Malaria

July 2015
On the Cover
Marianne North (1830–1890)
Foliage, Flowers, and Seed-vessels of a Peruvian Bark Tree (1870s)
Oil on card
20 x 11.5 in / 50.8 x 29.2 cm
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Quinine

Correction

International Conference on Emerging Infectious Diseases
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Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 21, No. 7, July 2015
Presenting the ongoing challenges that emerging microbial threats pose to global health
Disseminated Infections with *Talaromyces marneffei* in Non-AIDS Patients Given Monoclonal Antibodies against CD20 and Kinase Inhibitors


**SYNOPSIS**

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**Learning Objectives**

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

1. Distinguish the clinical and epidemiologic characteristics of *T. marneffei* infection, based on a case series report
2. Discuss the recent emergence of disseminated *T. marneffei* infection in non-AIDS patients with hematologic malignant neoplasms treated with targeted therapies
3. Identify possible mechanisms of action underlying disseminated *T. marneffei* infection in non-AIDS patients with hematologic malignant neoplasms treated with targeted therapies

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Infections with the fungus Talaromyces (formerly Penicillium) marneffei are rare in patients who do not have AIDS. We report disseminated T. marneffei infection in 4 hematology patients without AIDS who received targeted therapy with monoclonal antibodies against CD20 or kinase inhibitors during the past 2 years. Clinicians should be aware of this emerging complication, especially in patients from disease-endemic regions.

Talaromyces (formerly Penicillium) marneffei is a pathogenic, thermal dimorphic fungus that causes systemic mycosis in Southeast Asia. T. marneffei infection is characterized by fungal invasion of multiple organ systems, especially blood, bone marrow, skin, lungs, and reticuloendothelial tissues, and is highly fatal, especially when diagnosis and treatment are delayed (1,2). This disease is found predominantly in AIDS patients and occasionally those with cell-mediated immunodeficiencies involving the interleukin-12/interferon-γ (IFN-γ) signaling pathway, such as congenital STAT1 mutations or acquired autoantibodies against IFN-γ (1.3–6). The infection has rarely been reported among hematology patients, including those from disease-endemic regions (7,8).

At Queen Mary Hospital in Hong Kong, a 1,600-bed university teaching hospital that has a hematopoietic stem cell transplantation service, where a wide range of invasive fungal infections have been observed (9,10), only 3 cases of T. marneffei infection were encountered in >2,000 hematology patients in the past 20 years, despite the longstanding availability of mycologic culture and serologic testing (7,8,11,12). In contrast, the infection was commonly reported among AIDS patients (13).

In the past 2 years, we have been alerted by 4 unprecedented cases of disseminated T. marneffei infection among non-AIDS hematology patients given targeted therapies, including monoclonal antibodies (mAbs) against CD20 and kinase inhibitors, which are being increasingly used in recent years. We report details for these 4 hematology case-patients. The study was approved by the institutional review board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster in Hong Kong.

**Case-Patient 1**

Patient 1 was a 56-year-old Filipino man with Waldenström macroglobulinemia, idiopathic thrombocytopenic purpura, and primary biliary cirrhosis. He had fever, night sweating, productive cough, and left facial pain for 1 week and bloody diarrhea for 2 days. He had previously received fludarabine, dexamethasone, and rituximab (mAb against CD20, 18 months earlier) for treatment of Waldenström macroglobulinemia (Table 1). The idiopathic thrombocytopenic purpura was controlled with intravenous immunoglobulin and maintenance prednisolone and mycophenolate sodium. A chest radiograph showed a small cavitary lesion in the right lower lobe. His symptoms and signs did not resolve after he received empirical intravenous imipenem/cilastatin and metronidazole (Table 2).

A colonoscopy showed multiple shallow ulcers at the terminal ileum (Figure 1). Histologic analysis of an ulcer biopsy specimen showed slough of an acutely inflamed ulcer but no microorganisms. However, histologic analysis of a specimen from a nasopharyngeal biopsy performed for persistent left facial pain showed abundant yeast cells engulfed by foamy macrophages (Figure 2). Culture of terminal ileal ulcer biopsy specimens, stool samples, and nasopharyngeal biopsy specimens yielded T. marneffei. A contrast-enhanced cranial computed tomography (CT) scan showed 2 lesions (3–4-mm) with rim enhancement and perifocal edema at the right occipital and left parietooccipital lobes. A thoracic CT scan showed 2 cavitary lesions (4–8 mm) in the right upper and lower lobes.

Immunologic testing showed that the patient was negative for HIV and autoantibodies against IFN-γ. His CD3+ and CD8+ counts were within references ranges, but he had mild CD4+ lymphopenia (Table 2). His fever and symptoms resolved with after 2 weeks of treatment with intravenous liposomal amphotericin B, followed by oral voriconazole. Reassessment colonoscopy (at 2 months) and CT scan (at 6 months) showed complete resolution of all lesions.

**Case-Patient 2**

Patient 2 was a 44-year-old Chinese man who had fever for 2 days. He had previously received chemotherapy and mAbs against CD20 (rituximab, 14 months earlier; obinutuzumab, concomitant) for refractory chronic lymphocytic leukemia (CLL) involving bone marrow (Table 1). He was empirically given intravenous piperacillin/tazobactam and anidulafungin (Table 2). Histologic analysis of a trephine biopsy specimen showed persistent CLL with plasmacytic differentiation, and Grocott staining showed yeasts with central septa in small clusters. Culture of peripheral blood and bone marrow aspirate yielded T. marneffei. A change in antifungal treatment to intravenous amphotericin B led to defervescence and clearance of fungemia. He was given oralitraconazole as maintenance therapy. He remained well until 2 months later when he was hospitalized for deteriorating CLL complicated by neutropenic fever with multiorgan failure caused by opportunistic infections (Table 1). He died 5 months after the episode of disseminated T. marneffei infection.

**Case-Patient 3**

Patient 3 was a 63-year-old Chinese man with myelofibrosis and well-controlled diabetes mellitus. He had intermittent fever, right cervical lymphadenopathy, and productive cough for 4 months. He was given ruxolitinib (kinase
inhibitor) 6 months before symptom onset because of transfusion-dependent myelofibrosis despite splenectomy 4 years earlier (Table 1). A chest radiograph and thoracic CT scan showed multiple cavitary lesions and consolidation. Bronchoalveolar lavage was negative for bacteria, fungi, and mycobacteria. A serum cryptococcal antigen test result was negative. He was empirically given intravenous imipenem/cilastatin and oral doxycycline, but his symptoms persisted. A right cervical lymph node culture yielded T. marneffei. His symptoms and radiologic abnormalities resolved after treatment with intravenous amphotericin B for 2 weeks, followed by oral voriconazole for 6 months.

**Case-Patient 4**

Patient 4 was a 67-year-old Chinese man with acute myeloid leukemia and hypertension. He had fever and malaise for 2 days without localizing signs. He had been given sorafenib (kinase inhibitor) 8 months earlier for chemotherapy-refractory acute myeloid leukemia (Table 1). His fever did not respond to intravenous meropenem. Subsequently,

### Table 1. Characteristics of 4 case-patients with disseminated Talaromyces marneffei infection after targeted therapies*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case-patient 1</th>
<th>Case-patient 2</th>
<th>Case-patient 3</th>
<th>Case-patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y/sex</strong></td>
<td>56/M</td>
<td>44/M</td>
<td>63/M</td>
<td>67/M</td>
</tr>
<tr>
<td><strong>Concurrent conditions</strong></td>
<td>Waldenström macroglobulinemia, idiopathic thrombocytopenia purpura, primary biliary cirrhosis</td>
<td>Chronic lymphocytic leukemia</td>
<td>Myelofibrosis with splenectomy, diabetes mellitus</td>
<td>Acute myeloid leukemia, hypertension</td>
</tr>
<tr>
<td><strong>Targeted therapy</strong></td>
<td>Rituximab</td>
<td>Rituximab and obinutuzumab</td>
<td>Ruxolitinib</td>
<td>Sorafenib</td>
</tr>
<tr>
<td><strong>Action of therapy</strong></td>
<td>mAb against CD20</td>
<td>mAb against CD20</td>
<td>JAK-1/2 inhibitor</td>
<td>Multikinase inhibitor</td>
</tr>
<tr>
<td><strong>Time interval, mo†</strong></td>
<td>18</td>
<td>14 (rituximab) and concomitant (obinutuzumab)</td>
<td>Concomitant</td>
<td>Concomitant</td>
</tr>
<tr>
<td><strong>Cumulative dose before T. marneffei infection</strong></td>
<td>700 mg/dose iv x 4 doses</td>
<td>700 mg/dose iv x 13 doses (rituximab) and 1,000 mg IV x 3 doses (obinutuzumab)</td>
<td>10–20 mg 2×/d oral x 6.5 mo</td>
<td>400 mg 2×/d oral x 8 mo</td>
</tr>
<tr>
<td><strong>Other immunosuppressants (time interval, mo)†</strong></td>
<td>Fludarabine and dexamethasone (39), prednisolone 10 mg/d and mycophenolate sodium 360 mg 2×/d (concomitant)</td>
<td>Fludarabine and cyclophosphamide (48), CHOP (36), bendamustine (13)</td>
<td>None</td>
<td>Mitoxantrone and etoposide (21), daunorubicin (20), clofarabine (18), azacitidine (15), decitabine (15), cytarabine (14)</td>
</tr>
<tr>
<td><strong>Clinical manifestations</strong></td>
<td>Terminal ileitis, cerebral abscesses, nasopharyngitis, and multiple cavitary lung lesions</td>
<td>Marrow infiltration and fungemia</td>
<td>Right cervical lymphadenitis and multiple cavitary lung lesions</td>
<td>Fungemia</td>
</tr>
<tr>
<td><strong>Specimens positive for T. marneffei</strong></td>
<td>Feces, and terminal ileal and nasopharyngeal biopsy specimens</td>
<td>Blood and bone marrow aspirate</td>
<td>Right cervical lymph node</td>
<td>Blood</td>
</tr>
<tr>
<td><strong>Highest serum antibody titer against T. marneffei</strong></td>
<td>1:320</td>
<td>&lt;1:40</td>
<td>1:320</td>
<td>&lt;1:40</td>
</tr>
<tr>
<td><strong>Antifungal treatment (duration, mo)</strong></td>
<td>Amphotericin B (2 weeks) and voriconazole (&gt;21)</td>
<td>Amphotericin B (2 weeks) and itraconazole (5)</td>
<td>Amphotericin B (2 weeks) and voriconazole (&gt;6)</td>
<td>Amphotericin B (2 weeks) and voriconazole (&gt;5)</td>
</tr>
<tr>
<td><strong>Other opportunistic infections</strong></td>
<td>None</td>
<td>Bacteremia (Mycobacterium chelonae, Enterococcus faecalis, and MRCNS), fungemia (Candida glabrata), HSV oral mucositis, PJP</td>
<td>Bacteremia (Klebsiella pneumoniae)</td>
<td>Herpes zoster at right occiput</td>
</tr>
<tr>
<td><strong>Clinical outcome</strong></td>
<td>Responded to antifungal treatment</td>
<td>Clearance of T. marneffei fungemia but died of MODS and multiple infections 5 mo after T. marneffei infection</td>
<td>Responded to antifungal treatment</td>
<td>Responded to antifungal treatment</td>
</tr>
</tbody>
</table>

*†mAb, monoclonal antibody; JAK, Janus kinase; IV, intravenous; CHOP, cyclophosphamide, hydroxydaunorubicin, Oncovin, and prednisolone; MRCNS, methicillin-resistant coagulase-negative Staphylococcus; HSV, herpes simplex virus; PJP, Pneumocystis jiroveci pneumonia; MODS, multiple organ dysfunction syndrome.

