled several rat carcasses on the bedroom floor. On the day of his departure from Liberia, he developed fever, chills, joint pain of the knees and ankles, anorexia, sore throat, diffuse skin tenderness, and mild shortness of breath; he began taking amoxicillin and chloroquine before departing Liberia.

The patient's symptoms persisted upon arrival in the United States, prompting him to seek medical attention on day 5 of his illness. When he sought treatment, he had fever of 103°F, pulse of 99 beats/min, respiratory rate of 15 breaths/min, and blood pressure of 120/80 mm Hg (online Appendix Table, www.cdc.gov/EID/content/16/10/1598-appT.htm). His physical examination was notable for posterior cervical adenopathy and a palpable spleen tip (Table). He had no evidence of conjunctival, nasal, or oral petechiae; no skin rashes; and no signs of hemorrhage or other lesions. Initial laboratory data showed leukopenia and thrombocytopenia and minimal transaminase elevations (online Appendix Table). Empiric malaria treatment was initiated upon admission and was subsequently discontinued when *Plasmodium* spp. antigen testing was negative and thick and thin blood smears showed no evident parasitemia. By the next day, mild pharyngitis with slight tonsillar exudates had developed. On the third hospital day, substernal chest pain and profuse watery diarrhea developed. Increasing transaminases and a slight coagulopathy were noted. Lassa fever was considered in the differential diagnosis; contact precautions and, subsequently, airborne precautions were taken. Because of noted clinical improvement, he was not given empiric intravenous ribavirin.

On day 5 of hospitalization, Lassa virus was identified by real-time PCR by using samples collected 2 days earlier, and sequencing of the amplified fragment yielded a unique sequence similar to sequences from previous Lassa virus isolates from Liberia. Subsequent samples confirmed Lassa fever diagnosis on the basis of real-time PCR, viral culture, and serology (Table).

The patient's fever resolved by day 16 of his illness. After 2 successive negative blood real-time PCR results, he was discharged from the hospital on day 21 of his illness with instructions to avoid unprotected sexual intercourse for 2 months. No hearing abnormalities were noted at the time of discharge or during telephone conversations 2 weeks and 2 months later.

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Table. Day by day symptoms and clinical information for a man 47 years of age with Lassa fever, Pennsylvania, USA, 2010*

Date	Symptoms	Examination findings	Bowel movements	Clinical information	Clinical action
Jan 13–17	Fevers and chills, sore throat, arthralgias, diffuse abdominal pain	NA	NA	NA	NA
Jan 18	Fevers and chills, sore throat, watery diarrhea, diffuse abdominal pain.	Prominent parotids, posterior cervical lymphadenopathy, slight spleen tip	12		Contact isolation ordered.
Jan 19	Fevers and chills, sore throat, substernal chest pain with inspiration and when lying supine, diarrhea, diffuse abdominal pain. Arthralgias resolve.	Slight tonsillar exudates, slight spleen tip	≈18	Tests for Clostridium difficile, cryptosporidium, Giardia spp., thick and thin blood smears for malaria, Epstein-Barr virus, and respiratory virus panel; all negative	Contact precautions. Blood samples to test for Lassa virus drawn.
Jan 20	Fevers and chills; sore throat; substernal chest pain, worse when lying down; diarrhea. Abdominal pain improving.	Slight tonsillar exudates, slight spleen tip	3	HIV negative	EKG done, ribavirin requested, airborne precautions
Jan 21	Fevers and chills, sore throat, diarrhea. Chest pain resolves. Abdominal pain improving.	Slight spleen tip	5	Stool culture negative	Held off on ribavirin. CDC received specimen.
Jan 22	Fevers and chills, sore throat. Diarrhea resolves. Abdominal pain resolves.	Prominent sternocleidomastoid muscles	NA		Stopped IV fluids.
Jan 23	Fevers and chills, sore throat	Prominent sternocleidomastoid muscles	3, formed	PCR positive for Lassa virus	
Jan 24	Fevers and chills, sore throat	Prominent sternocleidomastoid muscles	NA		
Jan 25	Fevers and chills. Sore throat improves.	Prominent sternocleidomastoid muscles	3, formed		
Jan 26	Fevers and chills. Sore throat improves.	Neck less prominent	NA		
Jan 27	Fevers and chills. Sore throat resolves.	Decreased parotid enlargement and lymphadenopathy	NA		
Jan 28–29	Fevers and chills		NA		
Jan 30–Feb 3	Fever resolves		NA		

*NA, not available; EKG, electrocardiogram; IV, intravenous; CDC, Centers for Disease Control and Prevention.

A contact investigation was undertaken by the hospital and local, state, federal, and international health agencies. Exposed persons were identified as any persons who potentially came into contact with the patient or his body fluids during his illness. Because no contacts had direct exposure to body fluids (other than the patient's wife in Africa with whom he had sexual intercourse before becoming ill and who remained well, according to telephone follow-up with the patient), no patient contacts were considered high risk for secondary transmission (*10*). In total, 140 persons, including the patient's family in the United States, co-workers, and hospital workers who had contact with him (but did not have direct contact with bodily fluids) were identified as low-risk contacts. Health communication materials were developed on the basis of previous Lassa fever contact tracing activities (*10*). All hospital and community contacts were provided a Lassa fever fact sheet and asked to seek medical consultation if fever or other signs and symptoms of Lassa fever appeared. Upon completion of 21 days of follow-up, no secondary cases were identified.

Conclusions

The spectrum of Lassa fever can run from asymptomatic seroconversion to severe hemorrhagic fever with multiple organ failure and death (*1,2,6*). Factors supporting the diagnosis of Lassa fever in returning travelers include relevant epidemiologic exposure (travel to rural West Africa), signs and symptoms consistent with Lassa fever, and the absence of other infectious agents that can account for the illness. Although this patient did not seek treatment for hemorrhagic signs, his fever, pharyngitis, chest pain, and diarrhea (*1,6*), as well as thrombocytopenia and elevated transaminases, were consistent with Lassa fever (*6,12*). Early institution of ribavirin can dramatically decrease

death rates among patients with severe Lassa fever if given within the first 6 days of illness (*12*); therefore, empiric ribavirin should be considered for an ill patient suspected of having Lassa fever.

As in this case, early suspicion of Lassa fever should prompt isolation measures to avoid secondary transmission; laboratory testing should be limited to essential tests, and all laboratory specimens should be handled with appropriate biosafety precautions to avoid aerosolizing the virus (*13*). Experience in regions where Lassa virus is endemic suggests human-to-human transmission occurs through direct contact with blood and body fluids or large-particle inhalation; transmission through viral aerosolization is not seen; and generally, when universal precautions are undertaken, transmission is unlikely (*14*). Nonetheless, aerosol in addition to contact precautions were undertaken once Lassa fever was suspected, given the theoretical potential for acquiring infection through inhalation of airborne virus from respiratory secretions or, in this case, copious diarrhea.

The patient described in this report represents the sixth known occurrence of Lassa fever imported to the United States. Clinicians treating recent travelers to West Africa who are febrile should obtain detailed histories from patients to determine whether they have traveled into rural areas in which the potential for exposure to rodents exists. The symptoms, signs, and laboratory abnormalities of Lassa fever are nonspecific and can overlap with other tropical infections. Therefore, efforts should be made to promptly diagnose or rule out other infectious agents in a patient who has the appropriate travel and exposure history so that further diagnostic studies and empiric therapy with ribavirin can be undertaken rapidly. Moreover, as soon as Lassa fever is suspected, patients and their specimens should be handled with adequate precautions, the local health department and Centers for Disease Control and Prevention should be notified, and specimens should be sent for specific diagnostic testing.

Acknowledgments

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Type 2 Diabetes Mellitus and Increased Risk for Malaria Infection

Ina Danquah, George Bedu-Addo, and Frank P. Mockenhaupt

A case–control study of 1,466 urban adults in Ghana found that patients with type 2 diabetes mellitus had a 46% increased risk for infection with *Plasmodium falciparum*. Increase in diabetes mellitus prevalence may put more persons at risk for malaria infection.

In sub-Saharan Africa, infectious diseases remain the predominant cause of illness and death. *Plasmodium falciparum* malaria alone causes an estimated 1 million deaths annually (*1*). At the same time, sub-Saharan Africa faces the world's highest increase in type 2 diabetes mellitus; adaptation to Western lifestyles and genetic predispositions may accelerate this trend (*2,3*). A decade ago, type 2 diabetes mellitus prevalence in urban Ghana was 6.3% (*4*). By 2030, ≈20 million affected persons may live in sub-Saharan Africa (*2*). Type 2 diabetes mellitus increases susceptibility to common infections (*5*). In sub-Saharan Africa, the emerging co-occurrence of type 2 diabetes mellitus and tropical infectious diseases thus may have substantial implications. We describe prevalence of malaria infection in adults with and without type 2 diabetes mellitus residing in Kumasi, Ghana. Malaria transmission in Kumasi is low but patchy; mosquito breeding sites also occur in urban agricultural areas (*6*).

The Study

A case–control study of risk factors for type 2 diabetes and hypertension was conducted from August 2007 through June 2008 at Komfo-Anokye Teaching Hospital, Kumasi, Ghana. The patients' clinical and biochemical signs and symptoms were secondary objectives (I. Danquah et al., unpub. data). The study protocol was approved by the Ethics Committee, University of Science and Technology, Kumasi, and participants gave informed written consent.

Patients attending the diabetes (n = 495) or hypertension center (n = 451) were recruited. These patients promoted participation as preliminary (i.e., to be confirmed) controls to community members, neighbors, and friends (n = 222). Further preliminary controls were recruited from the outpatient department (n = 150) and among hospital staff (n = 148).

Participants were told to fast, abstain from alcohol and nicotine use, and avoid stressful and physical activities beginning at 10:00 PM the day before examination. On the day of examination, participants were asked about medical history and socioeconomic background, underwent physical examination, and provided venous blood and urine samples for laboratory testing.

Fasting plasma glucose (hereafter referred to as glucose concentration; fluoride plasma at 4°C) and hemoglobin (Hb) concentrations were measured (Glucose-201+, B-Hemoglobin; HemoCue, Angelhom, Sweden). Irrespective of symptoms, malaria parasites were counted per 500 leukocytes on Giemsa-stained thick blood films. *Plasmodium* infection and species were ascertained by PCR that included positive and negative controls (*7*).

Patients with type 2 diabetes mellitus were defined as those receiving documented treatment with antidiabetes medication or having a glucose concentration ≥7 mmol/L (*8*); patients with hypertension were defined as those receiving documented antihypertension treatment or having mean blood pressure ≥140/90 mm Hg for 3 measurements (*9*). Controls had neither condition.

Between-group comparisons were performed by the Mann-Whitney U, χ^2, and Fisher exact tests. Logistic regression produced adjusted odds ratios (aORs), and 95% confidence intervals (CIs).

Of the 1,466 study participants, 675 (46%) had type 2 diabetes (Table 1). Among these, 655 (97.0%) received antidiabetes treatment, but 317 (47.0%) had increased glucose concentration (≥7 mmol/L). The 414 patients with hypertension but not diabetes and 377 controls with neither condition were grouped despite differences, e.g., in age and socioeconomic parameters (data not shown); however, glucose concentration was similar for the 2 groups (mean 4.51 vs. 4.56 mmol/L; p = 0.53).

According to microscopic examination, 13 (0.9%) of all participants had malaria parasites at low density (median 880/µL, range 80–4,960/µL). Reexamination by PCR showed that 206 (14.1%) were infected with *Plasmodium* spp., largely *P. falciparum* (189, 12.9%). Infected persons were afebrile, but mean hemoglobin was reduced (–0.4 g/dL; p = 0.004).

More *Plasmodium* spp. infections were observed in persons with type 2 diabetes mellitus than in those without the disease (Table 1); most infections were caused by *P. falciparum* (16% vs. 10%; p = 0.001). This difference was

Table 1. Demographic and clinical characteristics of 1,466 urban residents of Kumasi, Ghana, 2007–2008*

Characteristics	Persons with type 2 diabetes mellitus, n = 675	Persons without diabetes, n = 791	p value
Age, y, mean (range)	54.7 (18–92)	47.1 (18–100)	<0.0001
Male gender	171 (25.3)	182 (23.0)	0.299
Wealth score <25th percentile†	265 (39.6)	271 (34.3)	0.044
Illiteracy	308 (45.8)	206 (26.1)	<0.0001
Formal education, none	240 (35.7)	130 (16.5)	<0.0001
Crowded living condition‡	177 (26.7)	120 (15.3)	<0.0001
Smoking, current or quit	49 (7.3)	35 (4.4)	0.024
Akan ethnicity	592 (87.8)	685 (86.6)	0.480
Residence			
Kumasi metropolitan area	476 (70.8)	603 (76.2)	
Kumasi suburbs	174 (25.9)	162 (20.5)	
Elsewhere§	22 (3.3)	26 (3.3)	0.048
Occupation			
Public servant	44 (6.5)	194 (24.6)	
Trader	198 (29.5)	190 (24.1)	
Farmer	65 (9.7)	48 (6.1)	
Unemployed	248 (36.9)	138 (17.5)	
Other¶	117 (17.4)	218 (27.7)	<0.0001
FPG, mmol/L, mean (range)	8.3 (1.3–37.1)	4.5 (2.9–7.0)	<0.0001
Hemoglobin, g/dL, mean (range)	12.9 (5.8–19.1)	13.6 (4.9–19.1)	<0.0001
Fever, $\geq$37.5°C	2 (0.3)	4 (0.5)	0.693
History of fever, preceding week	95 (14.1)	93 (11.8)	0.182
Respiratory tract infection	5 (0.7)	11 (1.4)	0.232
Urinary tract infection#	14 (2.1)	7 (0.9)	0.076
Plasmodium spp. infection, by microscopy	5 (0.7)	8 (1.0)	0.582
Parasite density, per µL, median (range)	1,160 (160–2,480)	860 (80–4,960)	0.770
Plasmodium spp. infection, by PCR			
Plasmodium spp.	117 (17.4)	89 (11.3)	0.001
P. falciparum	108 (16.0)	81 (10.3)	0.001
P. malariae	14 (2.1)	9 (1.1)	0.205
P. ovale	8 (1.2)	7 (0.9)	0.611

*Values are no. (%) unless otherwise indicated. p values were calculated by Mann-Whitney U test or Fisher exact test, as applicable. FPG, fasting plasma glucose concentration.
†<25th percentile of a calculated index of 11 markers of wealth: electricity, pipe-borne water, radio, fan, cupboard, television, bicycle, motorbike, refrigerator, car/truck/tractor, cattle.
‡>75th percentile of the number of persons living in the household.
§Hinterland and environs.
¶Includes casual laborer, artisan, and others.
#By nitrite-positive urine dipstick test (Combur 10, Roche Diagnostics, Mannheim, Germany).

not attributable to recent antimalarial medication (7 persons with type 2 diabetes mellitus vs. 13 persons without type 2 diabetes mellitus; p = 0.32), and, notably, 74/524 (14.1%) of the patients with type 2 diabetes mellitus who took metformin-based drugs were infected compared with 34/131 (26.0%) of those who did not (p = 0.01). Among controls and patients with hypertension, the *P. falciparum* prevalence was similar (35/377, 9.3% for controls; 46/411, 11.2% for patients with hypertension; p = 0.38), and in each case, it was comparatively higher among patients with type 2 diabetes mellitus (p = 0.003 for controls; p = 0.03 for patients with hypertension).

Several factors that differed between persons with and those without diabetes mellitus (Table 1) were associated with *P. falciparum* infection (Table 2). However, age-adjusted multivariate analysis confirmed that the odds of *P. falciparum* infection in patients with type 2 diabetes mel-

litus were increased (aOR 1.46; Table 2). This risk increase was still discernible in the same model comparing patients with type 2 diabetes mellitus with controls (aOR 1.68, 95% CI 1.06–2.65; p = 0.027) or patients with hypertension (aOR 1.38, 95% CI 0.94–2.02; p = 0.096), or when separating into metropolitan area (aOR 1.67, 95% CI 1.12–2.48; p = 0.01) and other residence (aOR 1.32, 95% CI 0.76–2.29; p = 0.33).

According to the multivariate model, exchanging type 2 diabetes mellitus with glucose concentration showed that each mmol/L increase in blood glucose increased the risk for *P. falciparum* infection by 5% (aOR 1.05, 95% CI 1.02–1.09; p = 0.002). Among patients with type 2 diabetes mellitus, a stepwise approach identified 8.6 mmol/L glucose concentration as the significant threshold of risk increase (aOR 1.63, 95% CI 1.07–2.48; p = 0.02).

Conclusions

This study provides evidence for increased risk for *P. falciparum* infection in patients with type 2 diabetes mellitus (Table 2). Most infections were detected by PCR exclusively, and all were asymptomatic.

Submicroscopic and asymptomatic *P. falciparum* infections are common in areas where malaria is endemic. In adults, PCR may identify up to 50% of infections, although only a few infections are diagnosed by microscopy (*10*). These submicroscopic infections tend to increase in areas of low endemicity and with patient age (*10*).

An increased risk for *P. falciparum* infection in persons with diabetes mellitus might become clinically relevant (and microscopically detectable) under several conditions. The impact of semi-immunity on controlling parasitemia may weaken with advancing type 2 diabetes mellitus and immune dysfunction (*5*), as suggested by the observed risk increase with increasing glucose concentration. Conversely, children who lack semi-immunity but have more severe type 1 diabetes mellitus may be particularly prone to malaria. Such vulnerability is also conceivable for women with gestational diabetes whose immune

Table 2. Univariate and multivariate associations with *Plasmodium falciparum* infection, Kumasi, Ghana, 2007–2008*

Parameter	Total no. patients	*P. falciparum* infection, no. (%)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	p value	aOR (95% CI)	p value
Diabetes mellitus type 2						
No	791	81 (10.3)	1			
Yes	675	108 (16.0)	1.67 (1.22–2.27)	0.001	1.46 (1.06–2.03)	0.021
Gender						
F	1,113	124 (11.2)	1			
M	353	65 (18.5)	1.80 (1.29–2.50)	<0.0001	2.13 (1.50–3.03)	<0.0001
Wealth score						
≥25th percentile	923	94 (10.2)				
<25th percentile †	536	94 (17.6)	1.88 (1.38–2.56)	<0.0001	1.76 (1.27–2.42)	0.001
Literacy						
Able to read	947	103 (10.9)	1			
Unable to read	514	85 (16.6)	1.63 (1.20–2.23)	0.002	1.59 (1.11–2.28)	0.011
Formal education						
Any	1,091	126 (11.6)	1			
None	370	62 (16.8)	1.54 (1.11–2.15)	0.010		
Living condition						
Uncrowded	1,147	133 (11.6)	1			
Crowded‡	297	52 (17.5)	1.61 (1.14–2.29)	0.007		
Smoking						
Never	1,380	171 (12.4)	1			
Current or quit	84	18 (21.4)	1.92 (1.11–3.32)	0.019		
Ethnicity						
Akan	1,277	156 (12.3)	1			
Others	188	33 (17.6)	1.52 (1.01–2.30)	0.045		
Residence						
Kumasi metropolitan	1,079	121 (11.2)	1			
Kumasi outskirts	336	64 (19.2)	1.87 (1.34–2.61)	<0.0001		
Elsewhere §	48	4 (8.3)	0.72 (0.25–2.03)	0.533		
Occupation						
Public servant	238	17 (7.1)	1			
Trader	388	50 (12.9)	1.92 (1.08–3.42)	0.026		
Farmer	113	34 (30.6)	5.74 (3.04–10.86)	<0.0001		
Other¶	335	38 (11.3)	1.66 (0.92–3.02)	0.095		
Unemployed	386	49 (12.8)	1.90 (1.07–3.39)	0.029		

*OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio. Age and gender were a priori included in the multivariate model. Further variables for inclusion in the model were identified by factor analysis excluding multicollinear parameters (1: retained diabetes, excluded occupation; 2: retained literacy, excluded education, smoking; 3: retained wealth, excluded living condition, ethnicity). The same model results from a logistic regression analysis initially including all above listed parameters, and then removing in a stepwise backward fashion all factors not associated with *P. falciparum* infection in multivariate analysis (p > 0.05). Inserting any of the excluded variables back into the model did not change the aOR of patients with type 2 diabetes mellitus by >7% each, suggesting the absence of substantial confounding. Leaving all parameters in the model yielded an aOR for patients with type 2 diabetes mellitus of 1.36 (95% CI, 0.98–1.90; p = 0.07). Alternatively, propensity score adjustment of that analysis, i.e. reducing covariates into a single variable, produced aOR = 1.41 (95% CI, 1.02–1.95; p = 0.04).
†<25th percentile of a calculated index of 11 markers of wealth.
‡Crowded living condition, >75th percentile of the number of persons living in the household, i.e., n>8.
§Hinterland and environs.
¶Includes casual labourer, artisan, and others.

systems are relatively naive with regard to pregnancy-specific *P. falciparum* (*11*). Moreover, low-level infections in patients with type 2 diabetes mellitus may constitute an unrecognized infectious reservoir in areas where malaria is endemic (*10*). The lowered *P. falciparum* prevalence under metformin medication accords with the biguanides' antimalarial efficacy (*12*).

Our data stem from a study that was not designed to assess influences on *P. falciparum* infection in a heterogeneous population. Multivariate analysis cannot exclude unmeasured confounders, and association does not mean causality. As a limitation, factors influencing infection were not specifically identified during recruitment and thus were not included in analysis. Also, despite adjusting for proxy indicators, e.g., wealth, exposure to infection might still have differed between the study groups, considering the patchy malaria transmission in Kumasi (*6*). Nonetheless, increased odds of *P. falciparum* in patients with type 2 diabetes mellitus were found after stratification by subgroups or residence. Ultimate corroboration would need a prospective, longitudinal study controlling for exposure (possibly monitored by serologic markers of transmission).

Although the actual reasons for the increase of *P. falciparum* infection are unclear, the risk increase with rising glucose concentration is a sign of biologic plausibility. Such risk could result from impaired defense against liver and/or blood-stage parasites and from prolonged persistence. In type 2 diabetes mellitus, decreased T cell–mediated immunity but limited impact on humoral responses are discussed (*5*). Mechanistically, increased glucose availability may feed *P. falciparum* growth as seen in vitro (*13*). Also, patients with diabetes might receive more infectious mosquito bites: olfactory signals mediate mosquito attraction (*14*), and these, including expiration, are subtly altered in persons with type 2 diabetes mellitus (*15*).

The rapid proliferation of type 2 diabetes mellitus in sub-Saharan Africa may put an increasing number of persons at risk for *Plasmodium* infection and malaria. Thus, the magnitude of both diabetes mellitus and malaria in sub-Saharan Africa warrants further investigation into the relevance and causes of our finding

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Epidemiology of Human Parvovirus 4 Infection in Sub-Saharan Africa

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Human parvovirus 4 infections are primarily associated with parenteral exposure in western countries. By ELISA, we demonstrate frequent seropositivity for antibody to parvovirus 4 viral protein 2 among adult populations throughout sub-Saharan Africa (Burkina Faso, 37%; Cameroon, 25%; Democratic Republic of the Congo, 35%; South Africa, 20%), which implies existence of alternative transmission routes.

Human parvovirus 4 (PARV4) was originally detected in plasma from a person at risk for infection with HIV through injection drug use (*1*). Genetic characterization of the complete genome sequence of the virus showed a distant relationship to existing genera within the family *Parvoviridae*, although viruses showing 61%–63% sequence similarity to PARV4 have recently been described in pigs and cows (*2*), together likely meriting the designation of

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a new genus within the family. Although infections with PARV4 are not followed by long-term viremia, viral DNA sequences can likely be detected in tissues lifelong after exposure (*3–6*), a form of latency or persistence shared with other human parvoviruses, e.g., human parvovirus B19, and adeno-associated viruses (*6–8*).

PARV4 differs strikingly from other parvoviruses in its epidemiologic associations and inferred routes of transmission. Initial studies of autopsy tissue demonstrated high DNA detection frequencies among injection drug users co-infected with hepatitis C virus (HCV) in the United Kingdom, Italy, and Germany (*3–5,9*). Infection frequencies were higher in those who were HIV seropositive but almost absent in low-risk, HCV-negative and HIV-negative control populations. Despite these new insights, studies based on autopsy or biopsy tissues are cumbersome and necessarily limited by sample availability and technical complexity.

The Study

To address gaps in knowledge about PARV4, we have recently developed an ELISA for antibodies to the viral protein 2 (VP2) of PARV4 genotype 1 to expand investigations of epidemiology and transmission of the virus (*10*). Larger scale screening confirmed the previously noted association between PARV4 infection and parenteral routes of exposure (injection drug use) in the United Kingdom and United States, much lower infection frequencies in HIV-infected gay men, and zero seropositivity in low-risk controls. We additionally found serologic evidence for high rates of PARV4 exposure among persons with hemophilia exposed to nonvirally inactivated factor VIII/IX concentrates but a virtual absence of infection in sibling controls occupying the same households.

To investigate further the epidemiology of PARV4 in sub-Saharan Africa, we assembled large sets of serum or plasma samples collected from a range of adult populations in several countries in Africa (Table). Samples were screened in duplicate by our previously described ELISA (*10*) by using protein purified in parallel from empty baculovirus constructs as control antigen to minimize assay nonspecificity. Low-risk orthopedic outpatient attendees (United Kingdom) and HIV-negative and HCV-negative nonremunerated blood donors (France) were used as negative control populations.