†Time interval between end of therapy and onset of symptoms for T. marneffei infection.
2 sets of blood cultures yielded *T. marneffei*. He was given intravenous amphotericin B for 2 weeks, followed by oral voriconazole. He remained well at follow-up 6 months after symptom onset.

**Discussion**

*T. marneffei* infection is an emerging complication in hematologic patients receiving targeted therapies. Historically, *T. marneffei* infection has rarely been seen in non-AIDS patients, even in disease-endemic regions. During 1994–2014, only 3 other cases were observed in our hematology patients (*7,8,11*). None of 47 patients with *T. marneffei* infection in another large local case series during 1994–2004 had hematologic disease (*13*). In the past 20 years, there has been no change in methods for laboratory diagnosis of *T. marneffei* infection or a marked increase

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**Table 2. Laboratory results for 4 case-patients with disseminated *Talaromyces marneffei* infection after targeted therapies**

<table>
<thead>
<tr>
<th>Laboratory parameter</th>
<th>Case-patient 1</th>
<th>Case-patient 2</th>
<th>Case-patient 3</th>
<th>Case-patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes, x 10⁹ cells/L</td>
<td>12.08</td>
<td>0.91</td>
<td>4.93</td>
<td>33.79</td>
</tr>
<tr>
<td>Neutrophils, x 10⁹ cells/L</td>
<td>11.01</td>
<td>0.45</td>
<td>3.11</td>
<td>8.45 (with blasts)</td>
</tr>
<tr>
<td>Lymphocytes, x 10⁹ cells/L</td>
<td>0.83 (CD4+ : 315/µL)‡</td>
<td>0.45</td>
<td>1.05</td>
<td>9.12 (with blasts)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>12.3</td>
<td>10.3</td>
<td>8.0</td>
<td>9.2</td>
</tr>
<tr>
<td>Platelets, x 10¹²/L</td>
<td>250</td>
<td>5</td>
<td>539</td>
<td>15</td>
</tr>
<tr>
<td><strong>Biochemical†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>136</td>
<td>135</td>
<td>139</td>
<td>138</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>3.5</td>
<td>4.1</td>
<td>3.7</td>
<td>4.4</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>101</td>
<td>111</td>
<td>78</td>
<td>92</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>40</td>
<td>32</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>34</td>
<td>36</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td>Total bilirubin, µmol/L</td>
<td>8</td>
<td>9</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>234</td>
<td>163</td>
<td>112</td>
<td>96</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>79</td>
<td>20</td>
<td>32</td>
<td>61</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>38</td>
<td>9</td>
<td>28</td>
<td>123</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>209</td>
<td>97</td>
<td>352</td>
<td>2,069</td>
</tr>
<tr>
<td><strong>Immunologic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined HIV antibody/antigen</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Autoantibody against IFN-γ</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Microbiologic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood culture</td>
<td>No bacteria and fungi</td>
<td>T. marneffei; Mycobacterium chelonae, Enterococcus faecium, MRCNS, and Candida glabrata§</td>
<td>Klebsiella pneumoniae§</td>
<td>T. marneffei</td>
</tr>
<tr>
<td>Bone marrow aspirate</td>
<td>ND</td>
<td>T. marneffei</td>
<td>Negative for pathogenic bacteria, AFB, and fungi</td>
<td>Negative for pathogenic bacteria, AFB, and fungi</td>
</tr>
<tr>
<td>Sputum culture</td>
<td>ND</td>
<td>T. marneffei</td>
<td>Negative for pathogenic bacteria, AFB, and fungi</td>
<td>Negative for pathogenic bacteria, AFB, and fungi</td>
</tr>
<tr>
<td>Urine culture</td>
<td>No bacteria and fungi</td>
<td>No bacteria and fungi</td>
<td>ND</td>
<td>No bacteria and fungi</td>
</tr>
<tr>
<td>Stool culture</td>
<td>T. marneffei; negative for pathogenic bacteria, including Clostridium difficile and AFB</td>
<td>No bacteria and fungi</td>
<td>No bacteria and fungi</td>
<td>ND</td>
</tr>
<tr>
<td>Serum CMV pp65 antigen</td>
<td>Stool for C. difficile toxin (negative); serum for Entamoeba histolytica antibody (negative); multiple blood smears for Plasmodium sp. (negative)</td>
<td>Negative for pathogenic bacteria, AFB, and fungi</td>
<td>Negative for pathogenic bacteria, AFB, and fungi</td>
<td>Negative for pathogenic bacteria, AFB, and fungi</td>
</tr>
</tbody>
</table>

*Reference ranges: leukocytes; 3.89–9.93 x 10⁹ cells/L; neutrophils, 2.01–7.42 x 10⁹ cells/L; lymphocytes, 1.06–3.61 x 10⁹ cells/L; hemoglobin, 13.3–17.7 g/dL; platelets, 162–341 x 10⁹/L; sodium, 136–148 mmol/L; potassium, 3.6–5.0 mmol/L; creatinine, 67–109 µmol/L; albumin, 39–50 g/L; globulin, 24–37 g/L; total bilirubin, 4–23 µmol/L; ALP, 42–110 U/L; ALT, 8–58 U/L; AST, 5–38 U/L; LDH, 118–221 U/L.|

†Results at presentation.

‡Reference range of CD4+ lymphocyte count: 415–1,418 cells/µL.

§Bacteremia caused by *M. chelonae, E. faecium, MRCNS,* and candidemia in case-patient 2, and bacteremia caused by *K. pneumoniae* in case-patient 3 occurred after recovery from *T. marneffei* infection and prolonged hospitalization.
Disseminated Infections with *Talaromyces marneffei*

in the number of hematology patients in our hospital. Therefore, these 4 cases indicate an increase in the incidence of *T. marneffei* infection in these patients. Although other immunosuppressants given to case-patients 1, 2, and 4 might have contributed to overall immunosuppression, none of these immunosuppressants, which have been used for years, have been associated with *T. marneffei* infection. Because use of targeted therapies is increasing in diverse patient groups, clinicians should be aware of this emerging complication, especially in patients from disease-endemic regions who have received these therapies with other immunosuppressants.

The exact mechanisms through which these targeted therapies lead to *T. marneffei* infection remain incompletely understood. Rituximab and obinutuzumab (used by case-patients cases 1 and 2) are mAbs against CD20 that predominantly target B cells. Unlike T cells, the role of B cell–mediated humoral response in *T. marneffei* infection is poorly defined. Although case-patient 1 had mild CD4+ lymphopenia probably related to concomitant use of prednisolone and mycophenolate sodium, *T. marneffei* infection is rarely seen in patients with CD4+ counts >300/µL (1). We postulate that B cell dysfunction might have impaired production of neutralizing antibodies against key virulence factors of *T. marneffei* or might involve impairment of cytokine-producing B cells, which are essential for T helper cell function (14).

More severe infections with fungemia and bone marrow involvement developed in case-patients 2 and 4, who had undetectable levels of serum antibodies against *T. marneffei*. Correspondingly, case-patients 1 and 3, who had antibody titers >1:320, did not have positive blood culture results (Table). Symptoms developed in case-patient 1 more than a year after he completed therapy with mAbs against CD20. This finding might be related to long-lasting B cell–depleting effects of mAbs against CD20 (15).

Regarding kinase inhibitors (used by cases-patients 3 and 4), ruxolitinib is a selective Janus kinase (JAK)-1/2 inhibitor that prevents signal transduction for type I/II cytokines, including IFN-γ, by interfering with the JAK-STAT signaling pathway. Use of ruxolitinib has been associated with opportunistic infections caused by intracellular pathogens, such as *Mycobacterium tuberculosis* and *Cryptococcus neoformans* (16,17). Similarly, patients with impaired JAK-STAT signaling, but not those with diabetes mellitus or splenectomy (case-patient 3), are predisposed to *T. marneffei* infection (6). Sorafenib is a multikinase inhibitor with various immunomodulatory effects, including impaired T-cell response and proliferation and reduced IFN-γ production (18). These immune defects have been associated with reactivation of latent tuberculosis and might also predispose patients to opportunistic infections caused by intracellular organisms such as *T. marneffei* (18).

The recognition of disseminated *T. marneffei* infection as an emerging complication in non-AIDS patients treated with targeted therapy has major public health implications.
SYNOPSIS

In regions to which *T. marneffei* infection is endemic, serologic surveillance for patients receiving targeted therapy might be useful in the early diagnosis of *T. marneffei* infection, as in the case of AIDS patients (19). In non-endemic regions, such as the United States, clinicians should be vigilant of this infrequent infection in at-risk hematology patients who have resided in or are returning from disease-endemic areas.

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References


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Macacine Herpesvirus 1 in Long-Tailed Macaques, Malaysia, 2009–2011


Macacine herpesvirus 1 (MaHV1; B virus) naturally infects macaques (Macaca spp.) and can cause fatal encephalitis in humans. In Peninsular Malaysia, wild macaques are abundant, and translocation is used to mitigate human-macaque conflict. Most adult macaques are infected with MaHV1, although the risk for transmission to persons who handle them during capture and translocation is unknown. We investigated MaHV1 shedding among 392 long-tailed macaques (M. fascicularis) after capture and translocation by the Department of Wildlife and National Parks in Peninsular Malaysia, during 2009–2011. For detection of MaHV1 DNA, PCR was performed on urogenital and oropharyngeal swab samples. Overall, 39% of macaques were shedding MaHV1 DNA; rates of DNA detection did not differ between sample types. This study demonstrates that MaHV1 was shed by a substantial proportion of macaques after capture and transport and suggests that persons handling macaques under these circumstances might be at risk for exposure to MaHV1.

Macacine herpesvirus 1 (MaHV1; also known as B virus) is a zoonotic pathogen that is enzootic among macaque (Macaca spp.) populations throughout Asia (1,2). MaHV1 is an α-herpesvirus related to human herpes simplex viruses (HSV) 1 and 2 (3,4) and to herpesviruses that infect other nonhuman primates such as baboons (5). Like HSV infection in humans, MaHV1 infection in macaques can clinically appear as vesicular lesions on the mucous membranes of the buccal cavity and genital area (6,7). However, macaques without clinically apparent lesions can still shed MaHV1 (6).