Serologic screening for PARV4 antibodies showed that the combined set of 360 blood donor and control samples from the United Kingdom and France were nonreactive by ELISA (Table). In marked contrast, high rates of anti-PARV4 reactivity were detected in populations from sub-Saharan Africa. The highest rates were observed in Burkina Faso, where a frequency of 37% was recorded among a screened HIV-negative and HCV-negative blood

Table. Seroprevalence of human parvovirus 4 antibodies in sub-Saharan African and control populations*

Country	Category	No.	Co-infection HIV†	Co-infection HCV‡	Mean year of birth (range)	Collection year	PARV4 positive, no. (%)
Burkina Faso	Blood donors	167	0	0	1982 (1951–1999)	2007	62 (37.1)
Cameroon	General population	238	0	0	1968 (1962–1972)	2007	59 (24.8)
Democratic Republic of the Congo	Military population	221	2§	0	1968 (1936–1986)	2007	78 (35.3)
South Africa	Blood donors (HIV-positive)	170	170	0	1976 (1945–1990)	2007	62 (36.4)
South Africa	Blood donors (HIV-negative)	180	0	0	NA	2009	8 (4.4)
United Kingdom	General population	161	ND	ND	1950 (1937–1977)	2005	0
France	Blood donors	199	0	0	1965 (1943–1989)	2008	0

*HCV, hepatitis C virus; PARV4, human parvovirus 4; NA, not available; ND, screening not done.
†HIV-1 screening methods: South Africa, France: fourth-generation ELISA.
‡HCV screening methods: Burkina-Faso, South Africa, France: third-generation ELISA, recombinant immunoblot assay confirmation of positive results; Democratic Republic of the Congo: third-generation ELISA, exclusion of reactive samples; Cameroon: PCR-based screening, exclusion of PCR-positive samples. United Kingdom: not screened (ND), low risk background and absence of parenteral or HIV risk factors.
§1 of 2 HIV-positive samples was seropositive for PARV4.

donor population. Frequencies of seropositivity were 25% and 35% in Cameroon and Democratic Republic of the Congo, respectively, and lowest in South Africa (4% in HIV-negative persons). However, within the latter group, HIV-1–infected donors were significantly more frequently seropositive for PARV4 than those who were not infected with HIV (36%; p<0.0001 by Fisher exact test). However, even with this risk factor, the overall prevalence was not as high as observed in the HIV-negative blood donors in Burkina Faso.

Conclusions

These findings provide new and unexpected information on the epidemiology and transmission of PARV4. First, although there is no evidence of PARV4 infection in nonpotentially exposed persons in Western countries (from the limited number currently surveyed), populations in sub-Saharan Africa, particularly in Central Africa, show high rates of exposure that cannot plausibly be accounted for by parenteral exposure. For example, the highest rate of seropositivity was observed among blood donors in Burkina Faso and Democratic Republic of the Congo who were uniformly negative for HCV antibodies, as well as for HIV-1 antibodies, by third-generation screening. In this setting, HCV infections are a frequent correlate of multiple blood transfusions and use of unsterilized needles in medical treatment or vaccination, as well as injection drug use. The high rate of seropositivity among HCV screen-negative samples from all 4 countries in Africa provides strong evidence for an alternative route of PARV4 transmission that is largely or entirely absent in Western countries.

These findings are consistent with PCR-based evidence for PARV4 viremia, presumably associated with acute infection, among young children in rural Ghana (11), a country adjacent to Burkina Faso where similar conditions for virus transmission may exist. In this study group, parenteral exposure was not identified, although infections were more frequent in families in low socioeconomic groups and those living near rivers and without a domestic water supply. In a separate study, autopsy samples from 2 HIV-infected African men (from Nigeria and Democratic Republic of the Congo) were PARV4 positive, despite not being infected with HCV and without a history of parenteral exposure (12). Neither study identified the specific risk factors and PARV4 infection sources.

It clearly is a challenge to conceive transmission routes for a virus that will not be directly transmitted among members of the same household, as demonstrated by the hemophiliac sibling data for the United States (10). Hypotheses such as possible arthropod-borne or parasite-associated transmission require careful evaluation, bearing in mind that parvoviruses, in common with other DNA viruses, are highly host species specific, and no other instances of vector-borne transmission in this virus family have been recorded.

The second major observation was a substantial difference in the rate of PARV4 seropositivity between those who were HIV infected and those uninfected (in South Africa, 36% and 4%, respectively). This association with HIV appears initially consistent with higher frequencies of PARV4 seropositivity reported among HIV (and HCV) co-infected injection drug users in Western countries. However, in the latter group, the association with HIV-1 was thought to reflect a greater frequency of illicit injection with shared needles (4,9). Furthermore, low frequencies or absence of PARV4 infections were observed in HCV-uninfected persons acquiring HIV-1 infection by sexual contact. How HIV-1 facilitates or becomes epidemiologically associated with PARV4 infection in HCV-negative South African blood donors thus remains unexplained in a population where HIV-1 infections are primarily acquired through sexual contact.

Although this study leaves many questions on the transmission of PARV4 unanswered, the striking differences in seroprevalence and risk-group associations between sub-Saharan Africa and Western countries provides the basis for future more detailed investigations of its transmission routes, epidemiology, and potential clinical outcomes of infections. The previously noted sequence homogeneity of PARV4 nucleotide sequences between variants detected in Western countries (4,13) is consistent with its recent global spread. A possible source in sub-Saharan Africa for PARV4 would contain many potential parallels with the emergence and global spread of HIV-1 and HCV in the 20th century.

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Artesunate Misuse and *Plasmodium falciparum* Malaria in Traveler Returning from Africa

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Plasmodium falciparum malaria developed in an African-born traveler who returned to Canada after visiting Nigeria. While there, she took artesunate prophylactically. Isolates had an elevated 50% inhibitory concentration to artemisinin, artesunate, and artemether, compared with that of other African isolates. Inappropriate use of artemisinin derivatives can reduce *P. falciparum* susceptibility.

Artemisinin derivatives were recently approved by the Food and Drug Administration for the treatment of *Plasmodium falciparum* malaria in North America and are available through the US Centers for Disease Control and Prevention and through Health Canada (*1–3*). Artemisinin-based combination therapy (ACT) remains the most effective therapy for *P. falciparum* malaria throughout the world, with the possible exception of the Thailand–Cambodia border (*4*). Because of the large numbers in the Toronto area of returning travelers and recent immigrants who have returned to countries of origin and visited friends and relatives, the Public Health Laboratory (Toronto) identifies ≈200 positive malaria smears annually; most *P. falciparum* isolates have come from sub-Saharan Africa. Evidence has indicated that such travelers tend not to seek medical advice before travel and are therefore at high risk of acquiring malaria (*5*).

The Patient

A 38-year-old Nigerian-born woman, who lived in the Toronto area (and has a good ability to recount her experiences), returned to Lagos, Nigeria, for a visit in January 2009. She did not seek pretravel advice. On arrival in Lagos, the woman purchased artesunate locally and began

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taking two 50-mg tablets weekly for the 4 weeks of her visit. Immediately on her return to Toronto, the patient experienced myalgia, nausea with vomiting, and chills, ≈7 days after she had taken her last dose of oral artesunate. She sought treatment at the emergency department of a community hospital. Physical examination showed that her temperature was 39.1°C and that she was dehydrated. Laboratory tests showed the following: leukocyte count 3,700 cells/µL, thrombocyte count 72×10^3 cells/µL, hemoglobin level 12.7 g/dL. Her chest radiograph showed that her lungs were clear. An examination of peripheral blood by thick and thin blood films showed a 0.7% parasitemia with *P. falciparum*. Her condition was treated with 1,250 mg of oral mefloquine as a single dose. She was treated as an outpatient, and she reported that symptoms promptly resolved over the next 48 hours without side effects.

A blood specimen was placed into culture in the Public Health Laboratory (Toronto), and the *P. falciparum* isolate was tested for drug susceptibility (*6*). The 50% inhibitory concentration (IC_{50}) was the following for certain antimicrobial agents (tested in triplicate): chloroquine 170.5 ± 7.8 nmol/L, mefloquine 16.6 ± 0.7 nmol/L, artemisinin 20.1 ± 0.6 nmol/L, artesunate 6.2 ± 1.4 nmol/L, dihydroartemisinin 1.8 ± 0.9 nmol/L, and artemether 21.4 ± 5.3 nmol/L. For this *P. falciparum* isolate, IC_{50} was significantly higher for artemisinin, artesunate, and artemether than for other representative *P. falciparum* isolates imported from Africa (Figure). Because of the short half-life of artesunate, the weekly doses of the oral drug may have led to development of a resistant strain when the patient was in Nigeria. Artesunate-containing drugs therefore should not be used for prophylaxis or single drug therapy. The purchased artesunate may also have been counterfeit and may have contained lower levels of active drug. Although these data suggest that this isolate has reduced susceptibility to artemisinin derivatives, the correlation between in vitro susceptibility and treatment outcomes does not appear to be consistent (*4*).

Previous studies have reported that resistance to artemisinin is mediated by an increase in gene copy number, mutations within the efflux pump of the *P. falciparum* multidrug resistance 1 (*pfmdr1*) gene, or mutations in the calcium transporter *pfATPase6* (*7,8*). When we examined each gene, using a combination of real-time PCR and DNA sequencing, we found that *pfmdr1* copy number was elevated in this isolate relative to that of the susceptible control strain 3D7. We also observed nonsynonymous mutations in both *pfmdr1* (Y184F) and *pfATPase6* (A623E, S769N), whereas other implicated residues remained in the wild-type form (*9*) (Table). Similar molecular analysis of other representative imported African clinical isolates demonstrated variable mutations for *pfmdr1* and *pfATPase6* and copy number in relation to IC_{50} values for key drugs (Table). A trend,
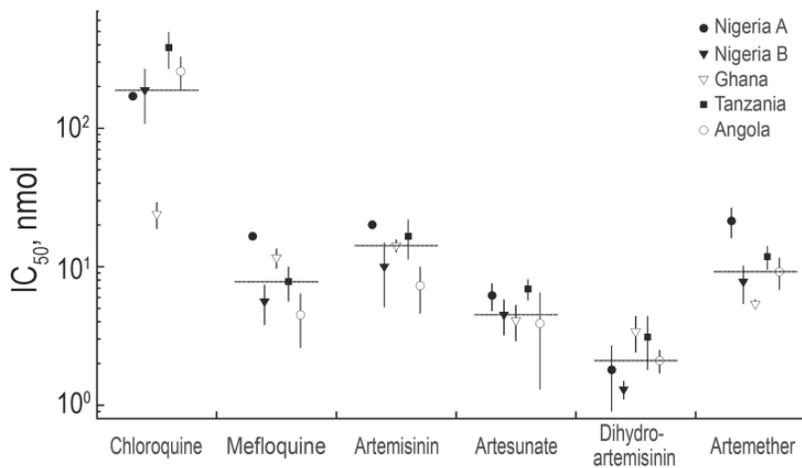
Figure. In vitro drug susceptibility of representative patient isolates from returning travelers who visited friends and relatives in Africa. The mean 50% inhibitory concentrations (IC_{50}) of chloroquine, mefloquine, artemisinin, artesunate, dihydroartemisinin, and artemether are plotted in nmol/L for each isolate, performed in triplicate (error bars indicate SD; n = 3). Nigeria A denotes the patient described in this report. The black horizontal line represents the median value.

albeit weak, was observed in which increased *pfmdr1* copy number was correlated with an elevated IC_{50} to mefloquine ($r = 0.52$) and artemisinin ($r = 0.42$). The presence of an asparagine (N) at position 86 of Pfmdr1, when coupled to an elevated *pfmdr1* copy number, appeared to correlate well with reduced susceptibility to artemisinin (Table). Chavchich et al. recently demonstrated that increased *pfmdr1* copy number occurred in a laboratory strain placed under drug selection pressure with artemisinin derivatives (*11*). However, Imwong et al. have indicated that genetic polymorphisms and copy number in *pfmdr1* do not predict treatment outcome with ACT (*10*).

Findings in the published literature vary in terms of use of artemisinin derivatives for in vitro drug susceptibility testing. Jambou et al. reported treatment failures with ACT in Cambodia, French Guiana, and Senegal (*8*). These authors used artemether for testing and showed IC_{50} values of ≈30 nmol/L in their "resistant" isolates from Senegal. Noedl et al. described treatment failures with ACT in Cambodia, for which IC_{50} values to dihydroartemisinin

were ≈10 nmol/L (*12*). Dondorp et al. showed IC_{50} values of 4–6 nmol/L to dihydroartemisinin and 6–8 nmol/L to artesunate in a region of Cambodia and Thailand where ACT treatment failures have occurred (*4*). Systematic molecular surveillance and standardized drug-testing methods with clinical isolates are required to establish the molecular correlates of reduced susceptibility to antimalarial drugs. In this regard, efforts are ongoing under the auspices of the Worldwide Antimalarial Research Network (*13*).

Conclusions

The patient's infection responded to mefloquine when she was back in Canada, possibly because of the high oral dose of mefloquine. Current guidelines from the US Centers for Disease Control and Prevention recommend quinine sulfate plus doxycycline, tetracycline, or clindamycin; or atovaquone-proguanil (Malarone; GlaxoSmithKline, Mississauga, Ontario, Canada) as first- and second-line treatment for uncomplicated *P. falciparum* malaria. Reduced susceptibility to artesunate is more likely to occur when it

Table. Results of sequencing single-nucleotide polymorphisms of *Plasmodium falciparum* isolate*

Strain	Pfmdr1					PfATPase		*pfmdr1* copy no.	CQ IC_{50}, nmol/L	MQ IC_{50}, nmol/L	ART IC_{50}, nmol/L
	86	184	1034	1042	1246	623	769				
3D7	N	Y	S	N	D	A	S	1.00	6.1	2.1	6.1
W2	Y	Y	S	N	D	A	S	0.97	252	3.2	7.3
Cameroon	Y	F	S	N	D	E	N	1.85	163	7.7	8.07
Congo	Y	F	S	N	D	E	N	1.51	355	10.7	10.9
Kenya	Y	F	S	N	D	E	N	1.75	282	11.7	10.1
Liberia	N	F	S	N	D	E	N	1.65	109	16.2	16.5
Nigeria C	Y	F	S	N	D	E	N	1.06	222	8.7	8.1
Nigeria A	N	F	S	N	D	E	N	1.52	171	16.6	20.1
Nigeria B	Y	F	S	N	D	E	N	1.09	188	5.6	10.0
Ghana	N	F	S	N	D	E	N	0.96	24.0	11.6	14.2
Tanzania	Y	F	S	N	Y	E	N	1.88	381	7.8	16.6
Angola	Y	F	S	N	D	E	N	0.81	258	4.5	7.3

*At Pfmdr1 and PfATPase6 residues previously implicated in artemisinin resistance and gene copy number of *pfmdr1* by quantitative real-time PCR in relation to mean IC_{50} (n = 3) data for key drugs (*7,8,10*). pfmdr1, *P. falciparum* multidrug resistance 1; CQ, chloroquine; MQ, mefloquine; ART, artemisinin; IC_{50}, 50% minimum inhibitory concentration; N, asparagine; Y, tyrosine; S, serine; D, aspartic acid; A, alanine; E, glutamic acid; 3D7, chloroquine-sensitive laboratory strain; W2, chloroquine-resistant laboratory strain; Nigeria A, clinical isolate described in this report.†

is associated with inappropriate use of artemisinin derivatives than because of circulating artemisinin-resistant *P. falciparum* in sub-Saharan Africa.

In an effort to achieve consensus that artesunate oral monotherapies should not be marketed, the World Health Organization convened the international pharmaceutical sector in April 2006. At that time, 15 companies agreed to cease manufacturing artesunate monotherapies. However, oral artesunate montherapies may still be purchased over the counter in malaria-endemic countries, as this report shows. Thus, strains of *P. falciparum* malaria are currently at risk of developing reduced susceptibility to artesunate derivatives.

Acknowledgments

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Severe *Plasmodium vivax* Malaria, Brazilian Amazon

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We describe a case series of 17 patients hospitalized in Manaus (western Brazilian Amazon) with PCR-confirmed *Plasmodium vivax* infection who were treated with chloroquine and primaquine. The major complications were jaundice and severe anemia. No in vivo chloroquine resistance was detected. These data help characterize the clinical profile of severe *P. vivax* malaria in Latin America.

During 2000–2007, in Latin America, a total of 7,554,993 cases of malaria were recorded; 5,507,167 (72.9%) of these cases were caused by *Plasmodium vivax* parasites. Of the *P. vivax* malaria cases, 3,833,477 were reported in Brazil, mainly in the Amazon Region (*1*). Official data from the Brazilian Ministry of Health identify Manaus as one of the leading cities in terms of number of *P. vivax* malaria cases in Latin America (*2*).

Manaus (population 1,738,641 in 2009), the capital of the state of Amazonas in the western Brazilian Amazon, is clearly part of a new frontier in the economic development of the Amazon. In 2009, a total of 19,698 cases of malaria were reported in Manaus (annual parasitary index 11.3/1,000 population; 92.6% caused by *P. vivax*). Since the mid-1990s, as *P. vivax* was becoming the predominant malaria species in Brazil (*3*), severe cases and even deaths attributable to *P. vivax* infection have been reported anecdotally (*3*). A concomitant trend of increased hospitalization of *P. vivax*–infected patients was seen in a Manaus tertiary care center (*4*).

In 2000, one of the authors noted the increased clinical severity of *P. vivax* cases seen in this same hospital; the frequency of hospitalization was very similar to that of pa-

tients infected with *P. falciparum* (M.G.C.A., unpub. data). Other reports from the same reference center in Manaus have been published regarding unusual complications of *P. vivax* infection, such as severe rhabdomyolysis (*5*) and immune thrombocytopenic purpura (*6*). At the same time, a cascade of reports from areas where *P. vivax* malaria is highly endemic confirmed the clinical severity of the infections (*7*). However, to date, data are lacking on the distribution of severe *P. vivax* malaria, the relationship of patient age, and the identification of possible risk factors. This study describes the clinical features of *P. vivax* malaria in a case series of patients who were hospitalized in a tertiary care unit in the Brazilian Amazon and their clinical response to treatment with chloroquine.

The Study

The Tropical Medicine Foundation of Amazonas is a tertiary care center for infectious diseases in Manaus (3°8′S, 60°1′W). In 2001 and 2002, a total of 13,056 cases of malaria were diagnosed in this institution (11,251 *P. vivax*), representing 65.1% of the total cases from Manaus. During the same period, 358 (3.2%) patients with *P. vivax* malaria were hospitalized. A retrospective analysis was performed of case-patients who fulfilled the malaria severity criteria of the World Health Organization (WHO) (*8*). These patients had an exclusive diagnosis of *P. vivax* malaria by thick blood smear (reviewed 2 times by experienced microscopists) and PCR, according to the technique described elsewhere (*9*).

PCR was performed on whole blood from all patients with *P. vivax* malaria, confirmed by microscopy and any *P. falciparum* severity criterion recommended by WHO. Blood specimens were routinely stored by the laboratory of the institution at –70°C. Full clinical information was available from the patients' charts, and serologic tests for dengue virus, *Leptospira* spp., and hepatitis A, B, and C viruses were performed on available serum samples stored at –20°C.

Each patient was monitored for 28 days in outpatient clinics after beginning antimalarial treatment. Patients were routinely discharged only after parasitologic clearance and clinical recovery. Until 2006, chloroquine was still prescribed for patients with severe cases at a dose of 10 mg/kg on the first day and 7.5 mg/kg on the second and third days, followed by primaquine (0.5 mg/kg/day for 7 days), according to the Brazilian Ministry of Health guidelines. In 2006, WHO formally recommended the treatment of severe vivax malaria to be the same as that for severe falciparum malaria, because of the risk for an unrecognized mixed infection (*8*).

Seventeen patients were included in the analysis, and their clinical and laboratory data are shown in Tables 1 and 2, respectively. All patients received chloroquine (orally or

Table 1. Clinical characteristics of 17 hospitalized patients who had parasitologic and molecular diagnosis of *Plasmodium vivax* infection, Manaus, Brazil, 2001–2002*

Patient no.	Year	Age/sex	WHO severity criterion	Duration of disease, d	Antimicrobial drug use	Erythrocyte transfusion	Concurrent condition	ICU	Death
1	2001	2 y/F	Severe anemia†	4	No	Yes	–	No	No
2	2001	9 mo/M	Severe anemia†	10	No	Yes	–	No	No
3	2001	3 y/F	Jaundice‡	3	No	No	HAV	No	No
4	2001	60 y/F	Acute renal failure§	7	No	No	Arterial hypertension	No	No
5	2001	1 mo/M	Severe anemia†	3	No	Yes	–	No	No
6	2001	40 y/M	Jaundice‡	9	No	No	–	No	No
7	2001	80 y/M	Acute renal failure§	6	No	No	–	No	No
8	2002	7 y/M	Hemoglobinuria/ jaundice‡	4	No	Yes	–	No	No
9	2002	5 mo/M	Severe anemia†/ ARDS	3	No	Yes	–	No	No
10	2002	28 d/M	Jaundice‡	5	No	No	–	No	No
11	2002	46 y/F	Severe anemia†/ ARDS	5	No	Yes	–	Yes	Yes
12	2002	48 y/F	Jaundice‡	7	No	No	–	No	No
13	2002	58 y/M	Jaundice‡	12	No	No	Diabetes	No	No
14	2002	47 y/F	Jaundice‡	8	No	No	–	No	No
15	2002	43 y/F	Shock¶/jaundice	4	Yes	Yes	–	Yes	No
16	2002	34 y/M	Jaundice	10	No	No	–	No	No
17	2002	50 y/M	Jaundice	7	No	No	–	No	No

*WHO, World Health Organization; ICU, intensive care unit; HAV, hepatitis A virus; ARDS, acute respiratory distress syndrome (tachypnea, shortness of breath, and signs of hypoxemia).
†Hemoglobin <7 g/dL in adults and <5 g/dL in children.
‡Total bilirubin >3.0 mg/dL.
§Creatinine >3.0 mg/dL.
¶Systolic arterial tension <80 mm Hg despite fluid therapy.

through a nasogastric tube) and primaquine. Acute respiratory distress syndrome (ARDS) (diffuse interstitial and alveolar infiltrate by chest radiograph and partial O_2 pressure 40 mm Hg by arterial gas analysis) developed in patient 11 two days after she received chloroquine, and she died

3 days later. This patient had a negative thick blood smear from day 3 of treatment with choloroquine. The other 16 patients were followed up after discharge until day 28. None had clinical symptoms of malaria, and all thick blood smears were negative at days 7, 14, and 28.

Table 2. Laboratory characteristics of 17 hospitalized patients who had parasitologic and molecular diagnosis of *Plasmodium vivax* infection, Manaus, Brazil, 2001–2002*

Patient no.	No. asexual parasites/mm³	Hemoglobin, g/dL	Total leukocyte count, cells/mm³	Thrombocytes, cells/mm³	Serum creatinine, mg/dL	Serum bilirubin, total/conjugated, mg/dL	Serum AST, IU/L	Serum ALT, IU/L
1	7,566	3.6	9,700	97,000	0.5	1.1/0.4	50	30
2	28,847	4.5	9,100	30,000	0.6	1.3/0.5	40	34
3	1,100	9.5	5,500	143,000	0.4	6.6/5.6	760	1,233
4	21,406	14.6	13,900	33,000	3.0	2.58/1.16	113	108
5	1,862	3.1	19,000	99,000	0.7	1.1/0.8	23	56
6	1,206	11.5	6,700	27,000	1.2	13.8/10.6	76	60
7	2,695	12.4	4,900	76,000	3.7	1.3/0.5	33	33
8	2,055	3.8	13,700	106,000	0.6	4.3/0.73	190	37
9	3,844	4.5	6,200	171,000	0.3	1.36/0.21	58	25
10	680	9.6	13,600	275,000	0.1	6.4/4.8	72	50
11	5,452	6.5	4,700	107,000	1.7	2.5/1.6	57	56
12	5,047	11.5	4,900	35,000	0.9	6.3/5.1	49	34
13	11,954	9.4	8,600	48,000	1.0	5.4/5.2	45	43
14	9,360	12.0	5,200	27,000	1.2	9.2/7.1	57	53
15	4,524	8.8	5,800	29,000	1.0	5.6/4.8	33	30
16	5,160	10.7	6,300	36,000	2.3	7.8/6.5	35	70
17	24,550	10.3	5,000	24,000	1.4	7.1/4.6	39	55

*WHO, World Health Organization; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

The patients in whom complications developed exhibited a remarkably wide age range (28 days–80 years). This age range is similar to that seen in other case series from Latin America, such as in hospitalized children from Venezuela with severe anemia that required blood transfusions (*10*) and in adults from Rondônia (a state in the western Brazilian Amazon) who had severe anemia, jaundice, acute renal failure, ARDS, and shock (*11*). *P. vivax* malaria with ARDS has been reported in travelers who acquired the infection in Manaus (*12,13*). The finding of severe anemia in 4 of 7 children highlights the relevance of this complication in *P. vivax* infection (Figure), as shown in a prospective study from Papua, Indonesia (*14*). Nine patients sought treatment for cholestatic jaundice; for 8, jaundice was the only complication. The mechanisms involved are unknown.

The concomitant diagnosis of hepatitis A virus infection in patient 3 (Table 1) indicates that other infectious diseases should be excluded when characterizing severe *P. vivax* malaria. Also of note is the presence of thrombocytopenia in 15/17 patients (none had clinical bleeding), which suggests that this hematologic complication may be a surrogate marker of severity.

Conclusions

The wide range of parasitemia found in our patients does not enable us to comment on the value of this variable as a determinant of severity. Sixteen patients recovered without the use of antimicrobial drugs; therefore, it is highly improbable that bacterial sepsis was a factor for severity in our case series. In unstable transmission areas (<0.1 autochthonous case per 1,000 persons per year), malaria in older patients may pose an additional problem because chronic diseases (e.g., arterial hypertension and diabetes) may predispose a patient to clinical decompensation.

In vivo chloroquine resistance was not detected in any of the cases that were followed up, despite recent confirmation of the phenomenon in this same locality (*15*). Because a reliable molecular marker of chloroquine resistance is lacking and parenteral artemisinin derivatives are recommended for treatment of patients with severe *P. vivax* malaria, studies that assess clinical severity and chloroquine resistance would be unethical. However, our findings suggest that chloroquine resistance would be a problem for individual patients and that the determinants of this resistance need to be clarified. Clearly, areas with chloroquine-resistant *P. vivax* also report severe *P. vivax* malaria, but we believe that these studies are not able to establish any firm causality. The finding of both phenomena in some areas may simply reflect high transmission of this species.

Our retrospective review illustrates the spectrum of severe *P. vivax* malaria in Manaus, and these results parallel the increasing clinical severity described in malaria-

Figure. Hand of a 2-year-old child (patient no. 1) with severe anemia (hemoglobin level 3.6 g/dL), showing intense pallor, compared with the hand of a healthy physician. Photograph provided by authors.

endemic areas such as Papua (Indonesia) and India. These severe *P. vivax* cases contribute to increased public health costs because of increased hospitalization and the need for intensive care and blood transfusions. The major complications in patients who required hospitalization were jaundice and severe anemia, although whether these complications were responsible for deaths is undetermined.

No clear severity criteria exist for *P.* vivax malaria. However, WHO criteria formerly defined for *P. falciparum* malaria seem to be applicable to most of the severe *P. vivax* malaria cases reported in hospital-based studies in the literature. PCR should be performed to rule out mixed infections and other common infectious diseases so that reports from different parts of the world are comparable. Despite the small number of patients, our data corroborate previous findings of severe disease found in areas where chloroquine-resistant *P. vivax* is being reported but suggest that establishing direct causality is not straightforward. We urgently need to know which clinical complications in *P. vivax* malaria are associated with death to validate severity criteria. A valid biomarker for chloroquine resistance would also enable associative studies to determine the association between resistance and severity.

Dr Alexandre is a malaria researcher at the Tropical Medicine Foundation of Amazonas and is a PhD candidate at the Uni-

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Erythema Migrans–like Illness among Caribbean Islanders

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Erythema migrans is the skin manifestation of Lyme disease and southern tick-associated rash illness. Neither disease is found in the Caribbean. We report 4 cases of erythema migrans of a possible emerging clinical entity, Caribbean erythma migrans–like illness.

Erythema migrans (EM), the pathognomonic rash for Lyme disease (LD), occurs in ≈90% of cases (*1*). During the past 30 years, an LD-like illness has been described in the southeastern and south-central United States (*2*). This illness resembles LD clinically, but patients show no evidence of infection with the etiologic agent of LD, *Borrelia burgdorferi* (*2*) or sequelae of LD (*2*). This clinical syndrome is called southern tick-associated rash illness (STARI). The vector for STARI is not found in the Caribbean. However, we report 4 cases of EM-like lesions in Caribbean Island residents.

Case Reports

In October 2007, a 28-year-old man (student) sought care for a rash of 6 months' duration and generalized joint pains of a few days' duration. He had a history of major depression but was not taking medication. He reported being well until 6 months earlier, when he noticed a rash on his left leg. It began as a lesion on his thigh and spread throughout his entire left lower limb and upper limbs. He had no other symptoms.

The patient's joints showed no signs of inflammation. On his left outer thigh was a large lesion (8 cm × 5 cm) with many similar lesions 1–5 cm in diameter, with central clearing on the rest of his lower limb and upper limb (Figure 1). No punctum was evident in any lesion. Two visits to a dermatologist resulted in prescriptions for topical ketoconazole and beclomethasone, which had no effect on the rash. A skin biopsy was eventually performed.

The patient grew up in St. Lucia but had traveled to Trinidad and Tobago 3 years earlier. In St. Lucia, he owned

several tick-infested dogs. In 2005, he had spent 2 weeks in New York, New York, USA. He did not recall being bitten by ticks in either place.

Because of the rash and the exposure to ticks, a tick-borne illness was considered. Serologic analysis for *B. burgdorferi* and histologic analysis of a skin biopsy specimen were undertaken, and the patient was treated with doxycycline, 100 mg 2×/d for 2 weeks. Within 1 week, his arthralgia disappeared, and by week 2, the rash had resolved. At 6-month follow-up, the rash had not recurred.

Results for immunoglobulin (Ig) M and IgG to *B. burgdorferi* by enzyme immunoassay and the C6 peptide
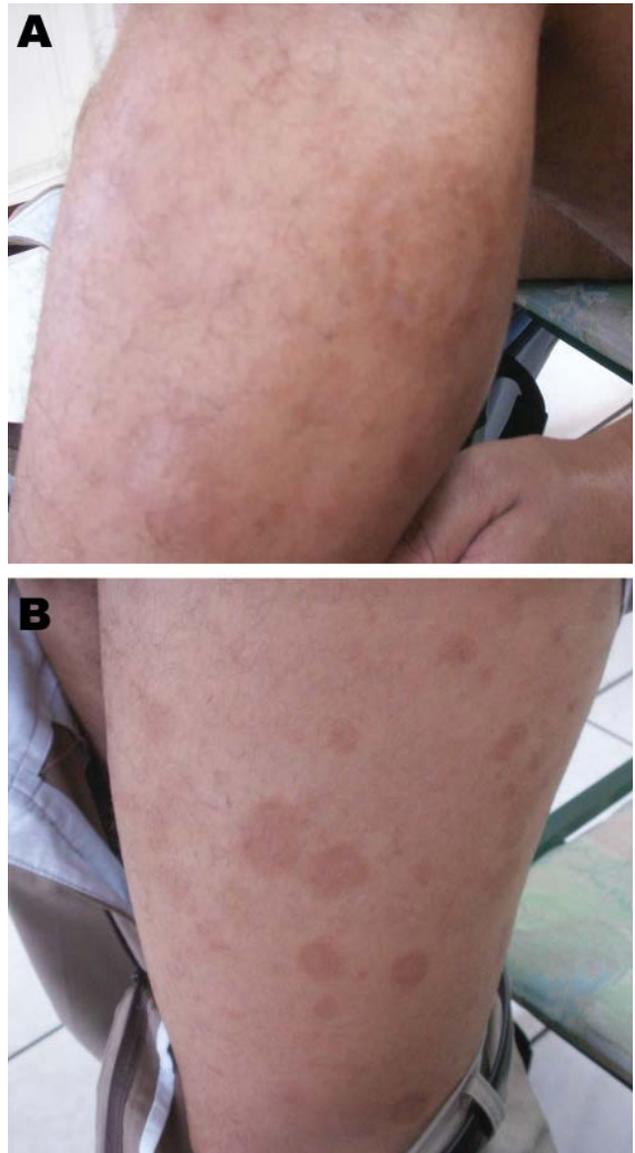

Figure 1. Erythema migrans–like rash on the left leg of a 28-year-old patient. A) Characteristic rash of erythema migrans (annular macular lesion that is erythematous with central clearing). B) Spread of the lesions to the rest of the leg.

antibody were negative. Histologic analysis of the skin biopsy specimen showed perivascular lymphocytic infiltrates with admixture of plasmocytes, consistent with EM (Figure 2). Culture and silver staining for spirochetes were not performed. On the basis of the clinical findings and negative serologic results for *B. burgdorferi*, an EM-like illness was diagnosed.

In March 2008, a 15-year-old boy from Trinidad and Tobago sought care for a red pruritic rash of 4 weeks' duration. Atopic eczema had been diagnosed 1 year earlier. Atopic eczema was again diagnosed and topical steroid cream prescribed, but the rash persisted. On examination in April 2008, the rash was present on his arms and legs. Multiple lesions 1–6 cm in diameter all had an erythematic border with central clearing. Histologic findings on skin biopsy were consistent with EM. After treatment with doxycycline for 14 days, the rash cleared. After 1 year, it had not recurred.
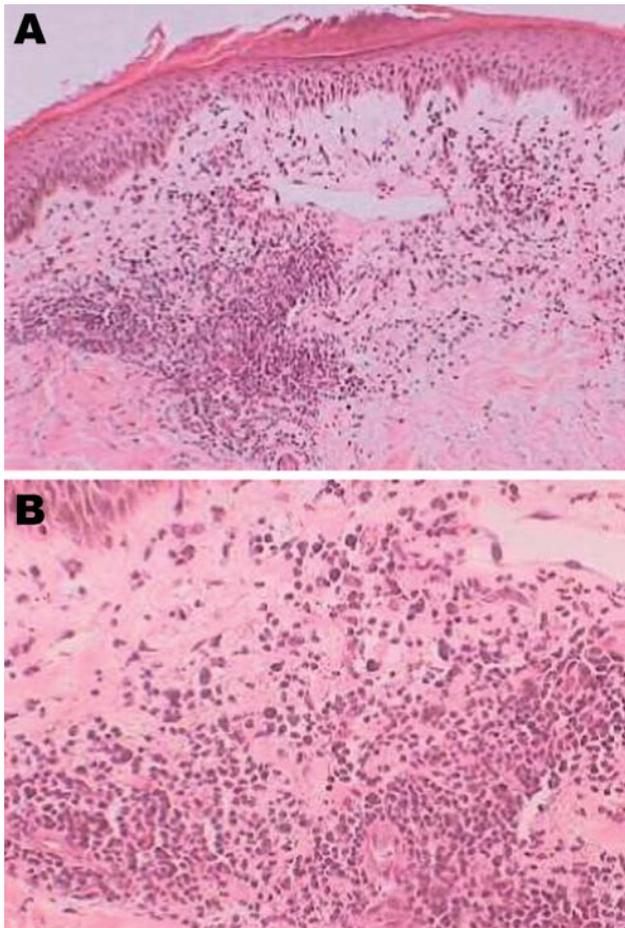
Figure 2. Histologic analysis of a skin biopsy specimen of a 28-year-old patient with erythema migrans, showing characteristics of erythema migrans. The epidermis shows parakeratosis, microvesicle formation, lichenoid interface, dermal edema, and perivascular chronic inflammatory cell infiltrates. Original magnification ×20 (A) and ×40 (B).

In April 2009, a 60-year-old woman from Grenada with diabetes sought care for an ongoing pruritic rash of 4–6 months' duration. The rash had an erythematous, flat border with a central clearing 7 cm in diameter but no punctum. It occurred only on her legs, first the left, then the right. Visits to several doctors resulted in prescriptions for vitamin A, vitamin E, and steroid and antifungal creams, all of which partially cleared the rash. She had no history of recent influenza-like symptoms or neurologic complaints. EM was diagnosed in October by biopsy. After treatment with doxycycline for 7 days, the rash resolved and had not recurred after 1 year.

In January 2010, a 48-year-old woman (veterinary laboratory assistant) from Trinidad and Tobago sought care for a circular, erythematic rash of 2 weeks' duration. The rash started on her right leg; had a flat, erythematous border with central clearing; and progressively increased to 5.1 cm in diameter. There was no evidence of a punctum. Two similar 2–3-cm lesions on her left leg appeared 3 days after the first lesion. She also reported fatigue, mild body and joint pains, and headaches relieved by acetaminophen. She had no history of travel to an LD-endemic region. EM was diagnosed and treatment with doxycyline was started. The rash began clearing after 2 weeks of treatment and had not recurred after 4 months.

Conclusions

STARI is characterized by expanding annular erythema, mild constitutional symptoms, spring–summer seasonality, recent antecedent tick bite at the site of the skin rash, absence of *B. burgdorferi* antibodies, and negative skin biopsy culture results for *B. burgdorferi* (*2*). Because none of our patients recalled an antecedent tick bite or had a punctum, their illnesses failed to meet the criteria for STARI.

In a prospective clinical evaluation of EM, lesions in STARI patients from Missouri were substantially smaller and more likely to have central clearing, and patients had a lower mean symptom score, compared with LD patients in a New York study (*3*). Although clinical findings of our patients were comparable with those of the Missouri STARI patients, our patients' multiple, widespread, and oblong lesions with no punctum and lack of known histories of tick bite were comparable with those of New York LD patients (*3*).

Unlike LD, for which *Ixodes* spp. ticks are the vector, STARI is thought to be spread by the Lone Star tick (*Amblyomma americanum*), the most common tick parasitizing humans in the southeastern and south-central United States (*4*). It is not an effective carrier of *B. burgdorferi*; however, 2% of the species are infected with another spirochete, *B. lonestari* (*5*). In 1 isolated case, *B. lonestari* was identified by PCR as the etiologic agent of STARI (*6*). In another report, no evidence of *B. lonestari* infection was found in 30 cases studied (*7*).

EM also has been associated with other infectious agents. In an imported human African trpanosomiasis case in France, the skin lesion resembled EM (*8*). In a study in Spain (*9*), *Dermacentor marginatus* ticks infected with *Rickettsia slovaca* produced EM-like rash and lymphade-nopathy in 22 patients studied. In addition, EM has been reported as a skin manifestation in Rocky Mountain spotted fever (*10*). EM can no longer be considered solely a sign of borrelial infection.

A. americanum ticks occur mainly in North America and certain areas of Mexico (*11*). *A. americanum* ticks and the LD vectors (*I. scapularis* and *I. pacificus* ticks) are not found in the Caribbean (*11*). The vector for the EM-like rash in our cases is therefore unlikely to be that of either STARI or LD. Other *Ambylomma* spp. ticks, however, have been found in Trinidad and Tobago (*12*). Whether these species are potential vectors for EM remains unknown; therefore, whether we are describing a new entity, Caribbean erythema migrans–like illness, remains to be determined.

We report EM-like skin lesions in 4 Caribbean nationals who did not have foreign exposure to ticks. The vectors for STARI and LD are not known to exist in the Caribbean region. Further research is warranted to isolate the responsible vector and etiologic agent.

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Dr Sharma is a researcher at the University of the West Indies. Her research interests include internal medicine, particularly commonly misdiagnosed conditions.

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Pandemic (H1N1) 2009 and Seasonal Influenza A (H1N1) Co-infection, New Zealand, 2009

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Co-infection with seasonal influenza A (H1N1) and pandemic (H1N1) 2009 could result in reassortant viruses that may acquire new characteristics of transmission, virulence, and oseltamivir susceptibility. Results from oseltamivir-sensitivity testing on viral culture suggested the possibility of co-infections with oseltamivir-resistant (seasonal A [H1N1]) and -susceptible (pandemic [H1N1] 2009) viruses.

Pandemic (H1N1) 2009 virus was first identified in mid-April 2009 (*1*), near the beginning of the Southern Hemisphere influenza season. The potential for reassortment of cocirculating seasonal influenza A viruses with pandemic (H1N1) 2009 virus within New Zealand generated considerable interest during the recent 2009 Southern Hemisphere influenza season (*2,3*). Of particular concern is the potential reassortment of neuraminidase gene segments leading to an oseltamivir-resistant pandemic strain.

Changes in the genome of pandemic (H1N1) 2009 virus by reassortment, recombination, or point mutation have the potential to alter the transmissibility, antigenicity, antiviral drug resistance, or virulence of the virus. Reassortment can occur when 2 viruses co-infect the same cell. The 8 influenza gene segments of each virus could then be exchanged, creating a reassortant virus. Pandemic (H1N1) 2009 is itself a reassortant virus containing gene segments of avian, human, and swine influenza virus origin (*4*). We report human co-infection with pandemic (H1N1) 2009 and seasonal influenza A (H1N1) viruses.

The Study

Influenza viruses were identified through the New Zealand national influenza surveillance system as part of the World Health Organization global program for influenza surveillance previously reported (*2*). Pandemic (H1N1) 2009 virus dramatically increased demand for influenza subtyping (*2*), necessitating a change in the standard real-time reverse transcription–PCR (rRT-PCR) algorithm. Samples were first screened with singleplex universal influenza A and pandemic (H1N1) 2009 assays (*5,6*). If negative results were obtained for both of these tests, samples were then tested for influenza B. If samples were positive for universal influenza A but not for pandemic (H1N1) 2009 virus, they were subtyped for seasonal H1 and H3 by rRT-PCR. Samples positive for pandemic (H1N1) 2009 virus were not subsequently assayed for other influenza viruses during testing but were tested at the end of the Southern Hemisphere influenza season as part of this study.

By testing viral cultures of pandemic (H1N1) 2009 viruses for oseltamivir resistance, by fluorometric-inhibition assay (*7*), putative co-infections of resistant seasonal influenza A (H1N1) and susceptible pandemic (H1N1) 2009 were discovered; i.e., pandemic (H1N1) 2009 viruses initially appeared to be resistant to oseltamivir because of a co-infecting oseltamivir-resistant seasonal A (H1N1) virus in culture. Within New Zealand, all seasonal influenza A (H1N1) viruses tested during 2009 were oseltamivir resistant, and all pandemic (H1N1) 2009 viruses were susceptible (*3*).

After the discovery of co-infection in viral culture, 1,044 clinical samples that were positive for pandemic (H1N1) 2009 were screened by rRT-PCR for seasonal A (H1N1) virus. Eleven co-infections were identified. Two additional samples indicated co-infections when viral culture was screened by rRT-PCR but could not be confirmed because our laboratory did not receive the original clinical specimen.

Laboratory contamination of viral culture could account for the presence of both influenza subtypes in viral culture samples. Co-infection was confirmed by using World Health Organization–recommended specific singleplex rRT-PCRs (*5*) on each of the 11 original clinical specimens (Table). The specific rRT-PCRs each are specific for the gene segment encoding hemagglutinin; 1 assay is specific for pandemic (H1N1) 2009, the other for seasonal influenza A (H1N1). The 2 assays were run in parallel for each sample with appropriate controls, including specificity controls.

Within this small number of cases, 10 of the 13 patients were female, and 6 patients were of Maori descent. Each figure was higher than the expected 51% and 14.6% representation in the New Zealand population, respective-

Table. Characteristics of 13 nonhospitalized patients co-infected with seasonal influenza A (H1N1) and pandemic (H1N1) 2009, New Zealand, June 2009*

	rRT-PCR results, Ct			Patient characteristics					
Patient no.	Sample type	Pandemic (H1N1) 2009	Seasonal A (H1N1)	Age, y/ sex	Ethnicity	Location	Date of onset	Received vaccination†	Received antiviral drug
1	Clinical	22.74	18.13	29/F	E	Manakau	14	No	Yes
2	Clinical	32.26	23.84	10/M	M	Rotorua	15	No	No
3	Clinical	23.97	17.87	13/M	M	Wellington	17	No	No
4	Clinical	26.04	32.96	18/F	E	Waikato	22	No	U
5	Clinical	22.67	34.68	22/F	ME	Wellington	22	Yes	No
6	Clinical	22.78	35.24	19/F	M	Bay of Plenty	23	Unknown	No
7	Clinical	18.03	33.58	36/M	E	Hamilton	23	No	Yes
8	Clinical	20.65	32.92	31/F	E	Hamilton	23	No	No
9	Clinical	23.69	31.5	24/F	M	Hamilton	24	No	No
10	Isolate	15.09	13.52	51/F	E	Hamilton	26	No	No
11	Clinical	30.77	22.76	21/F	U	Dunedin	28	No	Yes
12	Clinical	25.1	36.62	16/F	M	Rotorua	29	No	No
13	Isolate	29.93	12.54	17/F	E, M	Manukau	30	No	Yes

*rRT-PCR, real-time reverse transcription–PCR; Ct, cycle threshold; E, European; M, Maori; ME, Middle Eastern; U, unknown.
†Patient history of receipt of seasonal influenza vaccination.

ly, but co-infections were too few to draw any conclusions based on these characteristics (Table).

A vaccine for pandemic (H1N1) 2009 was not available when these samples were collected (June–November 2009), and only 1 of the 13 patients had a history of seasonal influenza vaccination. None of the 13 case-patients had severe illness or were hospitalized.

Eight of the 13 case-patients came from the central North Island; the remainder came from Auckland (2), Wellington (2), and Otago (1). All of these regions had high influenza activity during the 2009 New Zealand influenza season (2).

For each case, details of initial and ongoing transmission were unclear. Two cases occurred in a husband and wife, who had onset of symptoms on the same day; the remaining cases are not thought to be linked. All cases were reported after pandemic (H1N1) 2009 had become widespread in the community; therefore, contact tracing had ceased within New Zealand.

Dates of illness onset for all case-patients occurred within a 16-day period (June 14–30). This period coincided with the short period when both seasonal A (H1N1) and pandemic (H1N1) 2009 viruses cocirculated at approximately equal levels in the community, before the pandemic virus became the predominant strain (2) (Figure).

Conclusions

Results from oseltamivir-sensitivity testing on viral culture suggested the possibility of co-infections in patients with both resistant (seasonal A [H1N1]) and susceptible (pandemic [H1N1] 2009) viruses. This test required both viruses to grow sufficiently in cell culture and grow to similar titers. Only by this approach was co-infection discovered and later investigated by use of more sensitive and highly specific rRT-PCRs.

Co-infections of different influenza viruses are rarely reported; reports focus solely on co-infections of influenza A and B, not of 2 influenza A subtypes (8–11). Two recent studies, 1 examining 2,273 clinical influenza samples with multiplex PCR methods found no influenza co-infections (12,13); another study estimated influenza co-infections to be as high as 3% (14). The rate of co-infection determined in this study was 1.1% (n = 1,044), which may underestimate the actual rate because not all tests used in this study (either biochemical or molecular) screened for co-infection
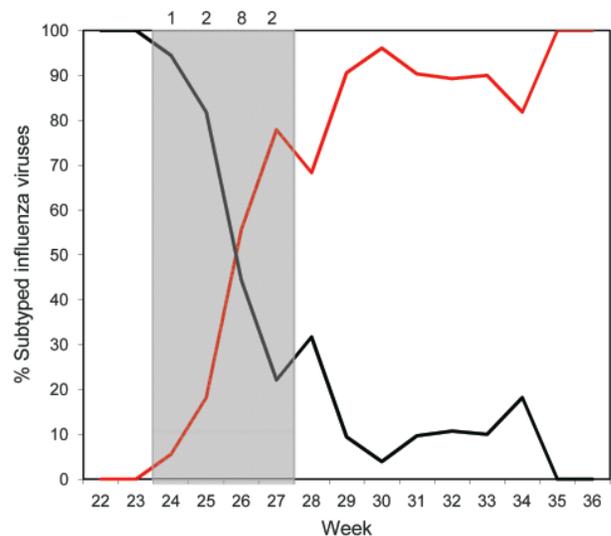
Figure. Co-infection during cocirculation of seasonal influenza A (H1N1) and pandemic (H1N1) 2009 viruses, New Zealand, 2009. Red line indicates pandemic (H1N1) 2009 viruses; black line indicates seasonal influenza A (H1N1) viruses. The gray shaded area indicates weeks in which the co-infections occurred; numbers above the graph indicate number of co-infections for that week: 1 co-infection in week 24, 2 in week 25, 8 in week 26, and 2 in week 27.

of pandemic (H1N1) 2009 with other viruses, such as influenza A (H3N2) or influenza B.

Although influenza co-infections are rare, we have shown that they occurred during the first stage of a pandemic when seasonal strains cocirculated. This cocirculation poses a risk for further reassortment for the pandemic strain, which could result in a new pandemic strain. Of particular concern is the potential generation of an oseltamivir-resistant pandemic strain. The genesis of a harmful influenza reassortant warrants further investigation in animal models or in vitro systems. Further analysis of natural co-infections may help elucidate a role for the human host in influenza reassortment.

Acknowledgments

Dr Peacey is a scientist at the Institute of Environmental Science and Research in New Zealand. His main research interests are viral infection and identification and the associated immunologic implications of viral infection.

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Chemokine Receptor 5 Δ32 Allele in Patients with Severe Pandemic (H1N1) 2009

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Because chemokine receptor 5 (CCR5) may have a role in pulmonary immune response, we explored whether patients with severe pandemic (H1N1) 2009 were more likely to carry the CCR5Δ32 allele than were members of the general population. We found a large proportion of heterozygosity for the CCR5Δ32 allele among white patients with severe disease.