Transmission of MaHV1 can occur transcutaneously (via bites) or permucosally (via exposure to macaque body fluids) (8,9). Among humans, ≈40 cases of MaHV1 encephalitis have been reported; all patients were laboratory workers who had come in contact with rhesus macaques (M. mulatta) only or with rhesus macaques and long-tailed macaques (M. fascicularis) or their tissues in the research environment (2,3). For these patients, signs and symptoms of MaHV1 infection included skin ulcers and lesions at the site of injury, influenza-like illness, and infection of the peripheral and central nervous systems (which can develop into brainstem encephalomyelitis and death) (7,9). The mortality rate for humans with untreated MaHV1 infection is >70% (7). This high case-fatality rate has led to strict regulations for handling macaques and macaque clinical samples in laboratories and resulted in the designation of MaHV1 as a Biosafety Level 4 (BSL-4) pathogen and, until recently, a select agent (2,7,9).

In macaques, MaHV1 frequently remains latent in the trigeminal and lumbosacral ganglia; however, in response to stress, it can be asymptomatically reactivated and shed in saliva and urogenital excretions (10). Macaques typically acquire MaHV1 at sexual maturity (11); previous studies have found IgG against MaHV1 in up to 100% of sexually mature wild or laboratory long-tailed and rhesus macaques (11,12). As with other viral infections, the presence of IgG indicates previous exposure or infection but does not indicate active virus shedding. During active infection, MaHV1 DNA can be detected in saliva or urogenital samples by use of PCR. Virus culture is also possible but is not routinely performed because doing so safely requires a BSL-4 laboratory (11). Using PCR as a diagnostic method has advantages over culture in that it can be performed under BSL-2 conditions, it produces results more rapidly, and its sensitivity and specificity are higher (13,14).

Little is known about the shedding rate of MaHV1 in macaques outside the laboratory setting, the frequency of transmission to humans, or the incidence of MaHV1 encephalitis among humans (particularly those with frequent contact with macaques). In Asia, at least 50% of cases of encephalitis are never diagnosed to the point of causative agent identification (15). Understanding the ecology of MaHV1 among macaques is essential for understanding
the potential for human infection. Macaques have adapted to urbanized human environments, and contact between humans and macaques can occur in a variety of contexts (e.g., feeding in public recreational areas, capture of wild macaques for the pet trade or biomedical research colonies, consumption, or population management by wildlife authorities). Human–macaque contact can result in bites, scratches, and indirect exposure to macaque body fluids (16,17). Simian foamy virus, a nonpathogenic retrovirus found in nonhuman primates including macaques, has been transmitted during occupational exposure to macaques via bites and scratches in many of the aforementioned contexts and in agricultural, suburban, and urban environments (18–20). Zoonotic transmission of simian foamy virus to humans has been demonstrated in Indonesia (18) and Bangladesh (19). Because exposure to MaHV1 can occur through similar routes (8), its transmission under circumstances similar to those of transmission of simian foamy virus in Asia is plausible.

In Peninsular Malaysia, conflict between humans and macaques in residential and public areas results from loss of macaque habitat, successful macaque adaptation to human environments, and subsequent macaque overpopulation. As a result, the Department of Wildlife and National Parks (DWNP) in Peninsular Malaysia implemented a macaque population management program, which includes the removal or translocation of macaques from a conflict area. The possibility of exposure to MaHV1 during macaque capture and transport presents a potential occupational hazard to wildlife personnel.

Our aim with this study was to describe the prevalence of MaHV1 shedding among wild-caught long-tailed macaques after capture and transport in Peninsular Malaysia. This study represents a step toward understanding the potential for zoonotic transmission of MaHV1 outside the laboratory.

Materials and Methods

Capture and Sample Collection

Independently of this study, DWNP, as part of their macaque management program throughout Peninsular Malaysia, captured and transported macaques from 6 states (Johor, Perak, Pahang, Pulau Pinang, Selangor, and Negeri Sembilan) to DWNP holding facilities. Capture and opportunistic blood sampling was performed by DWNP and EcoHealth Alliance during September–November 2009, July–October 2010, and July 2011. The macaques captured had been free ranging and lived in the peripheral vegetation of rural, suburban, and urban communities in several states of Peninsular Malaysia. Trapped animals were transferred into transport cages and taken to the nearest local DWNP facility, where they were held up to 72 h before being transported to the DWNP headquarters in Kuala Lumpur or relocated to a new area. Macaques were kept in groups in cages and provided with food and water throughout the holding period. Animals were captured in accordance with the protocols and guidelines of the Manual for Human–Macaque Conflict Management in Peninsular Malaysia (21). This study was conducted under Institutional Animal Care and Use Committee approval no. 18048 from the University of California (Davis, CA, USA). When handling and sampling macaques, personnel involved with this study wore personal protective equipment (PPE; e.g., eye protection, double-layered nitrile gloves, Tyvek coveralls, and P100 respirators) (22). Blood and swab samples were collected from each animal at its arrival at the headquarters or at the local DWNP facility before relocation. Macaques were immobilized with an intramuscular injection of a combination of 5 mg/kg ketamine and 5 mg/kg xylazine (21). After immobilization, oropharyngeal swab and urogenital swab samples were collected (when possible, urine was also collected by cystocentesis). The samples were placed in 2 mL cryovials (Nalgene Nunc International, Rochester, NY, USA) with 500 μL NucliSens lysis buffer (bioMérieux, Marcy l’Étoile, France) and immediately stored at -80°C. Macaque weight, body condition, sex, and approximate age were recorded. The age of the animals was determined by assessing their weight, body size, and the development of their incisors and genitals (23). Macaques were categorized as adult (>7 years), subadult (3–6 years), or juvenile (1–3 years). The sex and age of 2 animals and the sex of 1 adult animal were not recorded.

Molecular Testing

The samples were vigorously mixed; 100 μL of the sample was used for mechanical nucleic acid extraction by use of the NucliSENS miniMAG system (bioMérieux). The extracted nucleic acid was eluted with 60 μL of buffer. PCR was performed as previously described and validated by Scinicariello et al. (13). Briefly, MaHV1 primers (B virus 1, 5′-ACCTCACGTACGACTCCGACT-3′; and B virus 2, 5′-CTGCAGGACCAGTAGGAT-3′; 2.5 μmol/L) were each added to the extraction product and HotStarTaq Plus Master Mix (QIAGEN, Hilden, Germany). The product was placed in a thermocycler at 94°C for 5 min and then underwent 30 cycles as follows: 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. The products (10 μL) were then analyzed by electrophoresis on 1% agarose gels. Of the 10% of samples that were positive by PCR, 14 PCR products were randomly selected and purified with a PCR purification kit (QIAGEN) and sequenced by using the same primers to confirm identity. Sequences were 128 bp and were analyzed by using a BLAST search of GenBank (24). Because PCR is reported to be highly specific (13) and all 14 PCR products showed 93%–100% nucleotide homology to
MaHV1, we considered the other PCR products with identical amplicon size to also be positive for MaHV1. PCR is more sensitive than culture for detecting HSV (25,26), and we considered the detection of MaHV1 DNA in a sample as an indication of virus shedding, although viral load was not obtained through culture or quantitative PCR.

The positive control was produced in a BSL-4 facility and removed from containment by use of inactivation procedures approved by the Texas Biomedical Research Institute Biohazard Committee. Briefly, macaque herpesvirus 1 strain E2490 virion “mini-prep” DNA was generated as previously described (27). With the same B virus 1 and 2 primers, the region between nt 54886 and 54993 was amplified by PCR from the viral genome (13) by using the FailSafe PCR Enzyme Mix (Epicenter, Madison, WI, USA). This region corresponded to a region in the UL28 open reading frame. The resulting 128-nt fragment was amplified by PCR from the viral genome (13) by using the FailSafe PCR Enzyme Mix (Epicenter, Madison, WI, USA). This region corresponded to a region in the UL28 open reading frame. The resulting 128-nt fragment was cloned into pCR2.1-TOPO by using the TOPO TA kit (Invitrogen, Carlsbad, CA, USA) to generate the pMHUL28 gene. The insert was sequenced and confirmed. PCR sensitivity (limit of detection) was determined by using DNA from pMHUL28 and 2 confirmed-positive samples by diluting the DNA to copy numbers of $2.71 \times 10^4$ for pMHUL28 and $1.59 \times 10^1$ for the samples. The sensitivity limit for pMHUL28 by PCR was $9.13 \times 10^2$ molecules, and for the swab samples it was $\approx 1 \times 10^5$ molecules. The PCR sensitivity was previously determined to be $\approx 100$ gene copies by using purified viral DNA, and specificity was determined by SacII restriction enzyme analysis and Southern blot hybridization by using an MaHV1-specific internal probe (5′-GGAGAAGACGTCGCGGTCGTAC-3′) that discriminates MaHV1 from HSV (13).

**Immunoassay**

A subset of 149 animals, randomly chosen to represent each age group, were tested by the MaHV1 ELISA as described by Ohsawa et al. (28). Although the exact specificity and sensitivity of the original MaHV1 ELISA was not determined, it had been validated by testing of known MaHV1-positive (n = 14) and negative (n = 6) serum, and the assay correctly detected 100% of the positive samples and provided negative results for 100% of the negative samples (R. Eberle, pers. comm., 2013). In brief, MaHV1-infected and noninfected cell antigens were added to 96-well round-bottom plates and prepared as previously described (28). Wells were blocked with phosphate-buffered saline containing 5% bovine serum albumin and 0.05% Tween 20 (PBS-BSA-Tw) and were incubated at 37°C for 1 h, then rinsed with PBS-Tw. Serum samples were diluted to 1:100 with PBS-BSA-Tw, added to the plate (50 μL/well), and incubated at room temperature for 2 h. The wells were washed 5 times with PBS-Tw. Biotinylated anti-human IgG (Vector Laboratories, Burlingame, CA, USA) was diluted 1:5,000 with PBS-BSA-Tw (50 μL/well) and incubated at room temperature for 1 h. The wells were washed 5 times with PBS-Tw. A complex of avidin and biotinylated peroxidase was prepared according to the manufacturer’s instructions, diluted to 1:32, added to washed wells (50 μL/well), and incubated at room temperature for 1 h. The wells were washed 5 times with PBS-Tw. A 3,3′,5,5′-tetramethylbenzidine substrate solution was added (100 μL/well), and the plates were incubated without light for 8–12 min. The reaction was stopped with 2 mol/L sulfuric acid (50 μL/well), and the optical density at 450 nm was measured by using a microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA). An ELISA result was considered positive when the optical density was >0.1 (28).

**Statistical Analyses**

We calculated standard prevalence rates and 95% CIs (29) for differences in shedding prevalence based on macaque sample type, sex, and age. For pairwise analysis (2 parameters) of proportions, we conducted a z-test. A general linear model was used to investigate the effect of geographic location by state, sex, and age, and the Akaike Information Criterion was used to select the best-fit model (with no significant interactions between the variables). An analysis of variance of the general linear model with a post hoc Tukey HSD (honest significant difference) test was used to assess the significance of each factor. Statistical analyses were conducted by using the R Statistical package (R Core Team, Vienna, Austria). A p value of $\leq 0.05$ was considered statistically significant.