Chemokine receptor 5 (CCR5) is a protein that belongs to the β-chemokine receptor family and is expressed primarily on T cells, macrophages, and dendritic cells. CCR5 plays a role in mediating leukocyte chemotaxis in response to its ligands, which include RANTES, MIP-1a, and MIP-1b. It may help direct many immune cell subsets, including regulatory T cells and Th17 cells, to sites of infection. CCR5 is also 1 of 2 common co-receptors for HIV. Until recently, understanding the role of CCR5 in supporting the antiviral immune response was limited to appreciation of the role of receptor deficiency in protecting from HIV infection and disease progression. Persons who are homozygous for the CCR5Δ32 allele, a condition in which a 32-bp deletion in the CCR5 gene prevents its expression on the cell surface, have been shown to have reduced susceptibility to HIV infection; the heterozygous state delays HIV disease progression (1–3). However, homozygosity of the Δ32 allele has recently been shown to be associated with increased risk for symptomatic and fatal West Nile virus infection (4). This association was confirmed in a larger meta-analysis (5); CCR5 facilitated directed movement of lymphocytes during infection in a mouse model of West Nile virus infection (6). A case report of an adverse reaction to the yellow fever virus vaccine in a person heterozygous for CCR5Δ32 and a link between the CCR5Δ32 allele and severe tickborne encephalitis symptoms suggest that CCR5 may play a role in the immune response to other flavivirus infections as well (7,8). Several reports have suggested a potential effect of the CCR5Δ32 allele on the response to influenza viruses. In mouse models, CCR5 is pivotal in directing CD8+ T cells to lung airways during challenge with Sendai virus (9); similarly, deaths among CCR5$^{-/-}$ mice increase after infection with influenza A virus (10). Because of the range of severity of recent pandemic (H1N1) 2009 infections and the possible role for CCR5 in the pulmonary immune response, we sought to determine whether patients requiring intensive care admission and respiratory support for severe pandemic (H1N1) 2009 were more likely to carry the CCR5Δ32 allele than were members of the general population.

The Study

In response to the outbreak of pandemic (H1N1) 2009 in Mexico, we conducted an observational study of critically ill patients with this infection in Winnipeg, Canada. The research was approved by the local research ethics board. The study protocol is described in detail (11).

We examined blood samples from 20 patients with laboratory-confirmed pandemic (H1N1) 2009. Average patient age was 40.35 years. Ethnicity was nonwhite for 10 patients, white for 9, and unknown for 1.

Peripheral blood mononuclear cells were stored, and a subset of samples were thawed and resuspended in 200 uL phosphate-buffered saline. Genomic DNA was extracted by using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. DNA was amplified by using previously reported primers surrounding the 32-bp deletion in the CCR5 gene: 5′ primer, TCATTACACCTGCAGCTCTC; 3′ primer, TGGTGAAGATAAGCCTCAC. Wild-type CCR5 DNA results in a 197-bp product, but the Δ32 allele results in a 165-bp product. The genotype was determined by visual examination of the PCR product and of a known heterozygote used as a control.

The CCR5Δ32 allele was not found in the nonwhite patients, but it was found in 5 of the 9 white patients (Figure); overall allele frequency for white patients was 27.8%. Among the 5 who were heterozygous for the CCR5Δ32 allele, 1 died, 1 remained in the intensive care unit for >1 month, and 3 were discharged.

Conclusions

The outbreak of pandemic (H1N1) 2009 infection in Canada affected primarily young women; a preponderance

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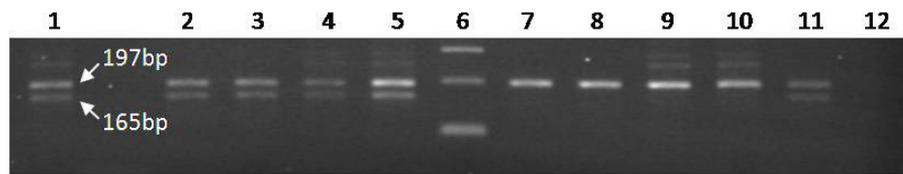
[1]These authors contributed equally to this article.

Figure. Amplification of the chemokine receptor 5 (CCR5) Δ32 locus in white patients. Lane 1, heterozygous positive control; lanes 2–5 and 7–11, patient samples; lane 6, 100-bp ladder; lane 12, negative control. CCR5Δ32 heterozygosity is observed in samples 2, 3, 4, 5, and 11.

were nonwhite and they had no major concurrent conditions. Risk factors identified included a history of lung disease or smoking, obesity, hypertension, and diabetes. The frequency of CCR5Δ32 heterozygosity among white populations has been reported to range from 10% to 15% (12,13); we found CCR5Δ32 heterozygosity at a higher than expected frequency (55.5%) among white patients with critical illness caused by pandemic (H1N1) 2009. Although deficiency of the receptor protects against acquisition of HIV, evidence is accumulating to suggest it plays a role in severity of illness caused by flavivirus infections (7,8). In animal models of influenza, CCR5 plays a role in directing CD8+ T cells to the site of infection, and its absence is associated with increased mortality rates (9,10); however, to our knowledge a similar association in humans has not yet been reported. Our observation suggests that CCR5Δ32 is 1 of the factors associated with increased severity of illness among white patients with pandemic (H1N1) 2009. Identifying genetic factors associated with greater risk for illness severity will help explain the unique pathogenesis displayed in the pandemic (H1N1) 2009 outbreak and may have public health implications. Further studies are required to illuminate the role of CCR5 in delivery of immune cells to the site of influenza infection.

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Klassevirus Infection in Children, South Korea

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To investigate prevalence and clinical characteristics of klassevirus in South Korea, we performed molecular screening in fecal and nasopharyngeal samples from hospitalized children with gastroenteritis. A total of 26 (8.8%) of 294 fecal samples were positive for klassevirus. Klassevirus may be a possible cause of gastroenteritis.

Identification of new picornaviruses (family *Picornaviridae*) in fecal samples has increased because of new molecular methods (*1,2*), but clinical significance is not clear. The genus *Kobuvirus* belongs to the family *Picornaviridae* and contains 3 species: *Aichi virus*, *Bovine kobuvirus*, and *Porcine kobuvirus*. Aichi virus was first identified as an etiologic agent of gastroenteritis, but the connection has not yet been proven (*3*).

In 2009, Holtz et al. (*4*) identified a new picornavirus, kobu-like virus (klassevirus), associated with feces and sewage, in a fecal sample from a child from Australia; the complete genome of this virus has been reported (*5*). However, because the prevalence of klassevirus-1 and its clinical role in gastroenteritis remain unclear, we investigated its prevalence and clinical characteristics in South Korea.

The Study

We analyzed 3 groups of samples. The first group (retrospective fecal group) comprised archived virus-negative fecal samples from 342 children <6 years of age hospitalized with gastroenteritis at Sanggyepaik Hospital during September 2007–April 2009 (*6,7*). The second group (prospective group) comprised 294 fecal samples prospectively collected during May 2009–February 2010 from hospitalized children <17 years of age at Sanggyepaik Hospital who had gastroenteritis. The third group (nasopharyngeal aspirate group) comprised 142 archived virus-negative nasopharyngeal aspirates from hospitalized children <6 years

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of age who had acute lower respiratory tract infections during September 2006–June 2007 (*8*). The ethics committee of Sanggyepaik Hospital, Inje University, approved the study protocol.

All fecal samples were tested for common bacterial diarrheal pathogens by routine microbiologic methods, as described (*9*). Rotavirus and adenoviruses 40 and 41 were identified by using the ELISA kits Rotaclone and Adenoclone 40/41 (Meridian Bioscience, Cincinnati, OH, USA). The seminested reverse transcription–PCR (RT-PCR) for norovirus, which used primers based on the capsid region and for human astrovirus based on open reading frame 1a were performed as described (*10,11*). PCR for human bocavirus 2, which used primers based on the nonstructured gene, and nested RT-PCR for Saffold virus (SAFV), which used primers based on the 5′ noncoding region, were performed as described (*1,6*). To detect Aichi virus, we conducted a nested PCR with primer sets based on the 3CD junction region, as described (*12,13*).

To detect klassevirus, we performed the first reactions of 2 nested RT-PCRs by using the following primers: LG0118 and LG0117 for 3D region and LG0119 and LG0136 for viral protein (VP) 0/VP1 gene, as described (*4*) (Table). The second reactions of each RT-PCR were conducted by using newly designed primers based on the Klasse mel1 sequence (GQ253936): KL3DF and KL3DR for the 3D region and KLVPF and KLVPR for the VP0/VP1 region at 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s. A third RT-PCR for the 2C region of klassevirus amplifying a 345-bp fragment was performed by modified primers KL2C-F1 and KL2C-R1 for the first reaction and KL2C-F2 and KL2C-R2 for the second reaction under the following conditions: 40 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 60 s. Amplicon was purified by using QIAquick (QIAGEN, Valencia, CA, USA) and sequenced in both directions with the BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were aligned by using BioEdit version 7.0 and presented in a phylogenic tree prepared in MEGA 4.1 (www.megasoftware.net). The tree was constructed by using the neighbor-joining method with Kimura 2-parameter estimation.

Among 342 children in the retrospective fecal group, 15 (4%) samples were positive for klassevirus by RT-PCR. All samples were positive by 3 kinds of RT-PCR for the 3D, VP0/VP1, and 2C regions. Klassevirus-positive samples in this group were frequently found in February 2009 (33%) and March 2009 (20%)

We tested 294 fecal samples collected prospectively for klassevirus RNA. Ages of children in the study were 109 (37%) <12 months of age; 159 (54%) 12–60 months of age; and 26 (9%) >60 months of age (median 18 months, range 1–174 months). The male:female ratio was 1.1:1 (155:139).

Table. Primers used in PCR for Aichi virus and klassevirus*

Virus/primer	Sequence (5′ → 3′)	Gene	Reference
Aichi virus			
6261	ACA CTCCCACCTCCCGCCAGTA (1st)	3CD	(12)
6779	GGAAGAGCTGGGTGTCAAGA		
C94b	GACTTCCCCGGAGTCGTCGTCT (2nd)	3CD	(13)
246k	GACATCCGGTTGACGTTGAC		
Klassevirus			
LG0118	ATGGCAACCCTGTCCCTG AG (1st)	3D	(4)
LG0117	GAAACCCAACCACGCTGTA		(4)
KL3DF	GTCTGGTCTATYGAYTACTCTTGCTTT (2nd)	3D	This study
KL3DR	AGGACGGAGTAGGGRGTRAA		This study
LG0119	GCTAACTCTAATGCTGCCACC (1st)	VP0/VP1	(4)
LG0136	GCTAGGTCAGTGGAAGGATCA		(4)
KLVPF	GTCACYCCMAACACCTCCACTGAAG (2nd)	VP0/VP1	This study
KLVPR	TTCTGCRCCATCRGCTCCCGA		This study
KL2C-F1	CTCGCYGAGGACATCACGGA (1st)	2C	This study
KL2C-R1	GTACAGGTACACRACCAGTGGCT		This study
KL2C-F2	AATCTGCTGCCCAGGCCGC (2nd)	2C	This study
KL2CR2	AGGGAGATGGCRGAGAGAGCTGT		This study

*VP, viral protein.

Enteropathogenic bacteria were found in 6 (2.0%) children, *Salmonella* spp. in 4 (1.0%), and enterotoxigenic *Escherichia coli* in 2 (0.6%). Rotavirus (13.7%) and norovirus (10.6%) were the most prevalent viral agents. Adenovirus, human bocavirus 2, sapovirus, and human bocavirus 1 were detected in 6 (2%), 3 (1%), 2 (0.6%), and 2 (0.6%) children, respectively. SAFV was detected in 1 child, and the virus belonged to SAFV-1 sublineage. Astrovirus and Aichi virus were not found.

Klassevirus was detected in 11 (4%) children in the prospective group, most frequently in June and August 2009 (27% and 36% of detections, respectively). All samples were positive by RT-PCR for the 3D and 2C regions, but the VP0/VP1 region could be amplified in only 8 samples, possibly because of the variance of the strains and sensitivity of the primer sets. Other viral agents were co-detected in 3 (12%). In 142 virus-negative nasopharyngeal aspirates, klassevirus was not detected.

The 26 klassevirus-positive patients ranged in age from 2 months to 175 months (median 31 months, mean 51 months). All klassevirus-positive patients had diarrhea; other symptoms manifested were fever, vomiting, cough, rhinorrhea, and skin rash. No patients had underlying medical problems, and all recovered completely.

Phylogenetic analysis showed that Korean isolates in this study clustered into reference strains, KV US/2002 (NC 012986) and KV AU/1984 (GQ 253930) (online Appendix Figure, www.cdc.gov/EID/content/16/10/1623-appF.htm), but the sequence variation was limited. The ranges of nucleotide differences and amino acid differences between Korean strains and reference strains were 5%–11% (online Appendix Figure) and 0%–14% (data not shown), respectively.

Conclusions

We found a higher prevalence of klassevirus in feces from children with gastroenteritis than has been found in previous studies (4,5), possibly because of the study population and different primers. Most (76.9%) klassevirus-positive children were <3 years of age, although the virus was detected in children >6 years of age. Co-infection of klassevirus with other viral agents was lower than co-infection with recently identified viruses, such as SAFV and cosavirus (1,2). These results suggest that klassevirus could be a possible cause of gastroenteritis in children; however, further studies that include asymptomatic control groups are needed to exclude the possibility of klassevirus being an innocent bystander. Recently, Li et al. (14) reported that salivirus, which has 90% nt similarity with klassevirus, might have a role in gastroenteritis. However, this virus could not be differentiated from klassevirus in the phylogenetic analyses based on 3 different regions (data not shown).

We did not detect klassevirus in nasopharyngeal aspirates despite respiratory symptoms in 26.9% of klassevirus-positive children. These results indicate that klassevirus might not be an etiologic agent of acute lower respiratory tract infections; additional studies are required to be conclusive. We detected SAFV in 1 patient; a recent study shows high prevalence of this virus in healthy children and in children with gastroenteritis (15), perhaps because of different assay sensitivities. In this study, the results of phylogenetic assay of the 3 different regions showed low genetic diversity of klassevirus, which suggest an outbreak by a single circulating klassevirus strain. A definite seasonality of klassevirus infection was not observed. In conclusion, we have detected klassevirus in children with gastroenteritis,

which suggests a possible association between this virus and gastroenteritis.

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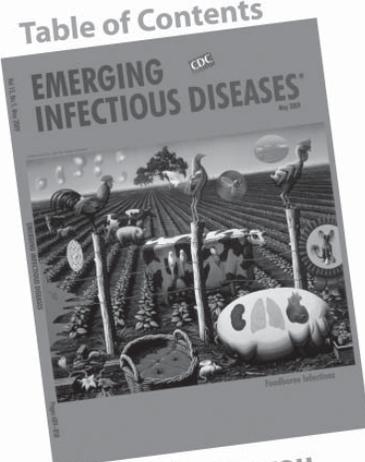
Human Cases of Methicillin-Resistant *Staphylococcus aureus* CC398 Infection, Finland

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Nationwide surveillance identified 10 human isolates of methicillin-resistant *Staphylococcus aureus* clonal complex (CC) 398. Further typing in comparison with animal isolates identified 4 clusters: 1 related to a horse epidemic and 3 to persons who had no direct contact with animals or each other. These findings may indicate unrecognized community transmission.

Animals may serve as a reservoir for methicillin-resistant *Staphylococcus aureus* (MRSA). The MRSA lineage clonal complex (CC) 398 has been reported to be common among pigs (*1–3*) and has also been found among other animal species. Occupational exposure to animals has been recognized as a new risk factor for MRSA (*4,5*). In Finland, MRSA has occasionally been detected in pets and farm animals. MRSA CC398 was first recognized in Finland in 2007 and involved a veterinary hospital epidemic of 13 horses and 1 employee. Since then, MRSA CC398 has also appeared in other persons and has been found in pig samples. The objective of this study was to recognize possible connections of emerging MRSA CC398.

The Study

Nationwide surveillance of MRSA in Finland, including notification for human cases and isolate characterization, was started in 1995 (*6*). From 1995 through April 2009, a total of 10,615 nonduplicate human isolates of MRSA were typed by using pulsed-field gel electrophoresis (PFGE); 1 in 2007, 6 in 2008, and 3 in 2009 (n = 10, 0.09%) were nontypable, a typical feature of MRSA CC398 isolates (*7*). Samples of animal origin are usually investigated clini-

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cally and through official surveys. No systematic national MRSA surveillance for animals exists, but it is possible to send suspected isolates for confirmation. After the horse epidemic in 2007, some admission screening of animals started at the veterinary hospital where the epidemic occurred. From January 2007 through April 2009, a total of 35 animal MRSA isolates were typed by PFGE; 20 (57%) were nontypable.

For this study, all human and animal isolates of MRSA nontypable by *Sma*I PFGE were further analyzed by testing antimicrobial drug susceptibility against 14 different antimicrobial drug groups, typing the *ccr* and *mec* complex regions within the staphylococcal cassette chromosome *mec* (SCC*mec*) by PCR (*8*), detecting the presence of Panton-Valentine leukocidin (PVL) genes (*9*), *spa* typing, and PFGE by using *Apa*I as the restriction endonuclease. *Apa*I PFGE was performed as described (*10*), except that the restriction digestion was performed with *Apa*I at 30°C for 4 h, and initial and final switching times of 5 s and 15 s, respectively, were used for a 20-h PFGE run. Multilocus sequence typing (MLST) (*11*) was performed on representative isolates of each different combination of origin (human vs. animal species), *spa* type, and SCC*mec*.

For persons whose MRSA isolate was nontypable by *Sma*I PFGE, a structured questionnaire was used to inquire about the presence of commonly known healthcare-related risk factors for MRSA (online Technical Appendix, www.cdc.gov/EID/content/16/10/1626-Techapp.pdf). These data were collected from infection control nurses at relevant healthcare districts or directly from patients by telephone interview.

PFGE-nontypeable isolates were found in samples from 10 humans, 13 horses, and 7 pigs. Based on combined results from *spa* typing, SCC*mec*, and PVL PCR, 1 single t2922 isolate and 4 clusters of isolates were identified: 14 isolates (t011, SCC*mec*IV) related to the horse epidemic; 3 human isolates (t011, SCC*mec*V) from 2 hospital districts (A and B); 5 human isolates (t034, SCC*mec*V, PVL positive) from 1 hospital district (C); and 7 isolates (t108, SCC*mec*V) from pigs (Table 1). MLST analysis identified sequence type (ST) 398 in all but 1 isolate. This isolate had ST1375, a double locus variant of ST398. In *Apa*I PFGE, 3 groups were identified (Figure). The human t034 isolates from 1 hospital district (C) fell into 1 group. Although all but 1 of the human t011 isolates were clustered together with the pig t108 isolates, subtype-level differences were still detected within this group. Isolates from the horse epidemic formed the third group, showing subtype-level difference from the t2922 human isolate. All isolates were resistant to tetracycline. Resistance patterns to erythromycin, gentamicin, tobramycin, and clindamycin (inducible or noninducible) varied but followed the same grouping as in *Apa*I PFGE (Table 1).

Table 1. MRSA CC398 clusters, Finland, January 2007–April 2009*

Cluster	Year	No. isolates	Origin	*spa* type	SCC*mec*	MLST	Antimicrobial drug resistance†	Healthcare district
1	2007	1	Human	t011	IV	398	tet, gen, tob	A
	2007	13	Horse	t011	IV	398	tet, gen, tob	
2	2008	3	Human	t011	V	1375	tet, ery, cli	A, B
Single	2008	1	Human	t2922	V	398	tet, ery, cli	A
3	2008–2009	5	Human	t034	V	398	tet, ery, cli (inducible)	C
4	2008–2009	7	Pig	t108	V	398	tet, ery, cli	

*MRSA, methicillin-resistant *Staphylococcus aureus*; CC, clonal complex; SCC*mec*, staphylococcal cassette chromosome *mec*; MLST, multilocus sequence typing; tet, tetracycline; gen, gentamicin; tob, tobramycin; ery, erythromycin; cli, clindamycin.
†All isolates were negative for Panton-Valentine leukocidin except cluster 3.

The human MRSA cases with CC398 isolates had no contact with each other. In 2 persons, the specimen was taken because of clinical symptoms, in 6 because of screening, and in 2 persons for unknown reasons (Table 2). Only the employee of the veterinary hospital had direct animal contact. For others, no direct contacts with horses or pigs were identified, and none owned a pet (Table 2).

Conclusions

Our nationwide population-based surveillance for MRSA showed the emergence of MRSA CC398 in 2007, and in total 10 findings in humans through April 2009. Molecular typing of both human and animal MRSA CC398 isolates identified 4 clusters; in only 1 was human carriage linked to occupation and related to the horse epidemic. Another cluster consisted of pig isolates, and 2 other clusters consisted of human isolates.

Animal contact is an established risk factor for MRSA CC398 (*4,5,12*). We identified direct animal contact for only 1 person. The other patients with MRSA lacked or had negligible animal contacts; instead, most of them had previous healthcare contacts. According to the national guidelines for MRSA control, screening is indicated when a patient has previously carried MRSA, has been exposed to a MRSA carrier, has been recently cared for at a facility where MRSA is endemic, or during an outbreak. Although some persons were possibly screened because of recent exposure to another MRSA carrier, direct transmission in these instances was unlikely because these persons carried different strains. Connections to Asia have previously been reported for persons with PVL-positive, t034 MRSA (*4*). Two cases of PVL-positive, *spa* type t034, MRSA infec-

tions in persons who had no contact with animals have also been reported from Sweden (*13*).

The proportion of CC398 of all MRSA in humans was far lower in Finland (0.09%) than in the Netherlands (30% in 2007) (*14*). Notably, the prevalence of MRSA in pigs is 40% in the Netherlands (*1*), whereas in Finland it is expected to be lower (*15*). The densities for pig and humans in the Netherlands are ≈60 and 30 times higher than those in Finland (http://epp.eurostat.ec.europa and www.stat.fi) respectively, and there are differences in screening policies. In the Netherlands, people in close contact with pigs or cattle are screened at hospital admission. It remains to be investigated whether the low occurrence of CC398 and lack of direct animal contacts among CC398 case-patients are related to lower occurrence of MRSA in animals, sparse pig and human populations, or screening policies in Finland.

Our questionnaire focused on healthcare-related risk factors and animal contacts. Although questions about traveling abroad were included, these data were difficult to interpret in relation to MRSA acquisition because travel is common. Furthermore, data for MRSA risk factors were not uniformly available for all patients. Another limitation was the lack of representative data for livestock, including pigs.

Except for the isolates from the horse epidemic, human and animal isolates were distinguished from each other by using a combination of molecular typing techniques. Additional typing by the *Apa*I–PFGE method may help in resolving outbreaks. Despite closely related and typical livestock associated *spa* types, *Apa*I PFGE distinguished the t108 pig isolates from the t011 human isolates at a possibly related level (4–5 band differences). In addition, the presence of 2

Figure. Dendrogram of *Apa*I–pulsed-field gel electrophoresis of methicillin-resistant *Staphylococcus aureus* clonal complex 398. Scale bars indicates percentage similarity.

spa type	No. isolates typed	Origin
t034	1	Human
t034	4	Human
t011	2	Human
t011	1	Human
t108	3	Pig
t011	1	Human
t011	13	Horse
t2922	1	Human

Table 2. Human MRSA CC398 infections, Finland, January 2007–April 2009*

Patient age, y/sex	MRSA strain	Reason for taking specimen	Sample source	Animal contact	Other information
27/M	t011	Screening	Nares	Direct: horses	Staff member of veterinary hospital, direct link to horse epidemic
63/M	t011	Clinical symptoms	Wound secretion	None	Dentist, history of skin grafts, healthcare worker in family, travel history to the Canary Islands
82/F	t011	Clinical symptoms	Wound secretion	None	Several previous hospitalizations, multiple diseases including basal cell carcinoma; son has a dog
65/F	t011	Screening	Nares	Limited, visit to a horse farm (but not to the stable) 2 mo before MRSA isolation	Retired nurse, hospitalizations in Finland and in Switzerland due to trauma
57/M	t2922	Screening	Skin	None	Atopic skin, hospitalizations within previous year
82/M	t034	Unknown	Wound secretion	Limited, not specified	Several previous hospitalizations
78/M	t034	Unknown	Skin	Limited, occasional visits by a dog, had parakeets 20 y previously	Several previous hospitalizations
96/M	t034	Screening	Nares	None	Several previous hospitalizations
39/M	t034	Screening	Nares	None	Several previous hospitalizations, homeless, alcoholic, contact sport activities
42/M	t034	Screening	Nares	Unknown	Unknown

*MRSA, methicillin-resistant *Staphylococcus aureus*; CC398, clonal complex 398.

different SCC*mec* types among t011 and nonexistent links in time and space between human cases may suggest unrecognized transmission chains in the community.

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Hepatitis E Virus Genotype Diversity in Eastern China

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We studied 47 hepatitis E virus (HEV) isolates from hospitalized patients in Nanjing and Taizhou, eastern China. Genotypes 1, 3, and 4 were prevalent; genotype 3 and subgenotype 4b showed a close relationship with the swine strains in eastern China, thus indicating that HEV genotype 3 had infected humans in China.

*H*epatitis E virus (HEV), genus *Hepevirus*, is a nonenveloped virus with a positive-stranded RNA genome of ≈7.2 kb (*1*). Researchers have hypothesized that zoonotic infection is involved in HEV transmission (*2,3*). HEV isolates have been divided into 4 distinct genotypes, and further classification of the 4 genotypes into 24 subtypes has been proposed (*4*). Genotypes 1 and 2 have been identified exclusively in humans, and genotypes 3 and 4 have been found in humans and several animal species. Genotypes 1 and 2 have been isolated in Asia, Africa, and North America; genotype 4 has been identified only in Asia; and genotype 3 has been found in almost every country. Although genotype 3 was found to be prevalent in swine populations in China in 2007 (*5*), this viral genotype has not previously been reported in humans in this country. Our study aimed to characterize the genotype diversity of the strains and to determine the full-length sequence of the most prevalent genotype in eastern China.

The Study

We studied 47 HEV isolates from patients (15 women and 32 men, 19–73 years of age) admitted to hospitals in Nanjing (26 patients) and Taizhou (21 patients) in eastern China and who were serologically confirmed to have HEV infection by commercial ELISAs (Wan Tai Pharmaceutical, Beijing, People's Republic of China). HEV RNA was detected by reverse transcription–PCR as described (*6*). A serum sample negative for HEV was included as a control for testing sample contamination.

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Sequence analysis based on the PCR-amplified products (primer sequences were removed) indicated that among the 47 HEV isolates, 31 belonged to genotype 4, 13 belonged to genotype 1, and 3 belonged to genotype 3, which indicated that genotype 4 is the main type prevalent in humans in eastern China. The 13 genotype 1 strains shared >98% sequence homology and showed 3 distinct nucleotide sequences. Figure 1 shows the phylogenetic tree constructed for the 37 distinct sequences in this study and their closest matching sequences in GenBank. To further classify the subgenotype of these strains, we included in the phylogenetic analysis some well-characterized subtype strains (*4*).

Results indicated that the 31 genotype 4 isolates found in this study were divided into 5 different subtypes. Seventeen of the genotype 4 isolates were subtyped as 4b, according to the reference sequences (GenBank accession nos. AJ344188 and AB116161), and they shared 93.2%–99.7% sequence homology among themselves. Strains ECh250 shared 100% sequence homology with FJ461766 and could be classified into subtype 4e, according to the
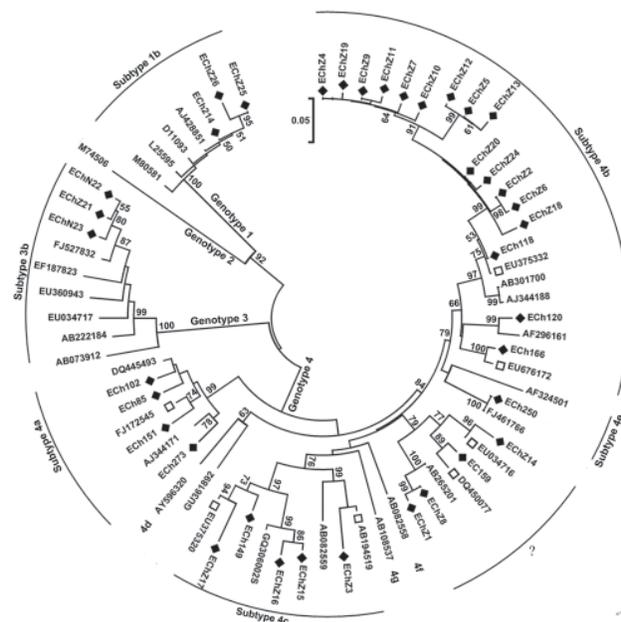
Figure 1. Phylogenetic tree of hepatitis E virus isolates in eastern China, 2007–2009. The phylogenetic tree was produced with a 348-nt open reading frame 2 sequence alignment of 37 isolates from this study and other 31 reference sequences, using the neighbor-joining method and evaluated by using the interior branch test method with MEGA4 software (www.megasoftware.net). Percentage of bootstrap support is shown by values at the branch nodes of the tree. Only nodes with a bootstrap value >50 are labeled; these values are the result of resampling the data 1,000 times. Black diamonds, isolates identified in the current study; white squares, GenBank sequences with the highest sequence homology to our sequences; ?, genotype 4 strains that could not be subtyped but closely clustered with each other, forming a new subtype. Scale bar indicates nucleotide substitutions per position.

reference sequence (AF234501). Five of the isolates, which shared 89.4%–91.2% sequence identities, belonged to subtype 4d, defined by reference strain AB082559. Four of the isolates shared 92.4%–95.7% sequence homology and could be defined as subtype 4a, according to the reference stsrain (AJ344171).

Notably, the other 4 genotype 4 isolates could not be subtyped on the basis of the reference strains described by Lu et al. (4) but closely clustered with each other, forming a new subtype (Figure 1). The 3 genotype 1 isolates identified in this study shared >98% sequence homology and could be defined as subtype 1b, a virus subtype mainly prevalent in China (4). The 3 genotype 3 isolates in this study shared 97.7%–98.6% sequence identities and were closely related (96.4%–97.9% sequence identities) to a swine isolate (FJ527832), found in swine in the Shanghai area (7).

Genotype 4 HEV has been found to be the dominant cause of hepatitis E in China and has been involved in zoonotic transmission in eastern and southern China (3,8). When a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search in GenBank was performed, we found that some genotype 4 isolates obtained in this study shared the highest sequence homology with previous swine strains (Figure 1). For subtypes 4a, 4c, and 4e and the new subtype groups, the swine strains that showed the closest relationship with our studied strains had been isolated from swine in other regions of China; this finding suggests that these isolates might not be indigenous to eastern China (Figure 1). Fifteen of the genotype 4 isolates in the 4b group had >96.3% sequence identity and closely clustered with a swine strain (EU375332) that had been isolated from swine in eastern China (9); strain ECh118 had 99.3% sequence homology to this swine strain (Figure 1).

Because the serum specimen from which ECh118 was identified was insufficient for complete genome amplification, EChZ20 was selected for complete genome sequencing using the primers described previously (10). The complete genome comprises 7,228 nt, excluding the 3′ poly(A) tail. The open reading frame 1 (ORF1) begins at nt 26 and ends at nt 5140 (5,115 nt); ORF2 (nt 5137–7161) comprises 2,022 nt and encodes 674 aa; ORF3 (nt 5165–5509) comprises 345 nt and encodes 114 aa.

The phylogenetic tree obtained for the complete genome of EChZ20 and other 16 representative genotype 4 isolates indicated that EChZ20 closely clustered with DQ450072, EF570133, and AB369690 and shared 90.7%, 91.7%, and 89.1% sequence identities with them, respectively (Figure 2, panel A). DQ450072 and EF570133 were isolated from swine in eastern China (10), and AB369690 was isolated from a patient from Japan who had traveled to Shanghai, China, before the onset of acute hepatitis E, according to information in GenBank. These results sug-

gested that subtype 4b virus isolates were involved in cross-species transmission from swine to humans in eastern China.

The genotype 3 isolates found in many countries, whether from swine or humans, have been reported to show a strong genetic relationship (11–13). Since 2007, genotype 3 has been found in swine groups in several areas in China (14); however, no reports have indicated that genotype 3 HEV infects humans in this country. In our study, genotype 3 was detected in humans in eastern China, and the strains found in humans showed high sequence homology (96.4%–97.9%) to the virus strains prevalent in swine in
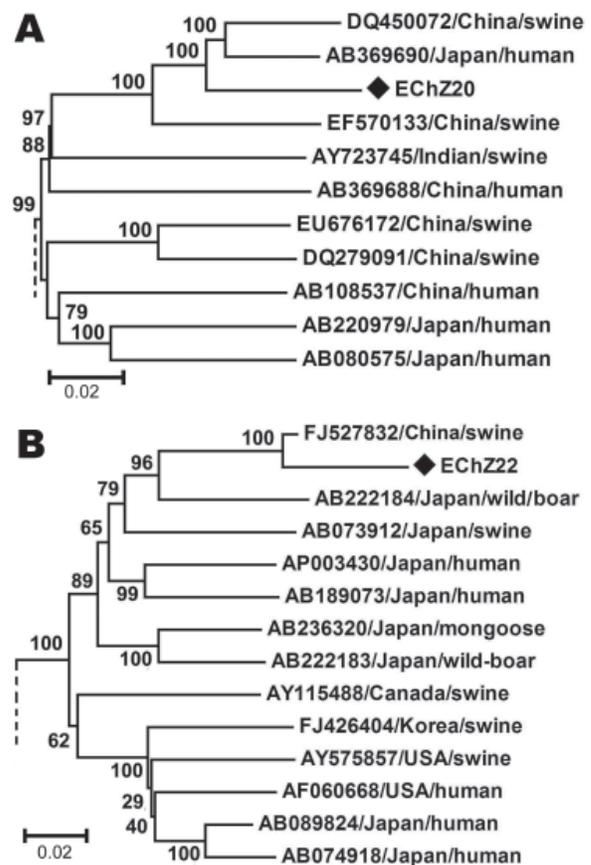
Figure 2. Phylogenetic tree showing alignment of the complete genome of EChZ20 of hepatitis E virus determined in the present study and the referenced genotype 4 isolates with complete genome available in GenBank (A), and the 1,681-nt partial open reading frame 2 sequence of EChN22 and referenced genotype 3 isolates with complete genome available in GenBank (B). The tree was constructed by using the neighbor-joining method and evaluated by using the interior branch test method with MEGA4 software (www.megasoftware.net). Percentage of bootstrap support is indicated at each node. GenBank accession number, source, and country of origin are indicated. Strains identified in this study are indicated by black diamonds. Only partial branches that were sufficient for elucidating the relationship between the study strains and their related strains are shown. Scale bars indicate nucleotide substitutions per site.

China. We intend to amplify the complete genome of this virus strain; however, because the quantity of serum in this study was limited, only a 1,681-nt partial ORF2 sequence of EChN22 was obtained.

Phylogenetic analysis, based on the 1681-nt sequence of EChN22 and other genotype 3 strains available in Gen-Bank, confirmed that EChN22 belonged to genotype 3b and that it clustered closely with a swine isolate from China (FJ527832) and shared 97.2% nt and 99.6% aa sequence homology with it (Figure 2, panel B). These results suggested that the genotype 3 virus strain prevalent in humans in eastern China might come from swine in this area. The sequences were deposited in GenBank under accession nos. HM439249–HM439285

Conclusions

Our study indicated that 3 genotypes of HEV (1, 3, and 4) are prevalent in humans in eastern China. The 31 genotype 4 strains could be further divided into 5 different subtypes, including a new subtype that, to our knowledge, has not been subtyped in previous studies. The complete genome of 1 representative strain of the most prevalent subtype (genotype 4b) was sequenced, and it showed a close relationship with swine strains prevalent in swine in eastern China.

Three genotype 3 HEV strains were identified, and one of them was selected and sequenced to obtain a longer sequence (1,681 nt). Phylogenetic analysis based on the 1,681-nt partial ORF2 sequence indicated the genotype 3 strain showed relatively high sequence homology (97.2%) to virus strains recently isolated from swine in eastern China. This finding suggests that the genotype 3 HEV strains prevalent in humans and swine in this area might come from a common infection source.

In the 1980s and early 1990s, genotype 1 was considered the predominant genotype in China; since 2000, genotype 4 HEV has become the dominant cause of hepatitis E disease in China (15). Our current study showed that genotype 3 HEV could be associated with human HEV infection in China. These results may provide a hint that China is transitioning from experiencing HEV infection that was primarily associated with HEV strains transmitted along the human-to-human route to strains transmitted zoonotically.

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Emergence of Oseltamivir-Resistant Pandemic (H1N1) 2009 Virus within 48 Hours

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An oseltamivir-resistant influenza A pandemic (H1N1) 2009 virus evolved and emerged from zero to 52% of detectable virus within 48 hours of a patient's exposure to oseltamivir. Phylogenetic analysis and data gathered by pyrosequencing and cloning directly on clinical samples suggest that the mutant emerged de novo.

Early descriptions of emergence of H275Y mutants in pandemic (H1N1) 2009 virus showed resistance after 11 and 23 days of therapy in immunosuppressed patients (*1*). Also in previous reports, transmission of mutant viruses occurred in immunosuppressed patients (*2*), although a cluster among healthy persons demonstrated that H275Y mutants could replicate and cause disease in the absence of drug pressure (*3*). Additional reports noted decreasing times to detection of resistance, from 14 to 4 days after therapy (*4–6*). We report development of oseltamivir-resistant pandemic (H1N1) 2009 virus in an infected woman in Singapore within 48 hours of drug treatment.

The Study

Pandemic (H1N1) 2009 virus was first detected in Singapore in May 2009. Infected patients were placed in isolation and offered oseltamivir, and respiratory samples were collected for screening for H275Y, the principal mutation associated with oseltamivir resistance in influenza A N1 viruses. H275Y was detected in a pandemic (H1N1)

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2009 virus isolated from a sample from a 28-year-old female patient on the sixth day of illness within 48 hours of her exposure to oseltamivir. (Written patient consent was obtained under Review Board approval no. E09-230.) A sore throat, myalgia, redness of the right eye, and a mild fever with a productive cough had developed on the day she returned to Singapore from Hawaii. Eleven close contacts, exposed before emergence of the mutant, were given oseltamivir prophylaxis on the patient's fourth day of treatment, and they remained well. By performing sequencing directly on 6 of her respiratory samples and on their viral isolates (online Technical Appendix, www.cdc.gov/EID/content/16/10/1633-Techapp.pdf), we investigated the origin of this H275Y mutant (the second earliest sample of this mutation to be deposited in GenBank).

Only wild-type sequences were detected in samples collected on the day before, the day of, and 14 hours after initiation of oseltamivir therapy (online Technical Appendix Table 1). Similarly, only wild-type sequences were detected in 192 clones generated from a sample collected a few hours before initiation of oseltamivir. Pyrosequencing directly on clinical samples collected 38 and 45 hours after initiation of therapy showed 24% and 52% mutant sequences, respectively (Figure). The relative amount of virus detected, as determined by the strength of PCR results (online Technical Appendix Table 1), increased from days 3 to 5 of illness by ≈1,000-fold. Oseltamivir treatment was initiated on day 4 of illness. On the same day, her maximum body temperature (38.8°C) was recorded, although no other signs or symptoms of clinical deterioration were observed. Her fever resolved on day 5 of illness, and she was allowed out of isolation on day 7 of illness.

When we compared the mutant drug-resistant isolate GN285 with the wild-type drug-sensitive isolate ON129, we found only 1 aa difference, the H275Y resistance-causing mutation in the neuraminidase gene, whereas a comparison of GN285 and ON129 with the reference strain A/Texas/05/2009(H1N1) showed several mutations (online Technical Appendix Table 2). Mutation PB1 I435V, shared between GN285 and ON129, did not occur in any of the other 7 drug-resistant strains included in the analysis. The whole genome maximum likelihood tree (online Technical Appendix Figure 1) showed that the wild-type and resistant viruses isolated from this patient were more closely related to each other than to any other virus in the analysis. Notably, GN285 and ON129 clustered together in 376 of the 500 bootstrap tests.

Conclusions

Our data indicate that oseltamivir resistance developed within 2 days. This time is similar to the interval for development of resistance to adamantanes in subtype H3N2 viruses when 30% of treated patients shed resistant, trans-

missible virus within 3 days of beginning treatment (*7*). Four cases of H275Y infection have been detected, with the use of sequencing, among 1,060 pandemic (H1N1) 2009 isolates tested (0.47%) in Singapore since June 2009 (not including the case reported here). Only 1 case was found in a pretreatment sample; the other 3 were identified after

treatment. The emergence of H275Y might also be affected by the timing of therapy. In the case reported here, oseltamivir treatment was begun on day 4 of illness when viral titers were almost maximal, which is probably the stage of illness best suited to select for resistant mutants because the presence of mutations is likely to be greatest when replica-
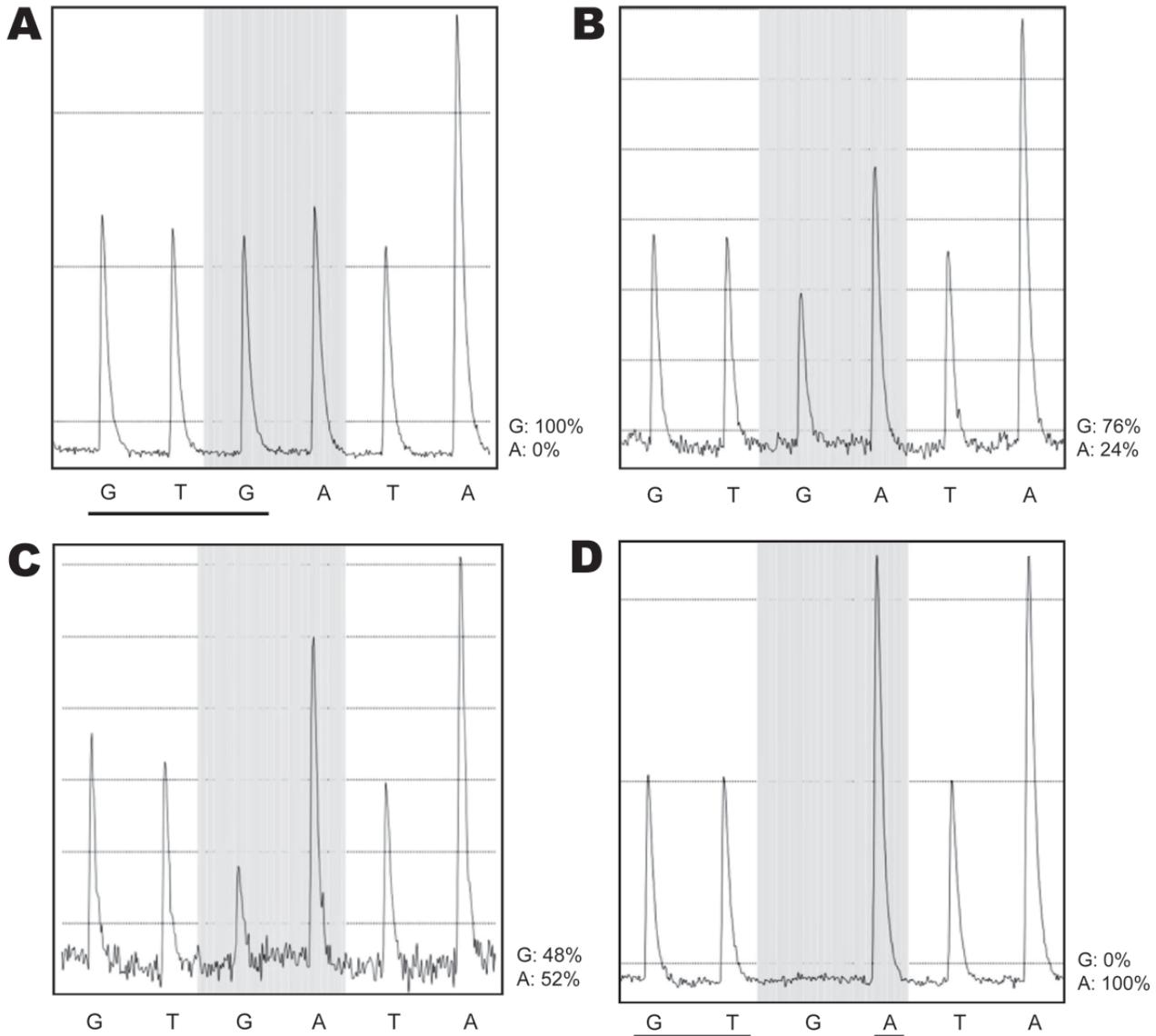
Figure. Pyrograms showing evolution of the H275Y mutation in pandemic (H1N1) 2009 virus, Singapore. A) May 29, sample 14 h after receiving oseltamivir shows 100% G. B) May 30, 38-h sample shows 76% G and 24% A. C) May 30, sample at 45 h shows 48% G and 52% A. D) May 30, virus isolated from 38-h sample is 100% A. The shaded area indicates the mutation site, showing the progressive loss of the third base, G, and its replacement by A. In panel A, all bases have peaks of equivalent height because each base is a singlet, except for the last peak, which is double the height and represents an A followed by another A, as in AA. In panel D, the G at the mutation site has disappeared and the signal of the next base, A, has doubled in amplitude, indicating the complete replacement of G by A. This reflects replacement of the complementary base C by T in the viral template. The 3 bases that constitute aa 275 in the neuraminidase protein are underlined in panels A and D. In panel A, the sequence is 100% GTGATAA. In panel D, it is 100% GTAATAA. In panel A, the wild-type 5′-GTG-3′ is equivalent to 3′-CAC-5′ in its plus-strand RNA, which codes for histidine (H). Similarly, the mutant 5′-GTA-3′ in panel D is equivalent to 3′-CAT-5′, which codes for tyrosine (Y). Therefore, the pyrograms show a mutation from H to Y at position 275—the H275Y mutant.

tion is greatest. Notably, rates of H275Y are high, reaching 13% (8) among immunocompromised groups in whom high viral titers might also be a contributory factor.

Pyrosequencing directly on clinical material allows the measurement of relative quantities of viral variants without introducing errors inherent in viral culture, which is known to favor the growth of H275Y mutants (6). Pyrosequencing cannot exclude the presence of subpopulations of <5%–10%, but if small proportions of mutant virus had been present, they would have been detected in the culture of the sample collected 14 hours after exposure to oseltamivir; however, only wild-type sequences were detected. Similarly, only wild-type sequences were detected in 192 clones derived directly from the sample collected a few hours before the first dose of oseltamivir. The phylogenetic data show, at the amino acid and nucleotide level, that the resistant and sensitive isolates cluster together, apart from other viruses. These data support the hypothesis that the H275Y mutant arose de novo from the wild-type virus from the same patient.

The fact that the effects of oseltamivir are likely to be greatest in severe disease (9–11), but of modest benefit in mild infections (12), has led to proposals for restricting the use of antiviral agents and the use of alternative antiviral agents and multidrug therapy to prevent the emergence of resistance (7,9) in severe cases. The proposed interventions may be of little consequence compared with the association and co-selection of H275Y with other genetic determinants. Although in isolation H275Y compromises seasonal influenza (H1N1) by reducing the amount of neuraminidase expressed on the cell surface, other mutations (R194G, V234M, and R222Q) may compensate and restore its expression to levels found in wild-type virus, without H275Y (13). This circumstance may explain the emergence and spread of H275Y in the absence of drug pressure in seasonal influenza (H1N1), which increased from being negligible in 2007 to 95% in March 2009 (7) despite a low consumption of oseltamivir (14). More than 99% of all pandemic (H1N1) 2009 neuraminidases have G at position 194, which corresponds to the R194G in seasonal influenza (H1N1). However, the effects of mutations are not easily transferable among different influenza (H1N1) types and may need to be tested separately (online Technical Appendix).

The clinical effects of the spread of H275Y in seasonal influenza (H1N1) have been minimal because seasonal influenza (H1N1) now accounts for an insignificant proportion of influenza (15). However, if H275Y mutants of pandemic (H1N1) 2009 emulate the expansion of resistant seasonal influenza (H1N1), the effect might be substantial because pandemic (H1N1) 2009 accounted for 90%–95% of circulating influenza A viruses in the Northern and Southern Hemispheres in late 2009 (15). We are speculat-

ing, however, because our laboratory data show that pandemic (H1N1) 2009 fell from 62% to 29% of 436 influenza cases from May 2010 to mid June 2010, Singapore's main influenza season, whereas the presence of influenza (H3N2) has risen from 23% to 53% (influenza B accounts for 15%–20%). As the next influenza season in the Southern Hemisphere approaches, the relative mixture of subtype H3N2 and H1N1 viruses will be under scrutiny again, not only to predict "best bet" vaccine components but also to ascertain their associated resistance patterns. Whatever the epidemiologic data exhibit, clinicians should consider resistance when patients do not respond to treatment for pandemic (H1N1) 2009 because H275Y can emerge literally overnight, as the case reported here reminds us.