**Results**

Samples from 392 long-tailed macaques from 6 states within Peninsular Malaysia (Figure) were screened by PCR; 149 of these were also screened by ELISA (Table 1). The overall detection of MaHV1 DNA in macaques, in urogenital and/or oropharyngeal samples (n = 392 tested), was 39.3% (95% CI 34.5%–44.1%). All 14 sequenced DNA samples displayed 93%–100% homology with those of MaHV1. Shedding status did not differ significantly among age groups: 37.6% (95% CI 31.2%–43.9%) among 221 adults, 38.8% (95% CI 28.1%–49.4%) among 80 subadults, and 43.8% (95% CI 33.5%–54.1%) among 89 juveniles (for 2 animals, age was not recorded). Male macaques were more likely than females to be shedding the virus at the time of sampling; prevalence was 44.1% (95% CI 37.5%–50.7%) among 220 males and 33.1% (95% CI 26.0%–40.2%) among 169 females; the sex of 3 animals was not recorded ($z$-statistic = 2.192466, degrees of freedom [df] = 1, p = 0.0001) (Table 1). Males were also significantly more likely than females to shed virus in saliva; prevalence was 26.4% (95% CI 20.5%–32.2%) among 220 males and 16.0% (95% CI 10.5%–21.5%)...
Among 169 females (z-statistic = 2.457458, df = 1, p = 0.007). Overall, the proportion of urogenital and oropharyngeal samples positive for MaHV1 DNA did not differ significantly: 24.7% (95% CI 20.5%–29.0%) of urogenital samples positive for MaHV1 DNA did not differ significantly: 24.7% (95% CI 20.5%–29.0%) of urogenital samples and 21.9% (95% CI 17.8%–26.0%) of oropharyngeal samples positive. We detected viral DNA in oropharyngeal and in urogenital swabs for 18.8% (95% CI 12.7%–25.0%) of the 154 macaques with positive results by PCR (Table 2).

Overall, IgG against MaHV1 was found in 73 (49.0%; 95% CI 38.5%–57.0%) of 149 macaques. We found that seroprevalence differed significantly among age groups: IgG was found in 70.0% of 50 adults, 46.0% of 50 subadults, and 30.6% of 49 juveniles (χ² = 15.6333, df = 2, p = 0.0004) (Table 3). Among macaques tested by ELISA, 24.2% (95% CI 17.3%–31.0%) were positive according to PCR but negative according to ELISA results, although these animals did not differ significantly by age, sex, or shedding site (oropharyngeal vs. urogenital) (Table 4).

The geographic origin of macaques that were MaHV1 positive by PCR was as follows: 4 (75.0%; 95% CI 32.6%–100%) were from Johor, 44 (54.5%; 95% CI 39.8%–69.3%) from Perak, 24 (54.2%; 95% CI 34.2%–74.1%) from Pahang, 8 (37.5%; 95% CI 4.0%–71.0%) from Pulau Pinang, 251 (37.5%; 95% CI 31.5%–43.4%) from Selangor, and 61 (27.9%; 95% CI 16.6%–39.1%) from Negeri Sembilan (Figure). An analysis of variance of the general linear model indicated that both sex and geographic location (state) were significantly associated with detection by PCR, but only the effect of sex (F-statistic = 3.97, df = 1, p = 0.047) had enough power to remain significant after a post hoc Tukey HSD (for categorical data) was applied (p = 0.049).

**Discussion**

We examined MaHV1 shedding among free-ranging macaques after capture and transport, a scenario under which occupational exposure could occur. In addition to the risk that capture and transport poses for handlers, macaques are probably under increased physiologic stress during capture and transport, which might result in increased virus activation and shedding.

Despite little published data for shedding prevalence in free-ranging or recently captured wild macaques with which to compare our findings, serologic evidence from wild-caught macaques transported from India to the United States in the 1950s for polio vaccine testing indicates that the stress of transport probably led to increased MaHV1 seroprevalence from 10% before transit to 70% after transit (30). These animals were young (1.0–1.5 years of age) and were kept in groups of 60. PCR studies of laboratory macaques (rhesus and long-tail) have reported a shedding prevalence range of 0%–71% (11,14,31). Sample sizes in these studies were generally very small, and most reported samples sizes were generally very small, and most reported

**Table 1. PCR results for macacine herpesvirus 1 in macaques, by age and sex. Malaysia, 2009–2011**

<table>
<thead>
<tr>
<th>Age group, y</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Unspecified†</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>positive</td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>Adult, &gt;6</td>
<td>120 (55.8; 36.9–54.7)</td>
<td>100 (28.0; 19.2–36.8)</td>
<td>1 (50.0)</td>
<td>221 (37.6; 31.2–43.9)</td>
</tr>
<tr>
<td>Subadult, 3–6</td>
<td>48 (39.6; 25.7–53.4)</td>
<td>32 (37.5; 20.7–54.3)</td>
<td>0 (0)</td>
<td>80 (38.8; 28.1–49.4)</td>
</tr>
<tr>
<td>Juvenile, &lt;3</td>
<td>52 (44.2; 30.7–57.7)</td>
<td>37 (43.2; 27.3–59.2)</td>
<td>0 (0)</td>
<td>89 (43.8; 33.5–54.1)</td>
</tr>
<tr>
<td>Unspecified†</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (100.0)</td>
<td>2 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>220 (97.4%; 37.5–50.7)</td>
<td>169 (56.1%; 26.0–40.2)</td>
<td>3 (13.3)</td>
<td>392 (39.3; 34.5–44.1)</td>
</tr>
</tbody>
</table>

†NA, not applicable.
‡2 macaques of unspecified age and sex and 1 adult macaque of unspecified sex were included in the study.
††Indicates a significant difference (p<0.05) between the 2 groups marked.
prevalence rates were <10%. Shedding prevalence determined in our study certainly falls within these ranges.

We observed that shedding prevalence was significantly higher among male than female macaques, although seroprevalence did not differ. This difference could be related to sociobiological dominance behavior by which females typically remain at the dominance level of their mother, whereas males lose that dominance rank when they leave the group at the time of dispersal. Thus, males continually must earn their ranking as they change groups (32). This behavior might predispose males to greater social stress during capture and transport. Unfortunately, we were unable to separate sex-based differences in physiologic stress from the potential effects of the stress of capture and transport, which might have affected male macaques differently than females.

We observed a significant difference in seroprevalence among macaques in different age groups; seroprevalence was highest among adults. This finding was consistent with findings of previous studies (11,12); however, we did not observe an age-based difference in shedding prevalence. One potential bias in our sampling strategy was that the age groups, which were composed of randomly selected individuals, did not reflect the age ratio of the overall group, which might have contributed to the lack of difference among age groups in shedding prevalence. We had expected to see a lower rate of shedding among juvenile than among adult animals because seroconversion is evident at sexual maturity for most laboratory and free-ranging macaques (33). It could be that younger animals were experiencing primary infection from exposure during capture and transport, which could explain the higher than expected shedding prevalence for this age group and in the overall study.

Our primary aim with this study was detection of MaHV1 DNA in macaques. However, we included serologic test results from a subset of animals to identify antibody seroprevalence among macaques in different age groups and to determine whether shedding occurs in the absence of detectable antibodies. We detected viral DNA in 36 seronegative macaques. This finding may have been the result of a recent primary infection before detectable IgG response. Some animals could have been infected by conspecifics during transport or just before capture. It is also possible that these animals were experiencing acute virus reactivation resulting from the stress of capture and transportation during the 6–72 h before sampling because previously infected animals would probably be seropositive. Unfortunately, data for the duration of time between capture and sampling were not available. The incubation period for HSV-1 or HSV-2 in humans is 2–12 days (34). Although the time between onset of stress and MaHV1 reactivation has not been determined for macaques, in mice, HSV can reactivate in as little as 14 h after exposure to a stressor (35). Approximately 80% of the animals shedding MaHV1 in the absence of detectable IgG were subadults or juveniles, suggesting that this infection was their first.

The low frequency of simultaneous MaHV1 detection in oropharyngeal and urogenital swab samples suggests a variable shedding pattern among individuals, which was not unexpected given the fact that the virus can sequester itself in the ganglia of the trigeminal nerve, sacral nerve (36,37), or both, which would probably affect the route of virus excretion. Other MaHV1 studies have also reported inconsistent detection of virus in oral and genital secretions from infected laboratory macaques sampled repeatedly over time (11,31). Sensitivity of the PCR assay we used was lower than that of the one used by Scinicariello et al. (33), which might have resulted in underdetection of viral DNA in macaque clinical samples. Use of real-time PCR (not available for this study), such as that developed by Huff et al. (38), which has a sensitivity of 10 viral particles,

### Table 2. PCR results for macaque herpesvirus 1 in macaques, by sample type, Malaysia, 2009–2011

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sex</th>
<th>Oropharyngeal positive</th>
<th>Urogenital positive</th>
<th>Oropharyngeal and urogenital positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>(%; 95% CI) positive</td>
<td>No.</td>
<td>(%; 95% CI) positive</td>
</tr>
<tr>
<td>M</td>
<td>220</td>
<td>58 (26.4; 20.5–32.2)†</td>
<td>220</td>
<td>56 (25.5; 19.7–31.2)†</td>
</tr>
<tr>
<td>F</td>
<td>169</td>
<td>27 (16.0; 10.5–21.5)†</td>
<td>169</td>
<td>41 (24.3; 17.8–30.7)†</td>
</tr>
<tr>
<td>Unknown‡</td>
<td>3</td>
<td>1 (33.3; 0–86.7)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>392</td>
<td>86 (21.9; 17.8–26.0)</td>
<td>392</td>
<td>97 (24.7; 20.5–29.0)</td>
</tr>
</tbody>
</table>

*For animals with positive results for both oropharyngeal and urogenital samples, percentages are of the total number of positive animals.
†Indicates a significant difference (p<0.05) between the 2 groups marked for oropharyngeal swabs.
‡33 macaques of unspecified sex were included in the results.

### Table 3. ELISA results for macaque herpesvirus 1 antibodies in , by age and sex, Malaysia, 2009–2011

<table>
<thead>
<tr>
<th>Age group, y</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Unspecified No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult, &gt;6†</td>
<td>32 21 (66.6; 49.2–82.1)</td>
<td>17 13 (76.6; 60.0–96.6)</td>
<td>1 1 (100.0; NA)</td>
<td>50 35 (70.0; 53.5–82.7)</td>
</tr>
<tr>
<td>Subadult, 3–6*</td>
<td>29 12 (41.4; 23.5–59.3)</td>
<td>21 11 (52.4; 34.5–73.7)</td>
<td>0 0 (NA)</td>
<td>50 23 (46.0; 28.1–59.8)</td>
</tr>
<tr>
<td>Juvenile, &lt;3</td>
<td>26 8 (30.8; 13.0–48.5)</td>
<td>23 7 (30.4; 12.7–49.2)</td>
<td>0 0 (NA)</td>
<td>49 15 (30.6; 12.9–43.5)</td>
</tr>
<tr>
<td>Total</td>
<td>87 41 (47.1; 36.6–57.6)</td>
<td>61 31 (50.8; 40.3–63.4)</td>
<td>1 1 (100.0; NA)</td>
<td>149 73 (49.0; 38.5–57.0)</td>
</tr>
</tbody>
</table>

*Indicates a significant difference (p<0.05) between the age groups marked.
would substantially improve sensitivity of future studies that screen macaques for MaHV1.