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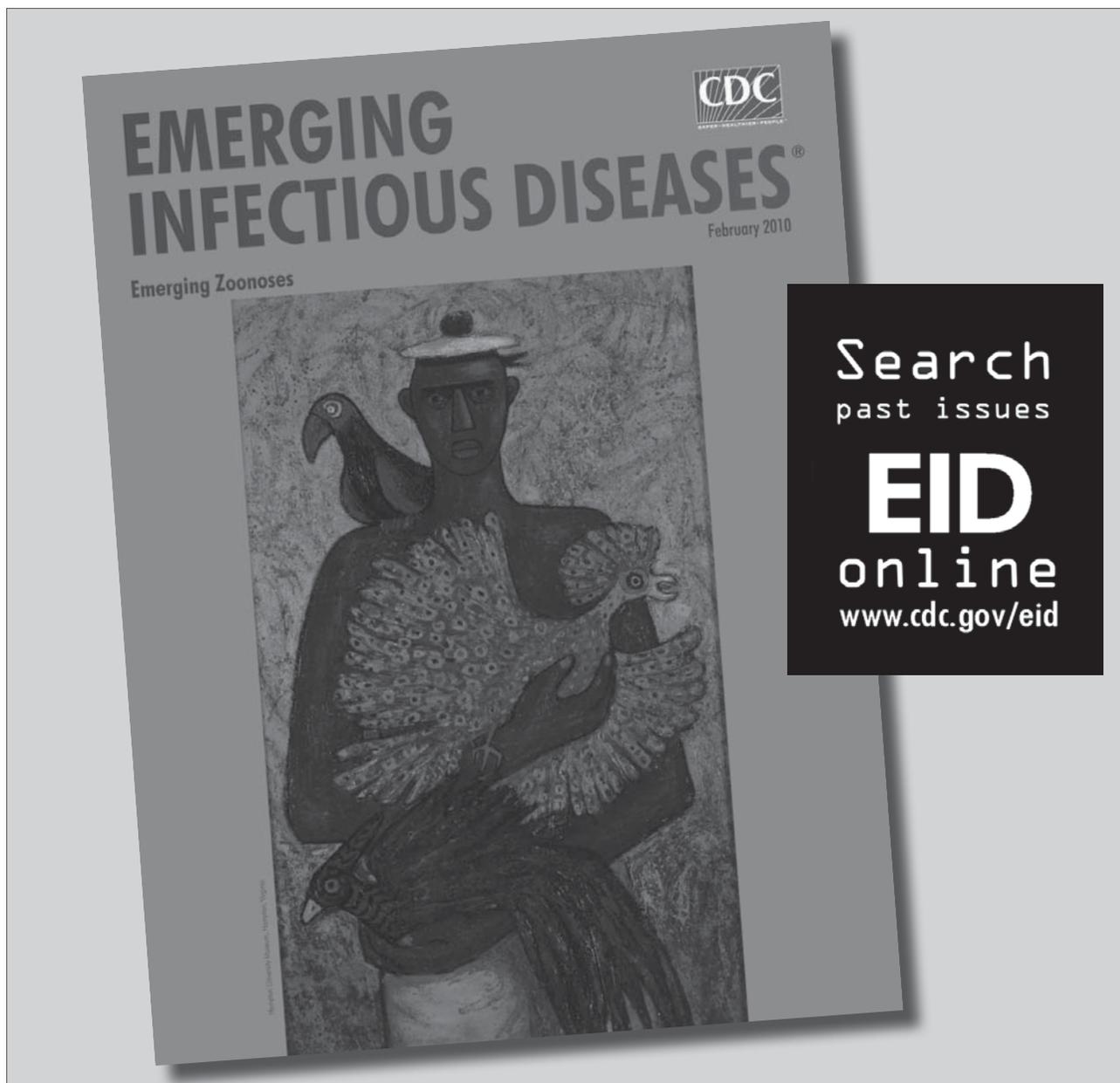
Avian Leukosis Virus Subgroup J in Layer Chickens, China

To the Editor: In recent years, cases of avian leukosis virus subgroup J (ALV-J) infection and tumors in commercial layer chickens and breeders of egg-type chickens have been emerging in the People's Republic of China. ALV-J was first isolated from meat-type chickens with myeloid leukosis in 1988. Although egg-type chickens have been experimentally infected with ALV-J to induce tumors (*1*), field cases of ALV-J infection and tumors in commercial layer chickens were not found worldwide until 2004 (*2*).

ALV-J has recently been found to have induced various tumors and caused production problems in commercial layer flocks and local chicken breeds in China (*2,3*). Many field cases of ALV-J infection and tumors have occurred in 15- to 29-week-old egg-type chickens in several provinces. Affected flocks had dramatically reduced egg production and hemorrhage in the skin surrounding the phalanges and feather follicles. Some birds had gray-white nodules in the liver, spleen, or kidneys, and liver and spleen were enlarged up to several times their normal size. Morbidity rates for some flocks reached 60%, and mortality rates for some flocks were >20%. Clinical samples from livers, spleens, whole blood, and tumors were collected from chickens in different provinces and sent for laboratory diagnosis. Results showed that the predominant virus in the samples was ALV-J.

During 2007–2009, we conducted an epidemiologic investigation of ALV in layer flocks in China. All virus isolation was performed in DF-1 cells. Briefly, 233 clinical samples were collected from 44 layer flocks in different provinces and used to inoculate subconfluent cell cultures containing Dulbecco modified essential medium supplemented with 10% (vol/vol) fetal bovine serum and antimicrobial drugs. After a 7–9 day incubation period, the cells were frozen and thawed 3×. A group-specific antigen-capture ELISA was used to identify ALV. After proviral DNA was extracted directly from infected cell culture or tumors, PCR with strain-specific primers was used to detect ALV-A, ALV-B, or ALV-J (*4*).

Of these samples, 150 (64.4%) were ALV-J positive, 28 (12.1%) were ALV-A positive, and 8 (3.4%) were ALV-B positive. Phylogenetic analysis showed an 87.3%–98.2% aa sequence identity of *env* genes in all ALV isolates compared with the HPRS-103 strain (*5*). All isolates had complete repeated transmembrane deletion and partial direct repeat–1 deletion but contained an intact E element. A mutation was found in the enhancer and promoter region of the U3 region in the 3′ long terminal repeat; this mutation is not found in ALV-J isolated from broiler chickens (*6*).

The newly isolated ALV-J strain from layer chickens was used to examine the pathogenicity in 1-day-old White Leghorn specific pathogen–free chicks soon after hatching in separate incubators and rooms in the experimental animal house facilities at Harbin Veterinary Research Institute, Harbin, China. The chicks were inoculated intraabdominally with a 1,000-unit 50% tissue-culture infective dose of ALV-J propagated in the DF-1 cells. Blood samples were collected to check for viremia at 10 weeks of age. Experimental birds were reared until 27–30 weeks of age.

Prolonged viremia developed in 15 (50%) of 30 chicks; hemangiomas developed in the skin surrounding phalanges and in the liver of 3 (10%); and myeloid leukosis, detected by gross or histologic examination, developed in 10 (30.3%). A previous study showed that meat-type birds infected with ALV-J retained a high level of viremia over their lifetime (*7*) but that layer chickens cleared the infection within a few weeks. Our study demonstrated that ALV-J infection can cause disease in layer chickens and can induce tumors and long-lasting viremia. For this reason, disease caused by ALV-J in layer chickens in China should be further investigated.

Because ALV-J is vertically transmitted from dam to progeny by the embryo, it represents a potential threat for humans who receive vaccines that are produced in chicken embryonic fibroblasts or embryonated eggs (e.g., yellow fever vaccine and measles and mumps vaccine) (*8*). An effective vaccine against ALV is not available. Eradication of ALV-J has been difficult because of substantial genetic and antigenic variation among ALV-J isolates as well as high levels of vertical and horizontal transmission (*9,10*). Therefore, effective prevention and elimination measures should be developed as soon as possible.

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Healthcare Worker Acceptance of Pandemic (H1N1) 2009 Vaccination, Morocco

To the Editor: In Morocco, the first case of pandemic (H1N1) 2009 was diagnosed on June 12, 2009 (*1*). Because a main determinant of public immunization success is healthcare workers' support and recommendations and because little is known about such with regard to pandemic (H1N1) 2009 vaccination in Morocco, our aim was to document healthcare workers' knowledge, attitudes, practices, and acceptance of pandemic (H1N1) 2009 vaccination in Morocco.

From January 15 through February 28, 2010, a structured, self-administered, anonymous questionnaire was distributed to a convenience sample of 1,332 healthcare workers in 5 public hospitals in Rabat, Morocco. Completed questionnaires were analyzed by using SPSS version 10.0 (SPSS, Chicago, IL, USA). The 1,002 responses gave a response rate of 75% (≈17% of the entire staff of the University Hospital of Rabat).

We found that the hospital staff had acquired basic knowledge about transmission and prevention of the pandemic (H1N1) 2009 virus. Responses indicated that 218 (22%) study participants had accepted vaccination (i.e., had been vaccinated) against this virus. Markedly more healthcare workers in Morocco were undervaccinated than were those in the United States; by mid-January 2010, estimated vaccination coverage among healthcare workers was 37.1% (*2*). Some evidence indicates that willingness of healthcare workers to be vaccinated with the new vaccine is poor: 48.0% in Hong Kong Special Administrative Region, People's Republic of China (*3*) and 22.3% in the United States (*4*). Vaccination coverage was significantly higher for those 20–30 years of age

than for those in other age groups (p = 0.001). The analysis by occupational category showed significantly higher coverage for paramedical staff (26%) than for physicians and pharmacists (19%) (p<0.01). The main causes for this reluctance were fear of adverse effects, concerns about the new adjuvant used, the short duration of clinical trials, and influence of the media.

The low acceptance rate of vaccination for pandemic (H1N1) 2009 among healthcare workers in Morocco is alarming because they serve as an example for their patients and the public. Vaccination is needed to keep the healthcare system operating at maximum capacity during a pandemic. The following factors appear to play a major role in acceptance: accessibility of the vaccine within the service; free vaccine; and a display explaining vaccination's benefits, protective value, and risk for adverse effects (*5,6*). Policy makers could use our findings to improve the vaccination strategy for healthcare workers in future vaccination campaigns.

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New Rural Focus of Plague, Algeria

To the Editor: Plague is a deadly rodent-associated flea-borne zoonosis caused by the bacterium *Yersinia pestis* (*1*). Human plague periodically reemerges in so-called plague foci, as illustrated by the 2003 reemergence of human plague in the Oran area, Algeria (*2,3*). We report emergence of a new plague focus in a remote region of Algeria.

In July 2008, three patients came to Laghouat University Hospital with signs of severe infection and painful, inflamed, enlarged lymph nodes suggestive of buboes. One additional patient became ill with pneumonia and coma after a bubo appeared. The patients were nomads living in a 24-person camp in Thait El Maa in the Laghouat area, 550 km southwest of Algiers (Figure). Plague was confirmed by culturing *Y. pestis* from 1 bubo aspirate. Ten days of oral doxycycline (4 mg/kg/d) combined with oral rifampin (20 mg/kg/d) and intramuscular gentamicin (3 mg/kg/d) cured the patients with bubonic plague, but the patient with pneumonic plague died.

In January 2009, eight individuals of the rodent species *Meriones shawii* (Shaw's jird) and 2 *Psammomys obesus* (fat sand rats) were trapped inside nomads' tents (H.P. Sherman Traps, Tallahassee, FL, USA). At time of capture, there was a cold wind with blowing sand, and, after visual inspection of the rodents, efforts to recover fleas failed. DNA from the rodents' spleens was extracted by using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) at the Medical Entomology Unit Laboratory, Pasteur Institute, Algiers, and subjected to PCR amplification of the plasminogen activator gene (*pla*) from 6 *M. shawii* jirds. Negative controls (DNA extracted from uninfected fleas maintained as colonies in Medical Entomology Unit Laboratory was used in the absence of negative animal tissue) remained negative.

After sequencing, the PCR amplicons showed 100% sequence identity with *Y. pestis* reference sequences. Identification was further confirmed in Marseille, France, by culturing 2 rodent glycerol-negative *Y. pestis* isolates (Algeria 1 and Algeria 2) and sequencing *pla*, *caf*, and *glp*D genes. The latter sequence was identical to the reference *Y. pestis* CO92, an Orientalis biotype. Multispacer sequence typing found the following combination: spacer Yp3, type 5; Yp4, 1; Yp5, 1; Yp7, 8; Yp8, 2; Yp9, 2; and Yp10, 1, a pattern that is typical for all Orientalis isolates investigated by this method but does not match the combinations observed for other genotypes. The original spacer Yp7 type 8 ruled out contamination (*4*).
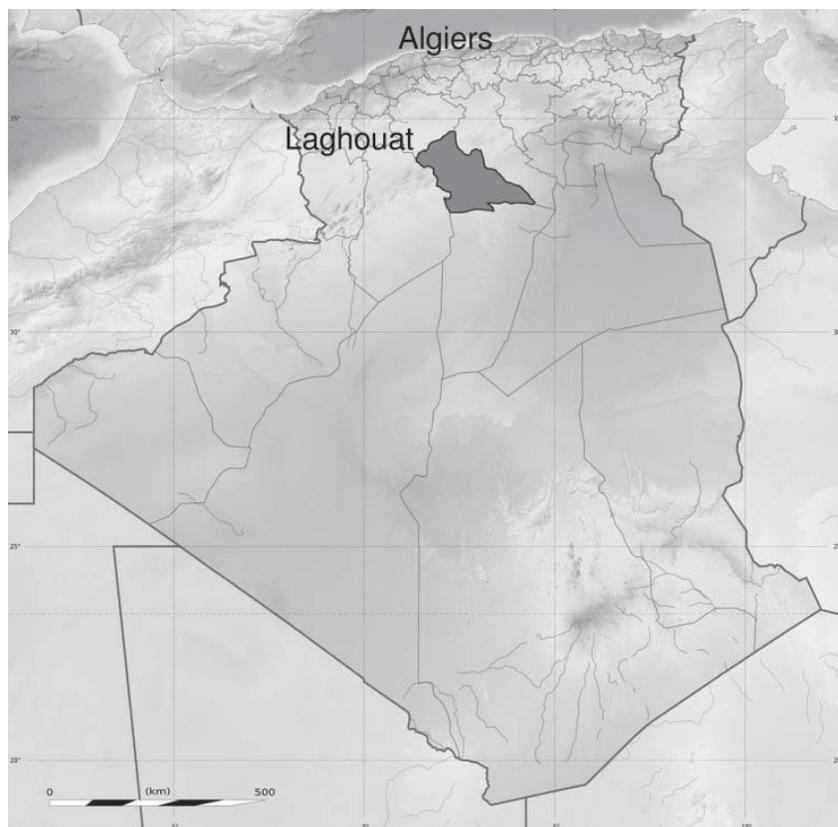

Figure. Location of a new rural plague focus in a nomad camp in Laghouat (dark gray shading; 35°29′N, 0°32′E), Algeria.

National health records indicate that plague foci have been known for decades in Algiers, Kahelia, Aumale, Philippeville, and Oran, where plague reemerged in 2003 after its abence for >50 years (*2,3*). In the Oran outbreak, it was not clear if reemergence resulted from importation through the international port of Oran or from a previously unknown rural focus (*3*). The Laghouat area was not previously known as a plague focus, and plague must therefore be regarded as an emerging disease in this region.

No patients with plague reported handling sick animals. Thus, the patients likely acquired plague from rodent flea bites. Because human ectoparasites were not found on the nomads, rodent ectoparasites must have transmitted the disease. It is unlikely plague had been imported into this region; the 2 rodent species from which *Y. pestis* was recovered are present in the area. To the best of the nomads' knowledge, there have been no reports of movements of commensal rats or other plague-susceptible rodents into the area near the sites where the patients acquired their illnesses.

We found *Y. pestis* in *M. shawii* jirds, a native rodent species living in close contact with human populations. *M. shawii* jirds have been shown to be a plague-resistant species (*5*) and thus are an efficient reservoir for *Y. pestis.* These data verify the presence of a new, rural zoonotic focus of plague. This situation is worrisome because nomads remain in close contact with rodents and fleas and the risk of further outbreaks remains high. In Oran and Laghouat, an Orientalis biotype sharing the same Yp8 and Yp9 spacer sequences was found, but limited multiple spacer typing of Oran strains hampered further comparisons beyond the biotype level (*3*).

A plague focus has been recently detected in a neighboring Libyan focus located at the same latitude as the Laghouat area (*6*). Our report suggests that extending surveillance to adjacent Libya and Mauritania, which also have natural foci of plague, is necessary. The reasons for emergence of plague in these regions are unknown, but *Y. pestis* can survive in the soil under laboratory conditions, possibly providing the opportunity for rodents to be infected and promoting reemergence of the disease (*7,8*).

Emergence of plague in an area of Algeria where it had never been reported illustrates the necessity to reinforce surveillance of plague in possible rodent hosts and their ectoparasites, which are in contact with humans, to prevent emergence and reemergence of this deadly infection. Surveillance should be maintained to monitor this natural focus and potential spread of plague that might occur because of climatic or habitat influences (*9*).

Acknowledgments

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Scrub Typhus Involving Central Nervous System, India, 2004–2006

To the Editor: Scrub typhus, caused by *Orientia tsutsugamushi,* is one of the most common infectious diseases of rural southern Asia, southeastern Asia, and the western Pacific. The disease is transmitted to humans by the bite of larvae of trombiculid mites harboring the pathogen. The disease often appears as a nonspecific febrile illness. The clinical picture of scrub typhus is typically associated with fever, rash, myalgia, and diffuse lymphadenopathy (*1*). Immunofluorescence assay (IFA) is the test of choice for serodiagnosis of rickettsial diseases (*2*). Scrub typhus has been reported from northern, eastern, and southern India, and its presence has been documented in at least 11 Indian states (*3–7*).

Our study's goal was to retrospectively analyze data of patients with scrub typhus involving the central nervous system. Scrub typhus was suspected on the basis of clinical signs such as febrile illness or fever with rash or eschar. The fever workup profile (Widal agglutination test, peripheral smear, blood, and urine culture) was noncontributory. Blood samples were obtained after patients gave informed consent. All patients with clinically suspected scrub typhus received

Table. Clinical features and laboratory investigations of patients who had scrub typhus with central nervous system involvement, India, 2004–2006*

Clinical and laboratory features	Results			
	Patient 1	Patient 2	Patient 3	Patient 4
Age, y/sex	52/M	50/F	30/F	47/F
Date of hospital admission	2004 Aug 22	2004 Aug 30	2005 Sep 9	2005 Sep 15
Fever duration before admission, d†	12	12	9	16
Chills	+	+	+	–
Rigors	+	+	+	–
Headache	+	+	+	–
Myalgia	+	+	+	+
Abdominal pain	+	+	–	–
Seizure	–	+	+	–
Altered sensorium	+	+	+	+
Conjunctival suffusion	+	–	+	+
Jaundice	+	+	+	+
Eschar	+ Axilla	–	–	–
Lymphadenopathy	+ Generalized	+ Cervical	+ Cervical	–
Meningeal signs	+	+	+	+
Urea, mg/dL	104	96	84	143
Creatinine, mg/dL	3.9	1.5	1.6	2.7
Bilirubin, mg/dL				
Total	3.5	2.7	4.6	3.0
Conjugated	1.0	1.7	3.6	2.6
Aspartate aminotransferase, IU	167	160	166	30
Alanine aminotransferase, IU	139	198	185	38
Alkaline phosphatase, IU	80	1,000	1,000	782
Proteinuria, mg/dL	+	–	+	+
CSF cytology	Lymphocytes, 54 cells/mm^3	Lymphocytes, 14 cells/mm^3	Lymphocytes, 60 cells/mm^3	Neutrophils, 38 cells/mm^3
Protein, mg/dL	125	69	34	118
Glucose, mg/dL	34	44	33	48
IFA titers‡				
IgG	512	512	Not done	Not done
IgM	<64	<64		
Drug treatment	Azithromycin, doxycycline	Ceftriaxone, doxycycline	Ceftriaxone, doxycycline	Ceftriaxone, doxycycline
Outcome	Died	Improved	Improved	Left hospital against advice

*CSF, cerebrospinal fluid; IFA, immunofluorescence assay; Ig, immunoglobulin.
†Fever defined as AM temperature >98.9° F or PM temperature >99.9° F.
‡IFA significant titers: IgG $\geq$128; IgM $\geq$64. The single serum samples, obtained at admission to hospital, were subjected to IFA by using a panel of 11 rickettsial antigens comprising spotted fever group rickettsiae (*Rickettsia japonica, R. helvetica, R. slovaca, R. conorii* subsp. *indica, R. honeï, R. heilongjangensis, and R. felis*), *R. typhi*, and *Orientia tsutsugamushi* (Gilliam, Kato, and Kawasaki strains).

antirickettsial drugs (doxycycline and/ or azithromycin) empirically. IFA and PCR of blood samples were performed to confirm scrub typhus following standard protocol (*3*). DNA was extracted from the blood sample (buffy coat) by using QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. A standard PCR specific for the 56-kDa protein with forward and reverse primers (OtsuF: 5′-AATTGCTAGTGCAATGTCTG-3′ and OtsuR: 5′-GGCATTATAGTAGGCTGAG-3′) was performed (*3*). PCR products were purified by using the QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. Sequencing reactions were done by using a DNA sequencing kit, dRhodamine Terminator Cycle Sequencing Ready Reaction Mix (Applied Biosystems, Foster City, CA, USA). Sequencing was performed on an ABI PRISM 310 DNA Sequencer (Applied Biosystems). The obtained sequences were identified by comparison with sequences available in GenBank by using the BLAST software (http://blast.ncbi.nlm.nih.gov) (*3*).

During 2004–2006, scrub typhus was confirmed in 27 patients; 4 had features of central nervous system involvement. All 4 had fever with altered sensorium and meningeal signs; 2 had seizures. No neurologic focal deficit was noted, but all showed cerebrospinal fluid abnormalities. One patient had an eschar, but none had a rash. Serum of 2 patients was subjected to IFA; both samples showed high titers (Table), and PCR for blood was positive for *O. tsutsugamushi* for all patients. Serum was not subjected to examination for leptospirosis. Patients were treated mainly on the basis of clinical grounds because results of serology were not available immediately. Some clinical features of scrub typhus and leptospirosis are similar, and dual infections have been reported (*8*); therefore, antimicrobial drugs active against both leptospirosis and

scrub typhus were included in treatment regimens. One patient received doxycycline and azithromycin, and the remaining 3 received ceftriaxone in addition to doxycycline. Two patients improved, 1 died, and 1 left hospital against medical advice. The clinical and laboratory details, treatments, and outcomes of all patients are given in the Table.

O. tsutsugamushi is an obligate intracellular parasite of professional and nonprofessional phagocytes that invades the central nervous system as part of systemic infection and is found in endothelial cells of blood vessels and in circulating phagocytes. A severe headache occurs almost invariably and has been used as a key clinical criterion for identifying suspected cases. Severe features of central nervous system involvement, such as neck stiffness, neurologic weakness, seizures, delirium, and coma, have been reported. Meningismus or meningitis has been found in 5.7%–13.5% of patients (*9*). The greatest degree of central nervous system involvement in rickettsial diseases occurs in Rocky Mountain spotted fever and epidemic typhus, followed closely by scrub typhus. The meninges are more commonly involved by *O. tsutsugamushi* than by other rickettsial infections, and the overall histologic picture in the central nervous system is best described as a meningoencephalitis (*9*). An exhaustive study of 200 cases of scrub typhus showed central nervous system involvement in most patients. However, focal central nervous system damage was rare, and during the encephalitis stage, few objective neurologic signs were apparent, other than those suggesting more generalized cerebral involvement, such as confusion, tremor, and restlessness (*10*).

Now that it is established that *O. tsutsugamushi* does invade cerebrospinal fluid, scrub typhus should be considered a cause of mononuclear meningitis in areas in which it is endemic. In our study 1 patient died

despite treatment with doxycycline and azithromycin, suggesting the possibility of resistance to these antimicrobial drugs as recently posited in a study conducted in southern India (*6*). Scrub typhus in these regions should be further investigated in prospective studies, and clinical isolates should be obtained to evaluate susceptibility to antimicrobial drugs.

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Pandemic (H1N1) 2009 and HIV Co-infection

To the Editor: We report a case of pandemic (H1N1) 2009 infection in a man with serologic evidence of HIV-1 infection. The clinical course was complicated by lung and brain involvement (respiratory failure and lethargy), severe leukopenia, and thrombocytopenia, but complications resolved after treatment with oseltamivir (150 mg 2×/d).

In November 2009, a 47-year-old man who had received a diagnosis of hepatitis C infection 8 months earlier sought treatment at Ospedale Santa Maria Nuova, Reggio Emilia, Italy. He had a 3-day history of fever, dry cough, and drowsiness. Eight days before being admitted, the man had resided in the hospital's inpatient detoxification unit, in which at least 10 influenza-like cases had been recorded. While in the detoxification unit, he had received methadone, 50 mg 1×/d. Computed tomography images of the brain and radiographs of the chest were normal; ultrasound examination showed upper lobe consolidation of the left lung. Hematochemistry showed high creatine phosphokinase levels, leukocyte count 1,380 cell/mm^3 (reference range 4,000–10,000 cells/mm^3), thrombocyte count 34,000 cells/mm^3 (reference range 150,000–450,000 cells/mm^3), partial pressure of oxygen 56 mm Hg, and partial pressure of carbon dioxide 53 mm Hg. Urinalysis results were negative for heroin, cocaine, and alcohol; cerebrospinal fluid (CSF) analysis results were within normal limits. Thrombocyte count returned to reference range after 2 days, and leukocyte count improved but remained <3,500 cells/mm^3 for 3 weeks. After admission to hospital, the man became lethargic and received noninvasive continuous positive airway pressure ventilation and treated with oseltamivir (150 mg 2×/d for 5 d), as well as with ceftriaxone, and levofloxacin. Reverse transcription–PCR on a throat swab confirmed influenza subtype H1N1 infection; blood cultures and urine were negative for pneumococcus, and *Legionella* spp. antigens. In addition, PCR of CSF for enterovirus and herpesvirus had negative results. The patient needed respiratory support for 4 days, after which his mental status and blood gases returned to reference levels. He was discharged from the hospital 2 weeks later.

On day 3 after admission, a nurse was accidentally exposed to the patient's urine through her eye. An ELISA was positive for HIV infection. Negative results for confirmatory Western blot tests on days 5, 15, and 23 showed the p24 and p41 bands; HIV RNA was >6 million copies/mL, CD4 lymphocytes 51% (reference range 29%–59%). Reverse transcription–PCR for influenza subtype H1N1 performed 2 months later on a stored CSF sample gave a negative result; PCR for HIV of the same sample indicated 25,000 copies/mL. In mid-December, because of a further drop in CD4 lymphocytes to 17% (214 cells/mm^3) and blood HIV RNA of 2.8 million copies/mL, the patient started highly active antiretroviral therapy and is being followed up as an outpatient.