The observed MaHV1 shedding patterns suggest that a substantial proportion of animals shed virus after, and potentially during, transport and that the risks for exposure to MaHV1 by wildlife personnel or others handling macaques under these circumstances should be seriously considered. Appropriate PPE, including coveralls, gloves, N95 or P100 respirators, and eye protection, are recommended for wildlife personnel when handling macaques (and any other nonhuman primate) under conditions in which stress and prolonged confinement with other macaques may contribute to increased shedding of MaHV1 and potentially other pathogens. Indeed, as a result of this study, DWNP is strengthening its existing policies requiring personnel handling macaques to wear PPE and use proper work area biosafety and disinfection techniques to reduce the risk for transmission of MaHV1 and other zoonotic pathogens, in accordance with established safety protocols (22). Personnel working with macaques have received additional training to increase their awareness of the potential risks for exposure to MaHV1.

Future studies should determine whether zoonotic transmission has occurred among those who have occupational contact with macaques during procedures such as capture, sample collection, treatment, and translocation (e.g., wildlife personnel) and should determine the incidence rate for infection among high-risk populations. Questions remain about the etiology of viral encephalitides throughout Asia and what proportion of these may be caused by MaHV1. Studies that examine the shedding prevalence of MaHV1 in free-ranging macaques will improve our understanding of shedding in the absence of anthropogenic stressors and, coupled with human surveillance, will enable further assessment of the potential risk for zoonotic transmission. These results will be of particular relevance to professionals who are occupationally exposed to macaques.

Acknowledgments
We express our gratitude to the wildlife officers and veterinarians of the DWNP in Peninsular Malaysia who provided access to macaques for this study. We thank Richard Eberle for generously providing ELISA plates and technical guidance, Nona Yeoh for facilitating the ELISA, and Parviez Hosseini and Andrew Huff for consultation on statistical methods.

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The prevalence and consequences of malaria among infants are not well characterized and may be underestimated. A better understanding of the risk for malaria in early infancy is critical for drug development and informed decision making. In a cross-sectional survey in Guinea, The Gambia, and Benin, countries with different malaria transmission intensities, the overall prevalence of malaria among infants ≤6 months of age was 11.8% (Guinea, 21.7%; The Gambia, 3.7%; and Benin, 10.2%). Seroprevalence ranged from 5.7% in The Gambia to 41.6% in Guinea. Mean parasite densities in infants were significantly lower than those in children 1–9 years of age in The Gambia (p=0.0001) and Benin (p=0.0021). Malaria in infants was significantly associated with fever or recent history of fever (p=0.007) and anemia (p=0.001). Targeted preventive interventions, adequate drug formulations, and treatment guidelines are needed to address the sizeable prevalence of malaria among young infants in malaria-endemic countries.

Infants are thought to be protected against malaria during the first 6 months of life, largely due to the transfer of maternal antibodies (1) and the presence of fetal hemoglobin (2). Thus, young infants have received little attention in terms of malaria research and treatment guidelines, and this age group has systematically been excluded from clinical trials. As a consequence, young infants are frequently given off-label antimalarial treatments at dosing schedules recommended for older infants and children (3). The lack of attention to case management in this age group is a cause of concern and should be addressed, particularly when considering the widespread use of artemisinin-based combination therapies (ACTs) (4) and ongoing antimalarial drug development.

The true prevalence of malaria in young infants is not well characterized, yet defining the prevalence is critical, especially in light of ongoing epidemiologic shifts in populations at risk for malaria (5). Data on the prevalence and clinical outcomes of malaria in young infants are limited and contradictory: some studies show minimal risk (6–8), and others report that the risk for malaria increases in the first months of life, according to the intensity of transmission (9). A few reports indicate that the prevalence of disease is higher than previously thought and that, after birth, the period of protection against malaria is shorter than the widely quoted 6 months (10,11). However, variations in study designs and challenges related to small sample sizes, lack of details regarding quality control, and varied procedures for sample selection make it difficult to interpret findings from earlier studies (3). A better understanding of the risk for malaria in early infancy is needed to develop antimalarial drugs and inform policy decisions for this age group (4). To improve our knowledge of malaria in young infants, we used standardized methods and more sensitive diagnostics to better characterize the prevalence of malaria among children ≤6 months of age in different epidemiologic settings.

Methods

Study Population and Sampling Design

This cross-sectional survey was conducted in 3 countries in western Africa: The Gambia, Benin, and Guinea (also known as Guinea Conakry), representing areas of low,
Malaria among Young Infants, Africa

moderate, and high malaria transmission, respectively. In each country, regions were selected to represent overall malaria transmission trends and surveys were conducted in the catchment areas of 3 health facilities selected by using simple random sampling. In The Gambia, the Essau and the Soma Major Health Centres and the AFPRC General Hospital in Farafenni were selected out of 6 available sentinel surveillance sites. In the southern part of Benin, Bethesda Hospital and the Dodji-Bata and Golo-Djigbe health facilities were selected from a total of 12 available facilities, and in the Farannah district in Guinea, the Nalia, Tiro, and Banian health centers were also selected from 12 available facilities (Figure 1).

Malaria transmission in The Gambia and Benin is seasonal, occurring during the rainy season, whereas transmission in Guinea occurs year-round. Surveys were timed to coincide with the peak of malaria transmission in each country: October–November 2011 in The Gambia and Guinea; July–August 2012 in Benin. Before conducting the surveys, we explained the study objectives to community members in the catchment areas and obtained community approval. We identified households with infants by reviewing delivery records to detect births in the 6 months before the survey; traditional birth attendants assisted with the reviews. An information sheet explaining the objectives of the survey and the study procedures was then distributed to the parents of the infants. After written informed consent was obtained from parents, identified infants were enrolled in the study. In households with ≥2 eligible infants, 1 infant was selected by using simple random sampling. Once the index infant was selected, 2 older children (1–9 and 10–15 years of age) living in the same household were also selected by simple random sampling and included in the study with the objective of estimating the force of transmission and differences in the local risk for infection between infants and older children. If children of the required age group were not available within the infant’s household, the nearest households were visited consecutively until eligible children were identified and enrolled.

Data Collection

Study participants underwent a physical examination; axillary temperature and weight were recorded for each child. Information on the use of bed nets, including long-lasting insecticidal nets, and history of fever in the previous 24 hours was collected by using a structured questionnaire. All study participants had a blood sample collected by finger prick (children 1–15 years of age) or heel prick (infants ≤6 months of age). A rapid malaria diagnostic test (RDT) (ICT Malaria P.f. Cassette Test [ML01]; ICT Diagnostics, Cape Town, South Africa) was performed, and children who tested positive were immediately treated according to treatment guidelines for the country in which they lived. Hemoglobin concentration was measured by using a HemoCue Hb 301 System (HemoCue AB, Ängelholm, Sweden) according to the manufacturer’s instructions. Thick-film blood slides were stained with 10% Giemsa for 10 min, and the presence of Plasmodium falciparum parasites was determined by reading 100 high-power fields under oil immersion. Slides were read independently by 2 microscopists, and parasite density was estimated by counting the numbers of asexual parasites per 200 leukocytes. Results were expressed as the number of parasites per microliter, assuming a total leukocyte count of 8,000 cells/μL. A 20%
error check was used to identify discrepancies between slide readers. All discordant results were read by a senior microscopist, and the result was used as the final read. Blood slides from Guinea and The Gambia were read at the Medical Research Council (MRC) Unit in The Gambia; blood slides from Benin were read, following the same protocol, at Entomological Research Centre of Cotonou. The first 99 slides from Benin were read again in The Gambia; results were comparable.

Molecular diagnosis of malaria parasites and speciation of Plasmodium species were conducted by using dry blood-spot samples (DBSs) collected on filter paper (Whatman 3MM; Whatman 3 Corporation, Florham Park, NJ, USA). DNA was extracted from 3 disks (6-mm diameter), which had been punched from DBSs by using a QIA Xtractor robot (QIAGEN, Venlo, Limburg, Netherlands) according to the manufacturer’s protocol, and analyzed by using nested PCR as previously described (12).

To determine the prevalence of malaria antibodies, we punched disks (6-mm diameter) from DBSs and placed them in 96-well plates. Serum that had been eluted after overnight (18 h) incubation at room temperature in 150 μL of reconstitution buffer (150 μL phosphate-buffered saline/0.05% [vol/vol] Tween 20/0.05% [wt/vol] sodium azide) was used to determine antibodies against the 19-kDa merozoite surface protein 1 (MSP119) by indirect ELISA, as previously described (13,14). MSP119 used in these assays was obtained from the London School of Hygiene and Tropical Medicine (London, UK).

Sample Size and Statistical Analysis
The sample size was computed on the lowest expected prevalence of infection, assumed to be 2% in The Gambia. For each country, we estimated that 750 children in each of the 3 age categories would be sufficient to determine the prevalence of malaria. Assuming infants <6 months of age made up ≈2%–3% of the total population, the required sample size would be found within a population of ≈40,000 persons.

Data from the case record forms were double-entered into an OpenClinica database (https://community.openclinica.com/). After being cleaned, the data were analyzed by using Stata Statistical Software, release 12.1 (StataCorp LP, College Station, TX, USA). Baseline data were analyzed by descriptive methods, and summary statistics were presented as means ±SDs for continuous data and frequencies and proportions for categorical data. The χ² test was used to analyze differences in proportions. Two-tailed p values and a 5% significance level were used. Results for infants ≤6 months of age from all 3 countries were pooled together, and univariate and multivariate logistic regression analyses were performed to determine features associated with malaria in this age group. A forward fitting logistic regression model was used to account for confounders and interaction. The odds for malaria with increasing age within the 0- to 6-month-old age group was determined and presented by country.

For the serologic tests, the distributions of log-transformed antibody titers were fitted as the sum of 2 Gaussian distributions, which were assumed to represent a narrower distribution of seronegative results to the left and a broader distribution of seropositive results to the right. The mean concentration of the seronegative distribution (the distribution with the smallest mean) +2 SDs was considered the seropositivity cutoff (15).

Ethical Considerations
The study was approved by The Gambia Government/MRC Joint Ethics Committee, National Committee of Ethics for Health Research (Benin), and the National Committee of Ethics for Health Research (Guinea). Written informed consent was obtained from the parents of each participant by a signature or thumbprint.