Influenza (H1N1) and primary HIV infection share many signs and symptoms, such as fever, cough, sore throat, joint or limb pain, and diarrhea. The infections also share uncommon complications of the central nervous system (CNS); e.g., drowsiness, coma, and seizures. We cannot confirm that CNS involvement in the patient reported here was caused primarily by pandemic (H1N1) 2009, as suggested by influenza-like symptoms and the apparent effect of oseltamivir. Nor can we attribute CNS involvement to primary infection with HIV-1 (*1*); CSF results within normal limits and PCR negative for influenza subtype H1N1 do not rule out a causal relationship with pandemic (H1N1) 2009. In fact, the few cases of pandemic (H1N1) 2009 encephalopathy described show similar characteristics among children and adults (*2–4*). Alternatively, some authors have attributed HIV in CSF to brain inflammation and damage (*5,6*). The severe leukocytopenia and thrombocytopenia in our patient have not been described, even in complicated influenza subtype H1N1 infections (*7*). Because lymphopenia and mild thrombocytopenia are the usual findings, we believe that they probably resulted from HIV-1 or the effect of both viruses.

HIV seroconversion may initially occur during an acute febrile illness resembling influenza, and CNS involvement can complicate both infections. During an epidemic, acute HIV infection should also be considered (*8*). Less frequently, as in the patient described above, the 2 infections can occur simultaneously. History of recent risk behavior for blood exposure and severe leukocytopenia and thrombocytopenia should alert clinicians to other causes and prompt them to offer an HIV test to the patient.

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Dictyostelium polycephalum Infection of Human Cornea

To the Editor: Although *Dictyostelium* spp. are used for studying signal transduction, cytoskeletal functions, endocytosis, and molecular pathogenesis of infectious and other diseases (*1*), human or animal infections caused by this organism have not been reported. We report a case of keratitis caused by *Dictyostelium polycephalum* in an immunocompetent person.

A 35-year-old man sought treatment for redness, pain, and watering in the left eye of 11 days' duration. He had no history of ocular injury or surgery. At the time of his medical visit, he was using ophthalmic solutions of 5% natamycin sulfate, 0.5% moxifloxacin hydrochloride, and 0.3% gentamicin sulfate, each instilled every hour, and 1% atropine sulfate, 3×/d.

The vision in his right eye and results of a clinical examination were within normal limits. His left eye visual acuity was expressed as the ability to count fingers at 1 m. The eyelids were edematous and the conjunctivae were congested. The cornea showed a large central epithelial defect with underlying stromal infiltrate and Descemet folds. The surrounding cornea had a mild cellular reaction. The anterior chamber was deep, and the pupil was round, regular, and dilated. Iris and lens details could not be distinguished because of corneal haze. We obtained corneal scrapings, and the material was subjected to a detailed microbiologic analysis (*2*).

Microscopic examination showed double-walled spherical cysts in potassium hydroxide with calcofluor white stain, Gram stain (Figure, panels A, B), and Giemsa stain. On the basis of this finding, a presumptive diagnosis of *Acanthamoeba* keratitis was made. The patient was advised to use 0.02% polyhexamethylene biguanide and

0.02% chlorhexidine eye drops every half hour and 1% atropine eye drops 3×/d and was asked to return for a follow-up visit the next day. However, the patient did not return and could not be located. After 48 hours' of incubation, a nonnutrient agar plate showed growth of double-walled, spherical cysts ≈6–7 μm in diameter that had different morphologic features than those of *Acanthamoeba* spp. cysts.

To identify the organism, we extracted DNA from the growth on nonnutrient agar and subjected it to PCR specific for *Acanthamoeba* spp. (*3*); results were negative. The extracted DNA was then subjected to 18S rDNA PCR for free-living amebas as described by Tsvetkova et al. (*4*). A PCR product ≈800 bp was obtained and subjected to bidirectional sequencing with fluorescent-labeled dideoxy nucleotide terminators by using ABI 3130 XI automated sequencer in accordance with the manufacturer's instructions (PE Applied Biosystems, Foster City, CA, USA).

The Mega BLAST search program (www.ncbi.nlm.nih.gov/blast/megablast.shtml) of GenBank identified the sequence as *D. polycephalum* (99% similarity with AM168056). We deposited the sequence of our isolate in GenBank (accession no. GU562439). The organism showed cytotoxicity after in vitro inoculation of a rabbit corneal epithelial cell line.

The patient sought treatment 4 months after his initial visit. The left eye visual acuity was now expressed as the ability to see hand movements near the face. Slit-lamp examination showed lid edema and conjunctival congestion. The cornea showed a ring-shaped infiltrate, central thinning, surrounding corneal edema, and pigments on the endothelium (Figure, panel C); these findings were identical to the clinical picture of *Acanthamoeba* keratitis. Repeat corneal scrapings showed organisms of same morphologic features seen on the first visit by microscopy and culture. Organisms
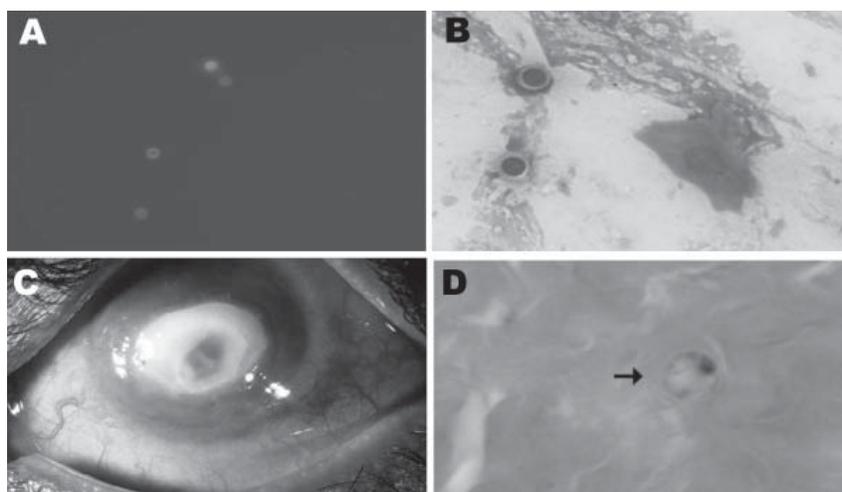
Figure. A) Spherical cysts of *Dictyostelium* spp. in potassium hydroxide (calcoflour white stain; original magnification ×40) preparation. B) Spherical double wall cysts of *Dictyostelium* spp. (Gram stain; original magnification ×100). C) Cornea of the patient's left eye, showing a ring-shaped central infiltrate and central thinning. D) Corneal button showing *Dictyostelium* spp. cysts (arrow; hematoxylin and eosin stain; original magnification ×100). A color version of this figure is available online (www.cdc.gov/EID/content/16/10/1646-F.htm).

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were reidentified as *D. polycephalum* by sequencing.

Because we were not aware of any drug treatment recommendations for infection by this organism, and the disease was advanced, surgical treatment was advised. Deep anterior lamellar keratoplasty was performed after 2 days. Histopathologic examination of the corneal button showed spherical cysts in mid stroma and inflammatory infiltrates (Figure, panel D). At the last follow-up (3 months after surgery), the corneal graft was clear with no evidence of infection.

Members of the genus *Dictyostelium* (social amebas or cellular slime molds) are divided into 4 high-level taxa with several species on the basis of DNA phylogeny (*5*). The life cycle of *Dictyostelium* spp. consists of an ameboid vegetative phase, a cyst phase, and a plantlike fruiting phase (*6*). *D. polycephalum* is ancestral and shows different characteristics than other species of *Dictyostelium* (*5,7,8*). In culture, it grows at a temperature of 34°C–35°C, which is higher than that for other species of *Dictyostelium* (*8*). Most myxamoebae aggregate to form sporocarps; however, some may round up in individual cells to form microcysts (*8*). The *D. polycephalum* isolated from our patient grew at 36°C on nonnutrient agar with an *Escherichia coli* overlay. The myxamoebae were seen after 24 hours, and the amebae had transformed into microcysts after 48 hours of incubation. However, on further incubation for 3 weeks at 36°C, no sporocarp formed.

Although we could identify the microorganism, the source of infection is unknown. Because the patient was a manual laborer, he could have become infected with the organism from contaminated water or soil. The clinical picture for keratitis caused by *D. polycephalum* was indistinguishable from that caused by *Acanthamoeba* spp. However, careful attention to cyst morphology in clinical samples and culture enabled us to identify this organism.

Underlying Medical Conditions and Hospitalization for Pandemic (H1N1) 2009, Japan

To the Editor: Early epidemiologic reports suggested that infection with pandemic (H1N1) 2009 virus most commonly occurred in teenagers and young adults (*1*). Although this infection appears to have a mild clinical course and the mortality rate has been relatively low in Japan (1.6 deaths/1 million population) (*2*), the reported number of patients with severe (requiring intubation or admission to an intensive care unit) cases of this disease has been increasing (*2*). The difference in severity of pandemic (H1N1) 2009 infection may be attributed to differences in underlying medical conditions or to age-related differences in susceptibility. To explore these differences, we investigated the incidence of laboratory-confirmed cases of pandemic (H1N1) 2009 virus infections resulting in hospitalization in Japan and the patients' age-specific

risks for hospitalization associated with underlying medical conditions.

During the outbreak in Japan, hospitals, local public health centers, and local governments were required to report hospitalizations associated with pandemic (H1N1) 2009 virus to the Ministry of Health, Labour and Welfare in Japan. Infection was confirmed by PCR at local public health centers. The data for hospitalized patients were integrated through a nationwide surveillance system, the interim National Epidemiologic Surveillance of Infectious Diseases.

We collected data on patients who were hospitalized in Japan for pandemic (H1N1) 2009 virus infection from July 28 through December 14, 2009. The number of new cases (incidence) increased in the middle of August and peaked at the end of November (*2*). We excluded from analysis those patients who had been hospitalized for the purpose of infection containment. Medical conditions were defined as diseases that are risk factors for severe illness associated with seasonal influenza: chronic respiratory diseases, chronic cardiovascular diseases, chronic renal diseases,

chronic liver diseases, neurologic diseases, hematologic diseases, diabetes, and immunosuppression caused by treatments or illnesses (including malignant neoplasm) and chronic childhood diseases (*3,4*). Data on the prevalence of the diseases among the population were obtained from the 2005 National Patient Survey and from population estimates obtained by the Ministry of Internal Affairs and Communications in May 2009.

During the surveillance period, 12,702 laboratory-confirmed cases for which the patients required hospitalization were reported; 110 of these patients died (Table). Hospitalization incidence was 10.0 admissions/100,000 persons (overall incidence of hospitalization in the general population in 2005 was 1,462.8 admissions/100,000 persons). Median age of hospitalized patients was 7 years (interquartile range 5–11 years). Of the 10,721 patients for whom type of care was known, 680 (6.4%) were admitted to an intensive care unit. Among 486 hospitalized women 15–44 years of age, only 42 (8.6%) were pregnant; this percentage is small compared with

Table. Age-specific incidence and risk for hospitalization with laboratory-confirmed pandemic (H1N1) 2009 virus infection, Japan, July 28–December 14, 2009*

Patient characteristic	Underlying medical conditions†		No underlying medical conditions‡		RR	AR
	No. (%) patients	Incidence/100,000§	No. (%) patients	Incidence/100,000§		
Age, y						
0–4	648 (22.1)	195.2	2,285 (77.9)	45.2	4.3	150.0
5–9	1,848 (32.6)	814.1	3,822 (67.4)	69.4	11.7	744.7
10–14	763 (35.2)	573.7	1,406 (64.8)	24.0	23.9	549.6
15–19	173 (38.9)	219.0	272 (61.1)	4.5	48.4	214.5
20–29	110 (43.8)	53.9	141 (56.2)	1.0	54.9	52.9
30–39	105 (49.8)	28.5	106 (50.2)	0.6	48.4	27.9
40–49	125 (61.6)	21.6	78 (38.4)	0.5	43.5	21.1
50–59	176 (76.9)	12.8	53 (23.1)	0.3	38.3	12.5
60–69	148 (77.5)	6.7	43 (22.5)	0.3	24.0	6.5
≥70	324 (81.0)	8.2	76 (19.0)	0.5	17.9	7.8
Gender¶						
M	2,857 (35.3)	NA	5,236 (64.7)	NA		
F	1,563 (33.9)	NA	3,046 (66.1)	NA		

*RR, relative risk for hospitalization; AR, attributable risk for hospitalization; NA, not available. Medical conditions defined as chronic respiratory diseases, chronic cardiovascular diseases, chronic renal diseases, chronic liver diseases, neurologic diseases, hematologic diseases, diabetes, and immunosuppression (including malignant neoplasm) and chronic childhood diseases.
†n = 4,420 (43.8%); 83 patients died.
‡n = 8,282 (65.2%); 27 patients died.
§Incidence = no. case-patients with pandemic (H1N1) 2009 virus infection and a particular medical condition/total population having the same medical condition (e.g., population 0–4 years of age with medical condition).
¶p value for difference in gender was 0.11, obtained by χ^2 test (2-sided) for testing the null hypothesis.

results of studies conducted in other countries (*5–7*). None of the pregnant women were in critical condition and none died of pandemic (H1N1) 2009 infection during the surveillance period. The overall lower prevalence of pregnant women in Japan (≈0.67% of the total population) compared with that in other countries (*8*) might account for the low number of pregnant women hospitalized for pandemic (H1N1) 2009 virus in Japan.

Of all 12,702 patients, 4,420 (34.8%) had underlying medical conditions. In terms of age, 1,848 (32.6%) of 5,670 patients 5–9 years of age and 324 (81.0%) of 400 patients >70 years of age had underlying medical conditions. For those with underlying medical conditions, incidence of hospitalization was relatively higher for children than for adults; rates for hospitalized children were 814.1, 573.7, and 219.0 per 100,000 persons for those 5–9, 10–14, and 15–19 years of age, respectively; whereas, the rate for hospitalized adults 60–69 years of age was 6.7/100,000 persons. In all age categories, the incidence of hospitalization for pandemic (H1N1) 2009 virus infection was notably higher for patients with underlying medical conditions than for those without. The attributable risk for medical conditions associated with hospitalization for pandemic (H1N1) 2009 virus infection was highest among patients 5–9 and 10–14 years of age (744.7 and 549.6, respectively), whereas the risk was lowest (6.5) among patients 60–69 years of age. The relative risk associated with underlying medical conditions was comparatively higher for patients in age groups 15–19, 20–29, and 30–39 years (48.4, 54.9, and 48.4, respectively) than among those >70 years of age (17.9). In contrast to seasonal influenza (*4*), persons <20 years of age had higher risk for hospitalization for pandemic (H1N1) 2009 than did older age groups.

Considering our findings regarding the attributable risk for hospitalization, interventions that aim to control pandemic (H1N1) 2009 virus infection among children, especially those with underlying medical conditions, can be considered key for minimizing the strain (financial, staffing, space) on the healthcare system. Our findings justify prioritizing the treatment of children and young adults by vaccination and early prescription of antiviral drugs.

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Internet Search Limitations and Pandemic Influenza, Singapore

To the Editor: In the past few years, several publications have reported that Internet search queries may usefully supplement other, traditional surveillance programs for infectious diseases (*1–3*). The philanthropic arm of Google offers Flu Trends, a site that provides up-to-date estimates of influenza activity in 20 countries of the Pacific Rim and Europe (*4*) by using data mining techniques to find good predictors of historic influenza indicators (*1*).

This service has yet to be extended to other countries and other diseases because access to official surveillance data is required, among other reasons. However, another Google service, Insights for Search, enables users to find and download time-series data of relative counts of arbitrary searches for a

large number of countries (*5*). Pelat et al. have shown that a few, well-chosen searches on Google Insights provide data that closely correlate with French surveillance data for seasonal influenza, chickenpox, and gastroenteritis (*3*). Although Internet searches appear to be a promising tool for public health surveillance, our experience from using Google Insights in the context of pandemic (H1N1) 2009 in Singapore suggests it has important limitations.

In Singapore, the recent pandemic caused an outbreak that peaked at the start of August 2009; the first confirmed importation was at the end of May and first confirmed unlinked case was at the end of June. However, the number Google searches for "influenza," "H1N1," "swine flu," and similar terms (in English and Chinese), as well as symptoms associated with the disease, peaked much earlier than did the number of cases (Figure). The number of searches surged after newsworthy events but was low during the epidemic itself and had declined to about 20% of maximum search volume by the time of the actual peak, as shown by traditional surveillance. Furthermore, no discernible local maxima were observed that corresponded to the peak in case data. In contrast, alternative traditional measures of influenza incidence—prevalence of the novel strain among viral samples and general practice surveillance (*6,7*)—provide a consistent description of the outbreak.

This finding echoes a major point raised by Carneiro and Mylonakis (*2*), namely, that without adjusting for spikes driven by disease publicity rather than the disease itself, Internet searches may lose much of their value in supplementing traditional surveillance measures. Our experience is that using Google Insights to survey a disease may not work well for diseases with considerable media exposure, in particular, emerging diseases such as pandemic (H1N1) 2009 or severe acute respiratory syndrome. Such outbreaks may require the more sophisticated approach used by Flu Trends, should it be extended to other diseases and more corners of the globe. We agree with Pelat et al. (*3*) that Google Insights may work well for less-publicized infectious diseases. The dividing line between well-publicized and unpublicized diseases may, however, remain ambiguous. Thus, to ensure that web search data reflect disease incidence requires validation against traditional surveillance, although in that situation, the availability of corroborating traditional methods of surveillance limits the value of web-query data.

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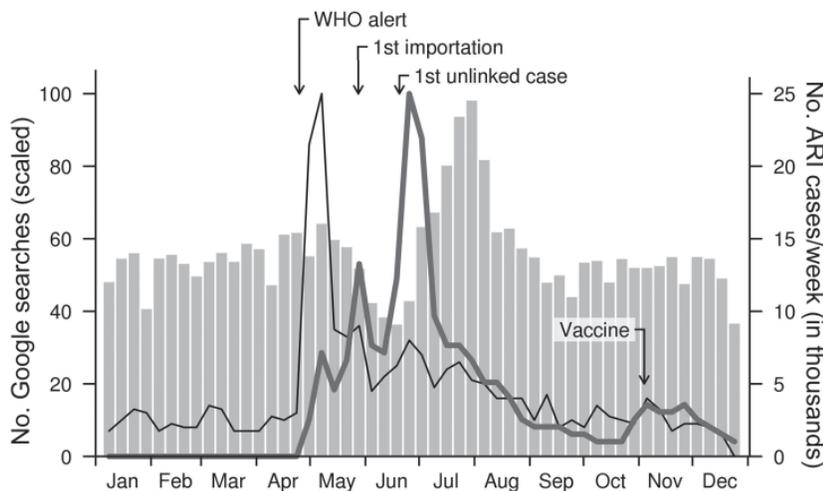
Figure. Number of Google searches conducted for "influenza" (black lines) and "H1N1" (gray lines) compared with number of acute respiratory infections (ARI, gray bars) reported in government clinics, Singapore, 2009. During the outbreak of pandemic (H1N1) 2009, Google search activity surged in response to newsworthy events (the World Health Organization [WHO] alert, first importation and unlinked local case, release of vaccine) but dropped substantially by the time most infections occurred in August. Other search patterns, such as for "swine flu" and simplified Chinese language terms for swine flu and influenza, were similarly disassociated with actual disease incidence.

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Body Lice, *Yersinia pestis* Orientalis, and Black Death

To the Editor: A scientific debate with public health implications wages: What caused the medieval European plague epidemics known as Black Death? Recent articles note inconsistencies between a rat flea–borne pandemic of *Yersinia pestis* (the bacterium that causes bubonic plague) and the documented characteristics of Black Death (*1*, among others). Ayyadurai et al. (*2*) acknowledge that a rat flea–only hypothesis does not fit Black Death observations, but they resolve theoretical transmission inconsistencies through a louse-borne hypothesis. Ayyadurai et al. base their surety of fact—that medieval "plagues" were caused by *Y. pestis* infection—on a 2007 study (*3*) in which 5 of 36 teeth of "plague" victims, none of which were dated to the Black Death era (1347–1351), contained biological evidence of *Y. pestis*. The 3 locations in that study were all port cities: 2 on the Mediterranean Sea and 1 on the Rhone River. As Duncan and Scott (*4*) note, bubonic plague most likely existed endemically near ship-borne trade, unlike the fast-moving epidemic fronts exhibited by medieval "plagues." Moreover, Gilbert et al. (*5*) found no *Y. pestis* DNA in 61 skeletons from primarily nonport locations in England, France, and Denmark.

We do not dispute the authors' claim that *Y. pestis* might have been present in some skeletons from port cities in France, or that body lice might, under certain circumstances, transmit the Orientalis biotype of *Y. pestis*; their work appears careful and considered. However, given the differences mentioned above and improved knowledge on the rapidity of virus mutation and worldwide transmission potential, we merely argue that the simplest explanation for medieval plagues has yet to be ruled out: that they may have resulted from a human-to-human transmitted virus. Adding complexity to an already complicated etiologic theory, and stating such as historical fact based on limited geography and sample size, does not seem congruent with Occam's razor.

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To the Editor: The letter of Ayyadurai et al. (*1*) reminded us of a little-known paper (*2*) on rats and Black Death by our colleague and mentor David E. Davis. He researched and wrote in his retirement after years of research and reflection on rat ecology and rodent-borne diseases (*3,4*). *Rattus rattus* is commonly recognized as the vertebrate host of flea-borne plague that swept through Europe in the 1300s, killing >50% of the population. Davis believed this explanation did not fit what he knew of the ecologic requirements of fleas and black rats. He studied reports of archeologic excavations and reviewed poems, medieval bestiaries, and paintings and concluded that these rats were scarce during the Black Death era.

His theory, based on historical information and investigative trips to Europe, was that invasive rats, if present, mostly occurred in low densities in port areas, not in rural inland areas. He noted that the expected rodent die-offs with bubonic plague were not associated with human epidemics and that rodent fleas would not have been active during winter to transmit plague. Flea-borne transmission from rodents usually causes a few deaths per household, but deaths of entire households commonly occurred in the medieval epidemics. Human-to-human transmission of pneumonic plague must have occurred, but as described by Ayyadurai et al., there was evidence of human bubonic plague, suggesting vector involvement. Davis did not present a viable reservoir/vector hypothesis for plague transmission; this and the later, well-known association of *R. rattus* and other rodents with plague throughout the world, may partially explain why his ideas received little attention. The finding that human body lice can be bubonic plague vectors suggests a mechanism for human-to-human transmission continuing during winter in inland areas and, as suggested by the authors, could also explain total deaths in households.

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In Response: Commenting on our recent demonstration that the human body louse was a likely vector of Black Death (the medieval European plague epidemics) (*1*), Welford and Bossak (*2*) point out that quantitative and qualitative inconsistencies in data for Black Death and modern plague argue against concluding that *Yersinia pestis* is the etiologic agent of Black Death (*3*). These authors acknowledge the paleomicrobiologic demonstration of *Y. pestis* in human remains collected at ports, yet they argue that such demonstration remains to be performed for human remains collected from inland burial sites (*2*).

Careful review of the literature indicates that 3 unrelated scientific teams have now demonstrated the presence of *Y. pestis*–specific biomolecules in 14th–18th-century human remains in 11 sites in Europe. These locations include 7 nonport, inland sites ≤650 km from the coasts (*4–6*) in addition to 3 Justinian (nonport) locations (*4,7*). Therefore, the fact that *Y. pestis* was the etiologic agent of Black Death can no longer be disputed; the inconsistencies correctly noted by Welford and Bossak actually question the reservoir and the vector of *Y. pestis* during the Black Death and the following epidemics rather than its cause. McLean and Fall remind us that the cumulative work of their mentor, David E. Davis, suggested that black rat ectoparasites could not have been likely vectors of medieval plague in Europe, based on the facts that expected die-offs of rats were not reported and that the rodents' fleas would not have been active during winter in medieval Europe (*8*).

McLean and Fall acknowledge that our experimental data pave the way toward an alternative scenario of body louse–borne transmission of the Black Death. Such transmission of *Y. pestis* was observed by Blanc and Baltazard during a cluster of bubonic plague cases in households in Morocco during World War II (*9*). These authors demonstrated that the body louse could be infected when living on a septicemic patient, could stay alive for 7 days with infectious feces, and could transmit plague (*9*). Demonstration of *Y. pestis* in human lice collected from Black Death burials would be a step toward understanding the epidemiology of Black Death; this technically demanding approach has been successfully used to assess the transmission of typhus in soldiers of Napoleon's Grand Army buried in Vilnius, Lithuania (*10*).

In agreement with these observations and those reported by Welford and Bossak (*3*), our work clearly indicates that *Y. pestis* could be efficiently transmitted by the human louse (*1*), a potential vector of Black Death because of its high prevalence in medieval Europe (*11*). Far from "adding complexity to an already complicated etiology theory," as Welford and Bossak stated, it seems to us that the cumulative evidence provided by paleomicrobiologic demonstration and by our recent work (*1*) clarifies the epidemiology of the Black Death and the subsequent epidemics. Louse transmission of *Y. pestis* also explains inconsistencies rightly noted by Welford and Bossak and provides a reason for the current plague cases in poor areas of the world where poor hygiene is common. A search for alternative hypotheses, including the previous viral hypothesis for Black Death, may not be necessary (*2*).