Results
Characteristics of the Study Population
A total of 6,761 children were included in the survey: 2,270 from The Gambia, 2,276 from Benin, and 2,215 from Guinea. The number of children categorized by age group, sex, and mean weight by age was comparable between countries (Table 1). Almost 40% (838/2,219) of the infants weighed <5 kg; no difference in weight was seen by country. In The Gambia and Benin, bed net coverage (defined as having slept under a bed net the night before the survey) was >90% in children 0–6 months and 1–9 years of age (Table 1). Conversely, bed net coverage was extremely low across all age groups in Guinea; only ≈30% of children <10 years of age and 14% of children 10–15 years age used bed nets (Table 1). Overall, the prevalence of fever was lower among children 10–15 years than among infants 0–6 months of age. The highest percentage of fevers (48.1%, 359/747) was seen by country. In The Gambia and Benin, bed net coverage was extremely low across all age groups in Guinea; only ≈30% of children <10 years of age and 14% of children 10–15 years used bed nets (Table 1).

Prevalence of Malaria
By all 3 diagnostic methods, malaria prevalence was lowest in The Gambia and highest in Guinea; Benin had intermediate values. In all 3 countries, malaria prevalence was generally lower in infants 0–6 months of age (Table 2). Results from the RDT and microscopy were comparable, although, with 1 exception, the RDT tended to identify more positive samples. The exception was that microscopy showed a much higher prevalence of malaria among young infants in The Gambia (Table 2).
By microscopy, all malaria cases identified in children from The Gambia were determined to be caused by infection with *P. falciparum* parasites. In Benin and Guinea, *P. malariae* and *P. ovale* parasite infections were also identified, predominantly as mixed infections. In Guinea, the prevalence of *P. malariae* parasite infections was 0.3% (2/724) in young infants, 12.0% (90/748) in children 1–9 years of age, and 5.8% (43/743) in children 10–15 years of age. Of these infections, 97% (131/135) were mixed infections with *P. falciparum* parasites. The prevalence of *P. ovale* parasite infection in Guinea was 3.1% (23/748) in children 1–9 years of age and 0.9% (7/743) in children 10–15 years of age; no cases were detected among young infants. In Benin, the prevalence of *P. malariae* parasite infection was 0.1% (1/761) in young infants, 1.7% (13/759) in children 1–9 years of age, and 2.8% (21/756) in children 10–15 years of age. In Benin, 34% (12/35) of the infections were mixed *P. malariae* and *P. falciparum* parasite infections. Overall, the mean parasite density per microliter of blood was 371.5 in The Gambia, 1,688.3 in Benin, and 2,037.9 in Guinea.

**Table 1. Characteristics of children in a study of malaria prevalence among young infants in different transmission settings, Africa**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>The Gambia, N = 2,270</th>
<th>Benin, N = 2,276</th>
<th>Guinea, N = 2,215</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>734 (32.3)</td>
<td>761 (33.4)</td>
<td>724 (32.7)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>768 (33.8)</td>
<td>759 (33.3)</td>
<td>748 (33.8)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>768 (33.8)</td>
<td>756 (33.2)</td>
<td>743 (33.5)</td>
</tr>
<tr>
<td>Sex, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1,222 (53.8)</td>
<td>1,189 (52.2)</td>
<td>1,159 (52.3)</td>
</tr>
<tr>
<td>M</td>
<td>1,046 (46.2)</td>
<td>1,087 (47.8)</td>
<td>1,056 (47.7)</td>
</tr>
<tr>
<td>Mean weight, kg (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>5.8 (3.6)</td>
<td>5.2 (1.5)</td>
<td>5.5 (2.5)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>12.8 (3.6)</td>
<td>14.3 (4.1)</td>
<td>14.4 (4.1)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>31.2 (7.5)</td>
<td>30.1 (7.8)</td>
<td>31.6 (7.6)</td>
</tr>
<tr>
<td>Bed net coverage, no./no. total (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>699/727 (96.1)</td>
<td>678/750 (90.4)</td>
<td>225/723 (31.1)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>724/765 (94.6)</td>
<td>656/722 (90.9)</td>
<td>222/746 (29.8)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>642/752 (85.4)</td>
<td>581/727 (79.9)</td>
<td>103/740 (13.9)</td>
</tr>
<tr>
<td>Fever or history of fever, no./no. total (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>136/732 (18.6)</td>
<td>129/758 (17.0)</td>
<td>282/724 (38.9)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>133/768 (17.3)</td>
<td>119/758 (15.7)</td>
<td>359/747 (48.1)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>56/760 (7.4)</td>
<td>83/756 (11.0)</td>
<td>221/743 (29.7)</td>
</tr>
<tr>
<td>Mean hemoglobin level, g/dL (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>11.8 (2.2)</td>
<td>11.3 (2.0)</td>
<td>12.1 (3.7)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>10.9 (1.5)</td>
<td>11.2 (1.4)</td>
<td>10.0 (1.7)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>12.4 (1.4)</td>
<td>12.3 (1.6)</td>
<td>11.8 (1.4)</td>
</tr>
</tbody>
</table>

*Defined as having slept under a bed net the night before survey.
†History of fever refers to fever in the 24 h before the structured questionnaire was completed.

**Table 2. Prevalence of *Plasmodium* species parasites, by testing method, among children in different transmission settings, Africa**

<table>
<thead>
<tr>
<th>Test method, age group</th>
<th>The Gambia, N = 2,270</th>
<th>Benin, N = 2,276</th>
<th>Guinea, N = 2,215</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid malaria diagnostic test*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>3/734 (0.4)</td>
<td>23/761 (3.0)</td>
<td>161/724 (22.2)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>11/768 (1.4)</td>
<td>254/759 (33.5)</td>
<td>667/748 (89.2)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>35/768 (4.6)</td>
<td>317/756 (41.9)</td>
<td>611/743 (82.2)</td>
</tr>
<tr>
<td>Microscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>25/734 (3.4)</td>
<td>25/761 (3.3)</td>
<td>133/724 (18.4)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>8/768 (1.0)</td>
<td>201/759 (26.5)</td>
<td>574/748 (76.7)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>21/768 (2.7)</td>
<td>284/756 (37.6)</td>
<td>621/743 (82.4)</td>
</tr>
<tr>
<td><em>P. falciparum</em> gametocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>1/734 (0.1)</td>
<td>7/761 (0.9)</td>
<td>61/724 (8.4)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>2/768 (0.3)</td>
<td>66/759 (8.7)</td>
<td>138/748 (18.4)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>7/768 (0.9)</td>
<td>70/756 (9.3)</td>
<td>91/743 (12.2)</td>
</tr>
<tr>
<td>PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Plasmodium</em> spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>27/734 (3.7)</td>
<td>78/761 (10.2)</td>
<td>157/724 (21.7)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>18/768 (2.3)</td>
<td>243/759 (32.0)</td>
<td>591/748 (79.0)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>35/768 (4.6)</td>
<td>324/756 (42.9)</td>
<td>577/743 (77.7)</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>9/734 (1.2)</td>
<td>41/761 (5.4)</td>
<td>139/724 (19.2)</td>
</tr>
<tr>
<td>1–9 y</td>
<td>10/768 (1.3)</td>
<td>193/759 (25.4)</td>
<td>531/748 (71.0)</td>
</tr>
<tr>
<td>10–15 y</td>
<td>25/768 (3.3)</td>
<td>234/756 (30.9)</td>
<td>502/743 (67.6)</td>
</tr>
</tbody>
</table>

*ICT Malaria P.f. Cassette Test (ML01) (ICT Diagnostics, Cape Town, South Africa).
For the 3 countries, *Plasmodium* spp.–specific PCR also showed a higher prevalence of malaria in all age groups with increasing malaria transmission intensity (Table 2). Prevalence of malaria in infants, as determined by molecular methods, was higher in Guinea (21.7%, 95% CI 18.7%–24.7%) than in Benin (10.2%, 95% CI 8.1%–12.4%) and The Gambia (3.7%, 95% CI 2.3%–5.0%) (Table 2). Species-specific PCR results, compared with microscopy results, showed a lower prevalence of *P. falciparum* infection only in young infants (1.2% [9/734] vs. 3.4% [25/734]; p = 0.005). Gametocyte prevalence by microscopy was lower in infants and increased with age and transmission intensity across the 3 countries (Table 2).

### Prevalence of Malaria Antibodies

Overall, the prevalence of MSP1<sub>19</sub> antibodies varied from 5.7% among young infants in The Gambia to 45.9% among 1- to 9-year-old children in Guinea (Table 3). Antibody seroprevalence generally increased with age and with transmission intensity across the 3 countries. With the exception of results for children ≥1 year of age in Guinea, antibody seroprevalence was higher than the prevalence of infection as determined by microscopy (Table 3). For young infants, antibody seroprevalence was also higher than parasite prevalence and increased with transmission intensity from 5.7% in the Gambia to 36.5% in Benin and 41.6% in Guinea.

### Malaria in Infants 0–6 Months of Age

The overall prevalence of malaria among infants was 8.2% (183/2,191) as determined by microscopy and 11.8% (262/2,191) as determined by PCR; the prevalence was substantially higher in Guinea than in Benin or The Gambia (Table 2). Mean parasite densities per milliliter of blood were significantly lower in infants than in 1- to 9-year-old children in The Gambia (68/μL [SD 168] vs. 26,708/μL [SD 32,074]; p<0.0001) and in Benin (6,894/μL [SD 20,567] vs. 12,933/μL [SD 49,895]; p = 0.0021) but not in Guinea (5,725/μL [SD 11,423] vs. 5,479/μL [SD 21,689]; p = 0.89).

Malaria in infants was significantly associated with fever or history of fever in the previous 24 hours (adjusted odds ratio [aOR] 1.65, 95% CI 1.15–2.37; p = 0.007), axillary temperature ≥37.5°C (aOR 2.07, 95% CI 1.08–3.98; p = 0.029), and anemia (aOR 5.54, 95% CI 3.91–7.84; p = 0.001) (Table 4). Infants weighing <5 kg had significantly higher odds for having malaria (aOR 3.45, 95% CI 2.22–5.26, p = 0.001).

In The Gambia and Benin, the odds for having malaria remained almost stable across the 0- to 6-month-old age group (Figure 2). In contrast, lower odds for having malaria was seen in infants 0–2 months of age in Guinea and markedly increasing odds for having malaria was seen in infants 2–6 months of age. The overall trend was lower odds for malaria in infants 0–3 months of age and subsequently increasing odds for malaria from ≥3 to 6 months of age (Figure 2). In Guinea and Benin, antibody titers were higher early in infancy, declined steadily over the 0- to 4-month age range, and then increased slightly after 4 months of age. Conversely, in The Gambia, infants had lower antibody titers and little evidence of increasing titers over the 0- to 6-month age range (Figure 3).

### Discussion

The prevalence of malaria among infants 0- to 6-months of age was not trivial (range 3.7%–22%, by PCR) and increased with transmission intensity, as documented by the prevalence among older children. This variability in prevalence may be due to differences in transmission intensity but may also be due to differences in the use of preventive measures, as illustrated by the extremely low use of bed nets in Guinea. Such low intervention coverage may be an indicator of weak health systems with limited access to...
other malaria control interventions (e.g., prompt and efficacious treatment and intermittent preventive treatment for pregnant women), which enable an efficient cycle of malaria transmission in local populations, including infants.

In the low-transmission setting in The Gambia, the risk for malaria did not vary substantially between age groups. This finding was in obvious contrast to those in Benin and Guinea, where prevalence among infants was substantially lower than that among older children. This suggests that in areas where transmission has decreased substantially to low levels, the risk for infection may be shared by the entire population, including infants, and in high-transmission settings, infection in infants may be relatively limited by passively transferred maternal antibodies or possibly by lower attractiveness of infants to mosquitoes. The prevalence figures reported are consistent with those of earlier studies, which were limited by smaller sample sizes, different selection criteria, and small geographic areas. The results of our surveys in these 3 countries in western Africa provide a regional estimate of the current prevalence of malaria among infants. The survey was conducted by using a relatively large sample size and robust methods of malaria diagnosis, factors that enhance the generalizability of the findings to other settings.

It is not surprising that MSP1_19 antibody seroprevalence was generally higher than parasite prevalence in young infants: this finding may be a reflection of maternal antibodies passively transferred to the fetus during the last trimester of pregnancy and not necessarily a reflection of the infant's own responses. Prenatal transfer of antibodies may also explain the dynamics of MSP1_19 antibody titers in young infants. In Guinea and Benin, high titers were observed in children in early in infancy, followed by a rapid decline in mean antibody titers until 4 months of age and then a subsequent slight increase. In contrast, infants in The Gambia had lower antibody titers and little evidence of an increase over the 0- to 6-month age range, indicating no substantial ongoing endogenous antibody production.

P. falciparum was the dominant parasite species in all age groups, but a few cases of P. malariae and P. ovale infection (mostly mixed infections with P. falciparum parasites) were found among infants in Guinea and Benin. Therefore, currently available ACTs should suffice for the management of these cases, although failed parasite clearance has been reported in some P. malariae and P. ovale parasite–infected persons treated with ACTs.

Malaria in infants was significantly associated with fever or with a history of fever in the 24 hours before the survey, but only 10% of infants with malaria had an axillary
RESEARCH

temperature ≥37.5°C at the time of the survey. Although parasite densities in infants were lower than those in older children, about half of the infants with malaria were symptomatic. This finding contrasts with the long-held belief that malaria in young infants is not associated with clinical symptoms (23, 24). The findings from this survey therefore provide evidence that malaria in young infants may be symptomatic and should be evaluated for and treated. In addition, malaria in this age group was significantly associated with anemia, indicating that malaria can have a major negative effect on the health of infants. Other previously reported clinical manifestations (e.g., splenomegaly, hepatomegaly, jaundice, vomiting, diarrhea, poor feeding, restlessness, drowsiness, pallor, respiratory distress, and convulsions) (25, 26) were not consistently documented in this survey. A study systematically investigating for malaria in all 0- to 6-month-old infants attending health facilities in the same areas as this survey has recently been completed and should provide more information on the clinical signs and symptoms of malaria in this age group; that study used RDTs, microscopy, PCR, and hemoglobin measurements.

We have shown that malaria in young infants is not rare, can be symptomatic, and has major health consequences, most notably anemia. Current World Health Organization guidelines recommend the use of ACTs in infants, but they specify that for young infants weighing <5 kg, the available evidence is insufficient to confidently recommend this treatment. Thus, many of the ACTs carry label restrictions saying they should not be used for infants weighing <5 kg (27). This restriction is problematic because a substantial proportion (40%) of the infants in these surveys weighed <5 kg and would therefore not meet standard criteria for treatment with ACTs. In addition, there are few pediatric ACT formulations, and the dosing is often difficult. Therefore, data on the efficacy and safety of ACTs in young infants is urgently needed to inform optimal treatment.

The tools used in our study provided comparable estimates of the prevalence of malaria in young infants, with the exception of RDTs, which greatly underestimated the prevalence of malaria in The Gambia, possibly because of the low parasite densities (28, 29). Prevalence estimates determined by microscopy and PCR were surprisingly similar, which may be due to the high sensitivity of microscopy readings conducted in a research institution with strict quality-control procedures. Using microscopy, we were able to detect parasite densities as low as 2 parasites/µL of blood; it is estimated, however, that in an average health care facility with standard microscopy, the detection threshold would be 50–100 parasites/µL of blood (30, 31). In some sites, the lower prevalence by PCR, compared with RDT, may be due to persistent antigenemia from past infections in the absence of current parasitemia.

The overall dynamics of infection across the 3 countries suggests that the period of protection against malaria may be the first 3 months of life, and thereafter the odds for malaria rise increasingly by age. Our findings therefore provide evidence that the period of perinatal protection may be shorter than 6 months and that the 0- to 6-month-old age group is not a homogenous group in terms of malaria susceptibility. Thus, the challenge is that young infants are not adequately protected against malaria because of their limited coverage by current preventive strategies, such as seasonal malaria chemoprevention and intermittent preventive treatment during infancy, which are not widely implemented. This inadequate coverage is critical because our findings show that young infants can be affected by malaria and subsequently become anemic, which would also potentially increase their vulnerability to other pathogens (32). Other interventions, such as the RTS,S/AS01 malaria vaccine, which will soon be registered for use, resulted in modest protection against clinical malaria in this age group and did not have any effect on preventing anemia (33).

In conclusion, the prevalence of malaria is sizeable among young infants living in malaria-endemic countries. This problem must be addressed through the development of adequate pediatric drug formulations, targeted preventive interventions, and treatment guidelines for young infants.

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Mr. Ceesay is a higher scientific officer and research coordinator at the Medical Research Unit, The Gambia. His research interests have focused mainly on the epidemiology of malaria in The Gambia and, more recently, on determining the risk for malaria in early infancy and in different transmission settings.

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Transdermal Diagnosis of Malaria Using Vapor Nanobubbles

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A fast, precise, noninvasive, high-throughput, and simple approach for detecting malaria in humans and mosquitoes is not possible with current techniques that depend on blood sampling, reagents, facilities, tedious procedures, and trained personnel. We designed a device for rapid (20-second) noninvasive diagnosis of Plasmodium falciparum infection in a malaria patient without drawing blood or using any reagent. This method uses transdermal optical excitation and acoustic detection of vapor nanobubbles around intraparasite hemozoin. The same device also identified individual malaria parasite–infected Anopheles mosquitoes in a few seconds and can be realized as a low-cost universal tool for clinical and field diagnoses.

Malaria control and elimination would benefit greatly from an efficient and universal diagnostic tool that is fast (provides results in seconds), noninvasive and safe (uses no blood sampling or reagents), simple to use (can be operated by nonmedical personnel), sensitive and specific (detects low-level asymptomatic infections), and inexpensive and that detects malarial infection in humans and in mosquitoes (1–21). We recently proposed a transdermal blood- and reagent-free approach based on hemozoin-generated vapor nanobubbles (H-VNBS) (22) in which malaria parasite–specific endogenous nanocrystals of hemozoin are optically excited in vivo with a safe and short laser pulse (delivered to blood vessels through the skin). The light is converted into nonstationary localized heat that evaporates the adjacent nanovolume of liquid and thus generates an expanding and collapsing vapor nanobubble inside the parasite. The nanosize and high optical absorbance of hemozoin provide higher malaria infection specificity of these H-VNBS than does any normal blood and tissue components (23–26). Their transient expansion and collapse result in a noninvasive pressure pulse that is easily detected through the skin with an ultrasound sensor. In our preliminary studies (22), H-VNBS detected parasitemia as low as 0.0001% in vitro (human blood), and 0.00034% in vivo (transdermal detection in animals), with no false-positive signals. Therefore, H-VNB might be able to detect extremely low parasite densities provided the method can be applied to humans or mosquitoes simply and inexpensively.

To determine the technical and medical feasibility of H-VNBS for malaria diagnosis and screening, we prototyped a diagnostic device and evaluated it in a patient with confirmed malaria and in noninfected persons as controls. We also evaluated the device in Plasmodium falciparum–infected mosquitoes.

Materials and Methods

Prototype Design and Algorithms

The laboratory prototype (Figure 1, panel A) comprised the newly designed compact low-cost pulsed laser (532 nm, 10 μJ, 200 ps; Standa, Vilnius, Lithuania). The laser pulse is delivered to the skin by the combination probe at the fluence 36 mJ/cm² (Figure 1, panel B). The probe was developed for transdermal diagnostics and includes an optical fiber guide and a custom acoustic sensor with a preamplifier that is integrated in 1 compact hand-held unit. In response to each laser pulse, the probe detects an acoustic pulse and generates an output electrical signal as an acoustic trace (Figure 1). Its output signals were collected and analyzed with custom-designed software (NI LabVIEW, Austin, TX, USA) by using a signal amplifier, digital oscilloscope (LeCroy 42X; Teledyne LeCroy, NY, USA), and computer. The peak-to-peak amplitude A of the acoustic trace obtained in response to each laser pulse was measured and presented as a histogram for 400 sequential laser pulses. A malarial infection–negative trace histogram was used to determine “the malaria threshold” T as the maximum amplitude for the malarial infection–negative signal. Any trace with an amplitude above that threshold was considered to be hemozoin (malarial infection)–positive. To quantify the infection, we counted the incidence rate of malarial infection–positive traces IR (the probability...
of the trace incidence with an amplitude above the malaria threshold calculated for 400 laser pulses) and calculated the Hemozoin Index (HI) (22): HI = IR(A – T).

**Monitoring of Transient Vapor Nanobubbles**

The direct monitoring of transient vapor nanobubbles in response to a single laser pulse uses our optical scattering method (27,28). This method used time-resolved optical scattering of a probe continuous laser beam of very low power (633 nm, 0.1 mW). The probe laser beam was focused on the blood sample collinearly with the excitation laser pulse. The axial intensity of the probe laser beam that passed through the blood sample was monitored with a photodetector. The response to the laser pulse included bulk heating and generation of a transient vapor nanobubble. The bulk heating of the exposed blood volume (without generation of a vapor nanobubble) was detected optically by using a thermal lensing effect that revealed the fast heating and gradual cooling of the exposed volume (Figure 2, panel A, black line in inset). The generation of an expanding and collapsing vapor nanobubble created a strong localized scattering of the probe laser beam by the vapor–liquid boundary, and this effect reduced the probe beam intensity with the bubble diameter (Figure 2, panel A, red line in inset). A vapor nanobubble–specific signal typically is shaped like an inverted bell and represents the growth and collapse of the bubble.

**Patient with Confirmed Malaria**

The patient was admitted to Ben Taub General Hospital, Harris Health System (Houston, TX, USA), with fever, myalgia, abdominal pain, nausea, and vomiting for the previous 4 days and no history of malaria chemoprophylaxis. Malaria identification and speciation was done by microscopy (thin, Wright-Giemsa–stained, peripheral blood smears) and a rapid malaria antigen test (BinaxNow Malaria; Alere Scarborough, Inc., Scarborough, ME, USA). Both tests confirmed a *P. falciparum* malaria infection. By the time of the H-VNB test, the patient had already received antimalarial drugs (doxycycline, malarone, quinidine, and quinine) for 24 h. During hospitalization, the patient had mild hemolytic anemia and thrombocytopenia, neither of which required transfusion of blood products. In addition to the antimalarial medications and to provide symptomatic relief, the patient received intravenous fluids, antiemetics, and antipyretics.