The analogic reasoning based on observations of current infectious diseases cannot be applied to the medieval Black Death. Paleomicrobiologic evidence and historical data force us to change the paradigm and to question the established dogma about the epidemiology of plague. McLean and Fall remind us that, even in science, alternative hypotheses have trouble challenging dogma (*8*). Black Death is one of many areas at the intersection of microbiology and history for which many hypotheses have been proposed and none has received confirmation; these hypotheses have been repeated for so long that they became accepted as demonstrated truths.

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Clostridium difficile Infections among Hospitalized Children, United States, 1997–2006

To the Editor: Zilberberg et al. described a notable increase in rates of *Clostridium difficile* infection (CDI)–related hospitalizations of children during 1997–2006 on the basis of analysis of data from 2 national administrative databases (*1*). As the authors acknowledge, they used administratively coded databases, which have inherent misclassification and testing biases.

Detection of *C. difficile* toxin indicates that bowel flora have been perturbed. However, the clinical role of toxin detection or isolation of *C. difficile* organisms in children is controversial. Although primary CDI is a recognized pathologic entity in children, one needs to consider whether another etiology related to a concomitant infection, antimicrobial drug administration, or alteration in enteral nutrition may be the precipitating event resulting in *C. difficile* toxin production.

It is our clinical observation that availability of testing for *C. difficile* and rapidity of assay results play a role in the submission of stool specimens for analysis. In 2007, we conducted a 5-month retrospective chart review of *C. difficile* testing practices at 2 local tertiary-care pediatric hospitals. Of 796 stool specimens submitted, 42 (5%) were notable for the detection of toxin A or B; these samples represented 35 patients (*2*). Medical coders likely face the same challenges as clinicians who must interpret toxin assay results and their clinical role with regard to hospitalized children. Although the ≈2-fold increase in CDI-associated hospitalization rates reported by Zilberberg et al. in their time series and cross-sectional analyses is notable, these results should be interpreted within the context of clinical and epidemiologic factors contributing to generation of this data.

Stephen M. Vindigni and Andi L. Shane

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In Response: I appreciate the letter by Vindigni and Shane pointing out the need for a cautious approach to treatment for infection with *Clostridium difficile* isolated from the stool of children, given their propensity for colonization by this organism (*1*). I could not agree more. In our report, we noted an increase over time in the rate of hospitalizations for not only *C. difficile* infections (CDIs) but also for rotavirus infections in children. This finding led us to acknowledge the possibility of a reporting bias for CDI (*2*). Other studies have detected a similar increase in CDIs among hospitalized children and have reported greater severity of associated disease (*3–5*). Such epidemiologic data, combined with emergence of the BI/NAP1/027 hypervirulent strain of *C. difficile* in the United States and abroad, support

a real increase in CDIs in a population for which the clinical definition is likely less specific than for adults. Although a more precise clinical definition for CDI in children (primarily those <2 years of age) is needed, studies like ours, which are necessarily limited methodologically, can serve to alert clinicians to be more vigilant to the possibility of disease caused by this evolving pathogen, even in a population thought to be at low risk.

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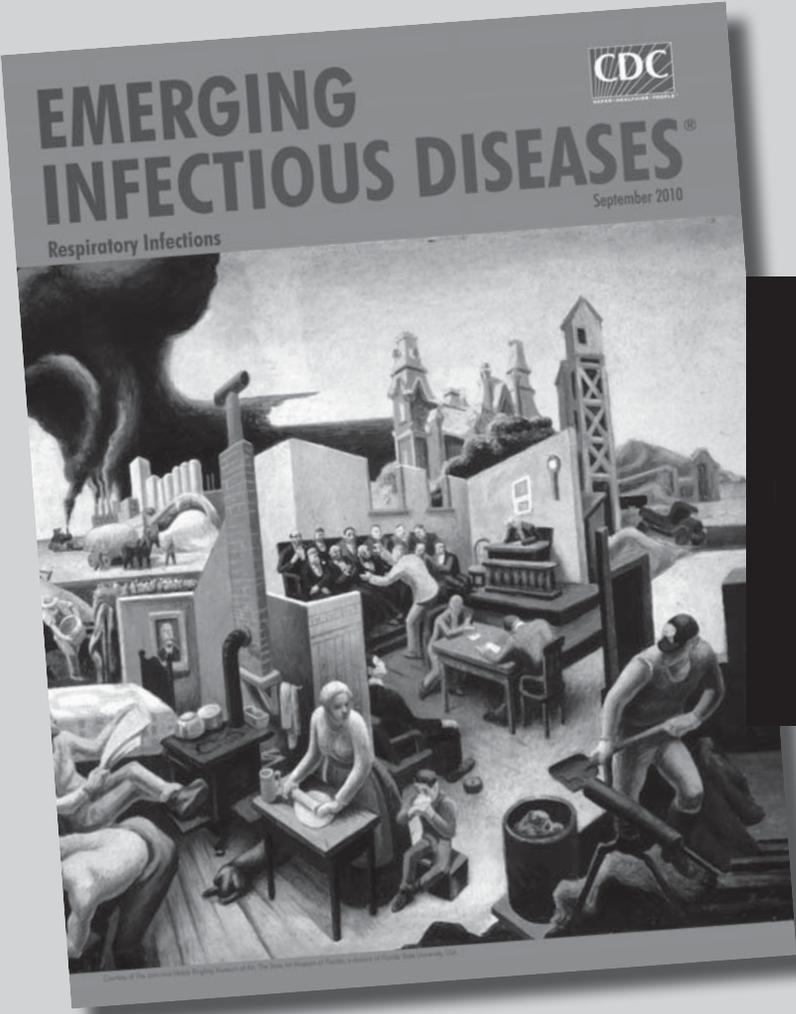
Living Weapons: Biological Weapons and International Security

By Gregory D. Koblentz

Cornell University Press, Ithaca, New York, USA, 2009
ISBN: 978-0-8014-4768-6
Pages: 272; Price: US $35.00

In the summer of 1996, as a (much younger) Army infectious disease physician, I headed off to a new assignment with the US Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Maryland. It was a heady time to be entering the niche field of biodefense. Five years before the events of 9/11 and the subsequent "Amerithrax" attacks would become an integral part of the world's consciousness, USAMRIID was (with the exception of a few veterinary laboratories studying anthrax, brucellosis, Q fever, and similar zoonotic diseases) the "only game in town." The institute had quietly wrestled with issues of medical biodefense since the United States shuttered its old offensive biowarfare program in 1969. As an assignee to its division of operational medicine, I was enamored of my ability to master the sparse literature and science of the field and soon became an "expert."

Such luxurious self-confidence is no longer warranted (if it ever was) or even possible. The field has, for better or worse, burgeoned in the decade since 9/11. Hundreds of texts and thousands of scientific articles now address every aspect of the daunting problems of biowarfare and bioterrorism defense, and mastery of such material is beyond the grasp of any one scientist, diplomat, or arms-control expert. Fortunately, several good reviews exist to guide those wishing to gain a basic understanding of these complex issues. Was yet another treatise thus necessary? My initial reaction was an emphatic "no," until I read Gregory Koblentz's book, Living Weapons.

Koblentz, deputy director of a graduate degree program in biodefense at George Mason University, tackles the myriad issues surrounding biodefense from a policy perspective. In so doing, however, he puts forth a readable, succinct, yet thorough review of the field. After a crisp introduction to the history of biowarfare, he focuses on the seemingly insurmountable obstacles to nonproliferation efforts aimed at state-sponsored weapons programs: treaty compliance verification, program oversight, and intelligence. He then addresses the parallel problems posed by nonstate actors and terrorists and closes with a prescription for reducing the dangers of uncontrolled biology.

A tour de force of bureaucratic hurdles and treaty-compliance issues would hardly seem compelling reading for all but a few die-hard policy wonks. Not so in the case of Koblentz's text. Even though his penchant for frequent summary makes for some redundancy, he provides an extremely readable, yet comprehensive, compendium of the implications for national security posed by wayward biology. Moreover, he does so without the ideological bias often found in other treatises on this subject. Specifically, Koblentz delivers a detailed review of the United Nations Special Commission inspections in Iraq and the lessons they provide. He similarly deals with the now-discredited 2002 National Intelligence Estimate, which provided much of the impetus for President George Bush's decision to invade Iraq. Yet, he analyzes these efforts and their implications for diplomats, policymakers, and arms-control experts without descending into partisan politics or anti-US demagoguery.

In summary, Living Weapons is a thoroughly readable book, filled with enough anecdotes to capture the reader's interest. But, more importantly, it provides a disturbing yet refreshing look at the myriad obstacles confronting those who would play a role—whether political, diplomatic, or scientific—in attempting to rein in the use of biology in war and terror. It is a must read for graduate students and experts alike.

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Superbug: The Fatal Menace of MRSA

Maryn McKenna

Free Press, New York, New York, USA, 2010
ISBN-10: 141655727X
ISBN-13: 978-1416557272
Pages: 288; Price: US $26.00

This book is an extensively researched and detailed review of methicillin-resistant *Staphylococcus aureus* (MRSA) by Maryn McKenna, a journalist and the former Centers for Disease Control and Prevention beat reporter for the Atlanta Journal Constitution. Although McKenna has a background in science reporting, she infused this work with drama, an approach that will draw in some readers but be off-putting to others. To the reader familiar with MRSA, the use of hyperbole coupled with factual inaccuracies leaves one wondering where truth stops and fiction begins. These shortcomings may keep this work off scholarly reading lists.

Most chapters include case presentations that emphasize the emotional toll wrought by MRSA infections. The cases effectively introduce topics such as MRSA in athletes and other risk groups, MRSA in animals, and postinfluenza MRSA pneumonia. The chapter on infections caused by the 80/81 strain of *S. aureus* in the 1950s is particularly useful because it demonstrates parallels between the 1950s epidemic and the USA300 clone of MRSA today. However, McKenna infers that the 80/81 strain disappearance was caused by use of antistaphylococcal drugs and not natural events. Although 80/81 did disappear after the introduction of methicillin, the cause of the strain's disappearance is largely unknown.

The community-onset MRSA epidemic of the past decade is not presented with a clear timeline. As a result, the reader is unclear if the incidence of disease is still increasing, has leveled, or is decreasing and could further parallel incidence of the 80/81 strain.

The chapter on healthcare-associated infections is MRSA centric and misses excellent opportunities to frame these infections and problems such as antimicrobial drug resistance and overuse in a broader context. The challenges of MRSA prevention are not balanced with other infection prevention priorities such as control of multidrug-resistant gram-negative pathogens and *Clostridium difficile*. Active surveillance to identify MRSA carriers is emphasized more than hand hygiene. Legislation mandating MRSA screening is discussed without explaining why major infection prevention organizations believe such legislation is unwise.

Some of the physicians, researchers, and other heroes in the MRSA story are appropriately praised. Failings of physicians and the healthcare establishments are deservedly criticized. However, there is no call to arms over some of the most egregious medical failures, such as poor hand hygiene compliance and unwise antimicrobial drug use. The reader is left frustrated about the inability of the medical establishment to control MRSA.

The book attempts to appeal to a broad audience, and although McKenna uses a lot of medical jargon, she effectively explains concepts such as antimicrobial drug mechanisms and molecular typing. Her style and the human interest stories will appeal to a lay audience, particularly consumer advocates. The historical background and scientific detail may appeal to healthcare professionals interested in infectious diseases or public health. However, the main goal of the book appears to be to scare the reader about the "Superbug." In this regard, McKenna succeeds.

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Clive Hicks-Jenkins (b. 1951), *The Prophet Fed by a Raven* **(2007)** Acrylic on panel (62 cm × 82 cm), Courtesy of the artist, private collection, www.hicks-jenkins.com

And the Raven, Never Flitting, Still Is Sitting, Still Is Sitting[1]

Polyxeni Potter

"At my studio back in Cardiff, the walls swarm with a cast of hermits, angels, penitents, devils, wild beasts, and anchorites," wrote Clive Hicks-Jenkins during a visit to Prague. "They are made of roughly painted card, jointed for articulation and capable of surprisingly varied and unlikely positions, rather like elaborate shadow puppets. They were constructed as studio aids to achieve a more expressive use of the human figure and free me from the choreographer's understanding of the body."

A native of Wales, Hicks-Jenkins spent the first 25 years of his working life as stage designer, choreographer, and theater director. The figures in Gothic Bohemian paintings in the National Gallery in Prague reminded him of the population on his own studio walls. He saw in these figures a kind of "postural distortion," affirming his notion that "This is not about flesh and the corporeal body."

He was born in Newport and showed early artistic talent, which he cultivated in London at the Rambert Dance Company and The Italia Conti Academy. He traveled widely in Europe and America, touring with dance and theater troops, but settled back in Wales in the 1980s to focus on his love of painting. His studio, the "battery," he shares

with Pipistrelle bats, which "roost in the roof space above and make frequent forays to pepper my paintings with their droppings. I don't mind. I enjoy their company."

Hicks-Jenkins' painting has been described as figurative, a term now encompassing any form of modern art characterized by references to the real world. "I am not at all captivated by 'likeness' or the capturing of it. Most of the figures in my paintings are of 'types' rather than specific, identifiable sitters."He has experimented with various styles, using elements from them in his own work, which is always changing. "I continue to lay myself open to the currents that carry me in new directions.... Every time I reach a point where skill becomes even close to practiced and reliable, I feel the urge to derail the train."

Hicks-Jenkins' artistic talent was nourished by his years in the theater. "The stage is a revealing space, much like a painting. All the attention is focused on the limited area, and everything within it becomes more important." The stage is a rich source of compositional elements. "I've developed a technique of making three-dimensional, articulated paper maquettes as part of my preparation for painting at the easel. These fragile little works are pieced together quickly from thin card, cut, and then worked in frottage, monoprint, conté crayon, and acrylic. The puppets are held together with hidden, brass paper fasteners." He

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[1]From "The Raven" by Edgar Allan Poe.

uses maquettes "not as convenient stand-ins for people" but to resolve a common dilemma of the artist pursuing an independent creation, an idea, instead of *mimesis*, an imitation of nature. These puppets, reminiscent of animation figures, distract him from the usual objects of observation, enabling him to concentrate on "Inventing dynamic though often anatomically impossible arrangements of limbs that a live model quite simply couldn't provide" and to fill space. "They've been crucial too in helping me flatten out and pile up shapes…. The paintings become almost like collages."

The creative effort of imagining and inventing codes and other means of communication goes back to prehistoric times and may be the factor distinguishing humans from other animals. We search for such codes throughout the millennia, trying to decipher human history. Many of the messages we find are from the visual arts, which appeal to the senses as well as the intellect. Mental and visual communications are integrated by details or cues to make characters or situations recognizable without captions. To this end, art often resorts to iconography, a tradition of communicating through images. In this tradition, recurrent figures may be portrayed with the same facial features or clothing, despite changing eras or styles. They may be painted in certain colors or be accompanied by recognized objects or animals. Once the subject becomes identifiable, other elements may be added that elevate the meaning from historical to universal.

Like many of Hicks-Jenkins' works, *The Prophet Fed by a Raven*, on this month's cover, contains elements of iconography placed in a contemporary setting and filled with timeless universal themes. The clues point to well-loved prophet Elias (also Elijah) of the Old Testament, an elusive figure of swift foot and obscure origins. The artist placed him on a ledge, as "He dwelled in the clefts of the torrents or in the caves of mountains" and lived the life of "a true son of the desert." "Nothing was too high for him, and when he was laid on sleep his body prophesied." He was "as fire, and his word burnt like a torch." He appeared abruptly on the historical scene, delivered his message against the corruption of his age, lived off charity under conditions of drought and famine, and vanished to the east of the Jordan, where legend has it, ravens "brought him bread and flesh in the evening, and he drank of the torrent."

Basic elements aside, the artist departs from iconography. The raven is true enough to form in its shaggy throat feathers, wedge-shaped tail, slender figure, narrow wings, and long, thin "fingers" at the wingtips. But it defies traditional color for flaming red and seems far more fiery than the prophet. The remote mountain backdrop is telescoped and manipulated into the top of the composition, placed within reach, virtually inside the window frame. Perched solidly on the edge, the bird turns one sharp eye toward its charge, Bowie knife of a beak parted as if in conversation with the figure slouched enigmatically in the foreground.

The raven, among the most intelligent of birds, is a frequent visitor in the arts where, as in life, it has a contradictory presence, signaling both life and death. Stately and handsome in its shiny uniform coat—black down to the legs, eyes, and beak—it is graceful and inquisitive. Despite its unsavory reputation as an indiscriminate eater, it struts, soars, and glides confidently in virtually all environments and around humans and other animals. "I have no interest in expressions of the stories where the nature of the wild is perverted from its true self," the artist says about his portrayal of animals. "For me the true miracle of relationships that break the usual mold is that the animal moderates its behavior because it's moved to."

In *The Prophet Fed by a Raven*, the bird breaks from 'likeness' to become a type, emblematic not only of what can happen between prophets and birds but of all animal–human interactions. In many of these, the roles are often reversed, as in this painting, where the bird takes care of the prophet living in the wild. Sometimes, the raven becomes the prophet, as in the case of West Nile virus spread in North America, when dying birds of the crow family, including ravens, foretold human infection in the New World. But ravens are not alone. With many infections emerging first in animals in remote and underprivileged settings, surveillance of animal health can forecast disease risks to humans. In Sri Lanka, field veterinarians used mobile phones to report animal health information, confirming that this type of animal population surveillance can work in isolated areas with limited resources. While the prophet–raven platform has changed with the times, the universal human–animal interface and its zoonotic consequences, including disease transmission, remain unchanged.

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Enhanced Surveillance for Coccidioidomycosis, Arizona, 2007–2008

Decrease in Shigellosis Death Rates without *Shigella*-specific Interventions, Asia

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Extended Spectrum β-lactamase–producing *Escherichia coli* in Neonatal Care Unit

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Estimating Number of Pandemic (H1N1) 2009 Infections, Beijing, China

Enterovirus 71 Infection with Central Nervous System Involvement, South Korea

Streptococcus pneumoniae Serotype 6D, South Korea

Dengue Virus Type 3 from Travelers returning from Tanzania and Côte d'Ivoire, Japan

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Complete list of articles in the November issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

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Article Title

Bloodstream Infections among HIV-Infected Outpatients, Southeast Asia

CME Questions

1. Based on the above southeast Asia study by Dr Varma and colleagues, which of the following statements about prevalence of overall bloodstream infections (BSIs) and of specific BSIs in HIV-infected persons is correct?

A. BSIs are commonly encountered in HIV-infected outpatients, noted in up to 5%
B. The most common bacterial infection was *Streptococcus pneumoniae*
C. *Mycobacterium tuberculosis* was the most common pathogen responsible for BSI
D. *M. tuberculosis* accounted for approximately one quarter of all BSI

2. Your patient is a 23-year-old Cambodian male recently diagnosed with HIV infection. Based on the above study, which of the following is most likely to be associated with increased risk for overall BSI?

A. CD4 = 150 cells/mm^3
B. Vomiting and abdominal tenderness
C. Generalized rash
D. Subnormal temperature

3. Based on the above study, which of the following statements regarding increased risk for specific BSI is most likely correct for the patient in question 2?

A. Hilar adenopathy on chest x-ray suggests tuberculosis infection
B. Difficulty breathing suggests fungal BSI
C. Jaundice was not a significant predictor of any class of pathogen
D. Loss of appetite was a specific predictor of bacterial infection

Activity Evaluation

1. The activity supported the learning objectives.

Strongly Disagree				Strongly Agree
1	2	3	4	5

2. The material was organized clearly for learning to occur.

Strongly Disagree				Strongly Agree
1	2	3	4	5

3. The content learned from this activity will impact my practice.

Strongly Disagree				Strongly Agree
1	2	3	4	5

4. The activity was presented objectively and free of commercial bias.

Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title
Changing Epidemiology of Pulmonary Nontuberculous Mycobacteria Infections

CME Questions

1. Which of the following patients is at most risk for pulmonary nontuberculous mycobacteria (NTM) infection?

A. A middle-aged female smoker with immune suppression with middle lobe infection
B. A middle-aged man with chronic obstructive pulmonary disease with upper lobe infection
C. An elderly woman with chronic obstructive pulmonary disease with diffuse nodules throughout the lung
D. An elderly man with lung fibrosis and immune suppression with bilateral lung disease

2. Which of the following diagnostic strategies is most appropriate for patients with suspect pulmonary NTM infection?

A. Multiple early-morning gastric aspirates
B. Lung biopsy
C. Multiple sputa and bronchoscopic sampling
D. Chest x-ray

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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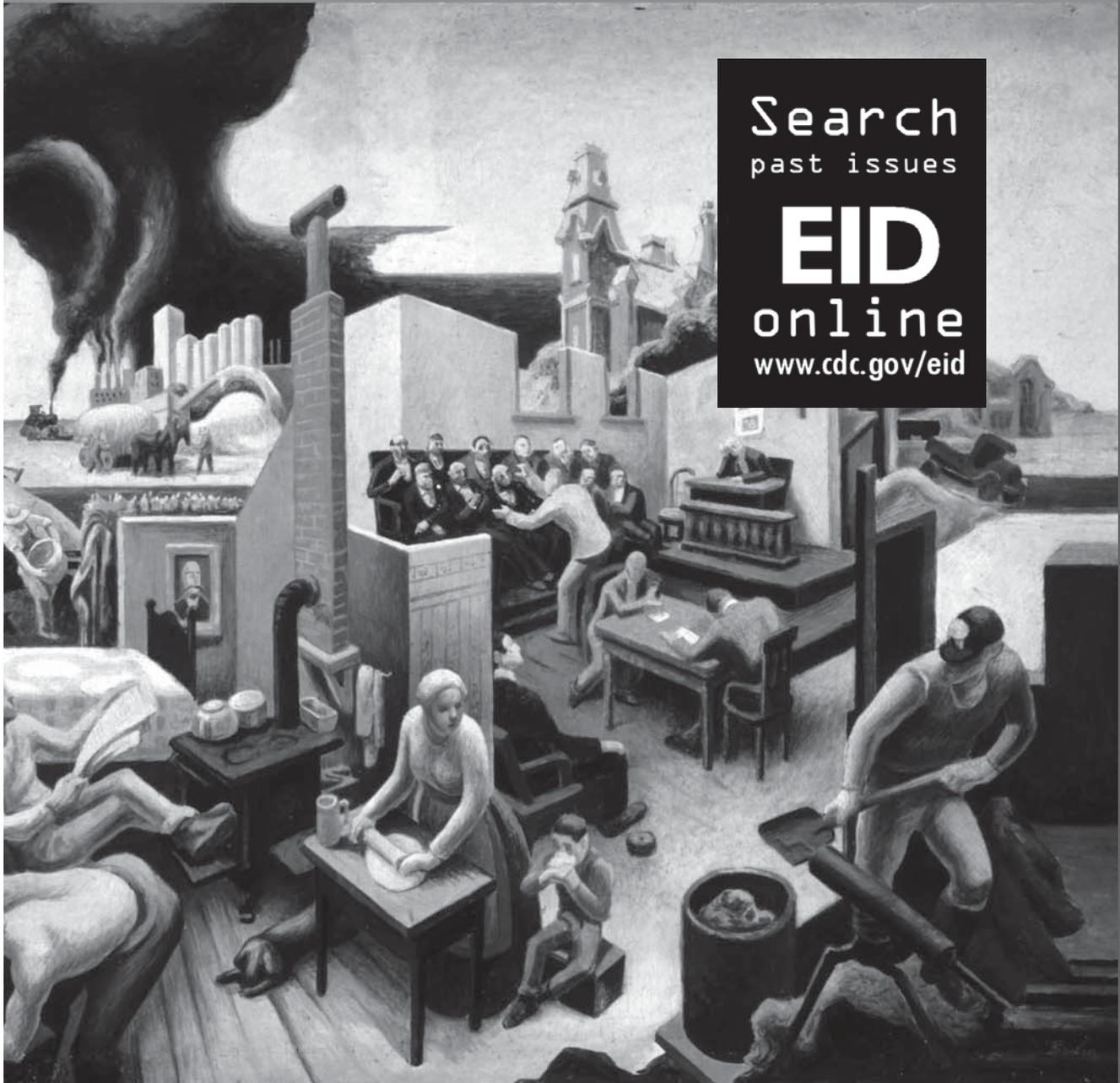
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Instructions to Authors

MANUSCRIPT SUBMISSION. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

MANUSCRIPT PREPARATION. For word processing, use MS Word. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, and figure legends. Appendix materials and figures should be in separate files.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

FIGURES. Submit figures as separate files, in the native format when possible (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpg or .tif files. Other file formats may be acceptable; contact fue7@cdc.gov for guidance. Figures should not be embedded in the manuscript file. Use color only as needed. Use Arial font for figure lettering. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced to print size. Large figures may be made available online only. Place figure keys within the figure; figure legends should be provided at the end of the manuscript file.

VIDEOS. High-quality video files are accepted in the following formats: AVI, MOV, MPG, MPEG, and WMV. The files should be no longer than 5 minutes in length.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only. 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.

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Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.