**Diagnostic Locations**

We found wrist and ear lobe veins to be the optimal location for the test. Fingertips were also explored but were inadequate because some persons develop very thick and rigid skin patterns that prevent efficient transdermal delivery of a laser pulse to the blood vessels.

**Influence of Skin Tone**

The difference between the amplitudes of the in vitro background traces from intact blood (Figure 2, panel B, black bars) and those in vivo from a malaria-negative volunteer (Figure 2, panel D, black bars) accounts for the additional contribution of the optical absorbance by melanin in dark skin. The increase in the bulk optical absorbance consequently increased the bulk transient heating (without the generation of H-VNBs) and thus increased the average trace amplitude of the background trace from 10.7 ± 1.7 mV in blood alone (in vitro) to 18.1 ± 5.4 mV in blood and skin (in human) as can be seen by comparing the black histograms in Figure 2, panels B and D. We further studied the influence of skin tone on the background trace in 5 healthy volunteers with different skin tones (Figure 2, panel F). For 532-nm wavelength light, the predictable increase in the background trace amplitude resulted from the higher concentration of the skin pigment, melanin, which determines skin darkness (tone). Therefore, in malaria diagnosis, reference malaria-negative data (histogram and threshold) should be linked to the same level of skin tone as in the malaria patient. Although the optical absorbance...
of skin pigment and hemoglobin is not as high as that for hemozoin (23,24) and is not sufficient to generate vapor nanobubbles under the laser pulse duration and fluence used (29), the bulk transient heat released by hemoglobin and melanin generates a background acoustic trace, which limits the signal-to-noise ratio for the H-VNB method and thus increases the detection threshold for malaria infection. This limitation will be alleviated by developing a “malaria-specific” pulsed laser with a wavelength ≈672 nm—the focus of our ongoing effort because such lasers are not currently available. The optical absorbance of hemoglobin and melanin at 672 nm is much lower than that at 532 nm, and the amplitudes of the background signals and their dependence on skin tone will be reduced. At the same time, the laser pulse energy efficacy of H-VNB generation at 672 nm is similar to that at 532 nm, as we demonstrated previously (22).

Mosquito Model
For infection in mosquitoes, female Anopheles gambiae mosquitoes were fasted for 6 hours and fed on infected blood by using jacketed membrane feeders warmed to 37°C by a circulating water bath. Briefly, cultured P. falciparum (NF54 strain) gametocytes were diluted to 0.3% gametocytemia, 50% normal human erythrocytes and human serum for 15 min. After removing unfed mosquitoes, blood-fed mosquitoes were maintained at 26°C and 70%–80% relative humidity on 10% dextrose. Ten days later, 15 randomly selected mosquitoes were dissected to determine the oocyst numbers, and 2 other groups were used for the device evaluation. Midguts were stained with 0.1% mercurochrome and oocysts counted microscopically. Each of the 15 mosquitoes had 9–151 oocysts (median 50). The remaining mosquitoes were killed by freezing at -20°C before analysis using the prototype device. Mosquitoes fed on uninfected blood and maintained as described were used as negative controls (uninfected and oocyst negative). For further use as positive controls, mosquitoes were fed on uninfected blood containing 60 μg/mL hemozoin.

All procedures were approved by the corresponding internal review board committees at Rice University and Baylor College of Medicine (for Ben Taub Hospital). The patient and the volunteers provided informed consent.

Results and Discussion
We have prototyped a diagnostic device (Figure 1) and evaluated it in a malaria patient and uninfected controls and in malaria infection–positive mosquitoes. Initial in vitro validation of the designed prototype used samples of whole human blood without (uninfected blood) and with hemozoin (#tlrl-hz; InvivoGen, San Diego, CA, USA).
(a proxy for malaria parasite–infected blood [22]). The sample cuvette modeled the blood vessel by using a skin-colored film, a channel with blood 1 mm deep, and an acoustically dampening bottom. The bulk optical absorption of the laser pulse at 532 nm by normal whole blood (mainly by hemoglobin) produced the background trace (Figure 2, panel A, black line) associated with the thermo-elastic effect (30) (a heat-driven transient pressure rise). However, no vapor nanobubbles were generated because the laser fluence applied was well below the vapor nanobubble generation threshold for any normal blood components (22). The absence of vapor nanobubbles was confirmed experimentally by monitoring the laser pulse–exposed sample volume with an optical scattering method (22,27) by using a low-power probe laser beam at 633 nm and monitoring its time-response to the excitation laser pulse (Figure 2, panel A, black line in inset). In normal blood, the optical scattering time-response to a single laser pulse indicated incremental transient bulk heating without generating a vapor nanobubble. Previously we have shown that such a bulk photothermal effect does not cause any detectable detrimental effects at the molecular and cellular levels (22). Adding hemozoin nanocrystals to the blood at a concentration of 23 μg/mL, (which corresponds to ≈0.8% of parasitemia [9]), resulted in a completely different acoustic trace under the same excitation and detection conditions (Figure 2, panel A, red line). This trace was attributed to H-VNBs, which were directly detected in the same sample by optical scattering with optical time-responses of 50–100 ns duration and the H-VNB-specific shape, which revealed the vapor bubble expansion and collapse without any recoil (Figure 2, panel A, red line in inset). Unlike the background acoustic traces obtained from the normal blood, the H-VNB acoustic traces yielded 5-fold higher peak-to-peak amplitudes and thus were easily differentiated from the blood background traces in the trace amplitude histograms (Figure 2, panel B).

To obtain proof of the device feasibility, the prototype was further tested in human volunteers who did not have malaria and on a malaria patient with a similar skin tone (dark). We applied the probe to wrists and earlobes and positioned it over subcutaneous vessels. Thus, laser pulses were delivered to blood through the skin. No blood samples were taken, and no reagents were applied. Acoustic traces in response to each of 400 laser pulses (of the same fluence as described earlier) were collected simultaneously with laser irradiation (within 20 seconds total) and analyzed statistically in real time. In the patient with malaria, the parasitemia (percentage of infected erythrocytes) was determined by microscopy (thin blood film) and varied from 2% (corresponding to ≈69,000 parasites/μL) 4 hours before the device test to 0.3% (corresponding to ≈8,600 parasites/μL) 9 hours after the device test. As malaria-negative controls, we used healthy volunteers with similar skin tone and under the conditions and procedure applied to the malaria patient. Acoustic traces from the wrist of the malaria patient (Figure 2, panel C, red line) showed the H-VNB–specific pattern similar to that of the hemozoin-positive samples in vitro (Figure 2, panel A, red line) and had much higher amplitudes than those obtained from a healthy volunteer with a similar skin tone (Figure 2, panel C, black line). The amplitudes for the traces from the malaria patient were significantly higher than those from the control, and the 2 histograms barely overlapped (Figure 2, panel D). Therefore, these acoustic traces indicated malarial infection in a clinically ill patient. Similar traces were obtained when the device was applied to the earlobes of the malaria patient and volunteers (3 volunteers with dark skin tone were studied) (Figure 2, panel E). The similarity between the wrist and earlobe results further validates the successful detection of malarial infection by the H-VNB method. A quantitative analysis used the volunteers’ histograms to determine the malaria threshold amplitude and revealed HI values as 42.4 mV and 1.3 mV in the malaria patient for the wrist and earlobe, respectively.

The safety of H-VNB generation in humans is ensured by the safe level of the laser fluence applied, 36 ml/cm², which is considered to be skin-safe according to federal regulations (31). In addition, no short-term (10 min) or long-term (3–4 d) signs of skin damage or irritation were observed in the study participants. These observations are also in line with our previous observation of no damage to the laser-exposed malaria parasite–negative blood cells (22). Therefore, the device and procedure developed appear to be safe, and coupled with their blood- and reagent-free nature, deliver a completely noninvasive diagnosis of malaria in humans.

We further studied the influence of skin tone on the background trace for 5 healthy volunteers with different skin tones (Figure 2, panel F). For 532-nm wavelength light, we observed a predictable increase in the background trace amplitude resulting from the increase in the concentration of melanin.

In the third model, we evaluated the device for the rapid noninvasive transcuticle analysis of individual malaria-infected (oocyst-positive) *Anopheles* mosquitoes. Ten acoustic traces were obtained for each mosquito (7 in each group) by scanning the body across the probe under the same laser pulse fluence as described for the human studies. The negative control group (fed with uninfected human blood, no oocysts) returned acoustic traces similar to those for hemozoin- and malaria parasite–negative human blood (Figure 2, panel G, black line). In the malaria-infected mosquitoes, the trace shape and amplitude (Figure 2, panel G, red line) were similar to those obtained for hemozoin- and malaria parasite–positive traces. The histogram
of malaria-infected mosquitoes significantly shifted to the right, compared with the negative control group (Figure 2, panel H). Finally, the positive control group of mosquitoes fed with the blood mixed with hemozoin (60 μg/mL) also showed traces of a high amplitude (Figure 2, panel H, green bars). This experiment demonstrated the feasibility of the rapid noninvasive detection of individual oocyst-carrying mosquitoes. We propose that vapor nanobubbles can be generated around residual hemozoin in developing oocysts or similar dense forms of heme formed by the malaria parasites at this stage (32,33).

These results provide a proof-of-principle for the H-VNB technology as a unique noninvasive transdermal diagnostic tool for malarial infection in humans and mosquitoes. The next step is to optimize the prototype with a malaria-specific laser operating at 672 nm for a better sensitivity (22). Such prototype will be evaluated in large-scale studies in humans in clinical and field settings in malaria-endemic countries. Although the estimated cost of a battery-powered device (size of a shoebox) is US $15,000, a single unit will be able to test ≥200,000 persons each year without any additional cost (e.g., specialized staff, facilities, and diagnostic reagents); thus, the cost of the individual test may be well below that of a rapid diagnostic test. The device may be able to diagnose asymptomatic and low-density infections, undetectable by microscopy and rapid diagnostic tests and could be deployed for mass screening and treatment or at border control points (a major advantage would be the speed at which results will be available). The presence of hemozoin in all blood stages and types of malaria parasites (25,26) ensures the broad and universal application of our method, even without differentiation of the malaria species. The rapid and simple detection of malaria-infected mosquitoes could provide an easy tool to estimate the transmission intensity, contributing to the efforts of malaria transmission reduction and local elimination.

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etymologia

Quinine [kwin’in]

From the Quechua kina, “bark,” quinine is an alkaloid of cinchona that has antimalarial properties. In the 1620s, Jesuit missionaries living in Peru learned of the healing powers of the bark of “fever trees” that grew in the high forests of Peru and Bolivia.

*Cinchona officinalis 001* by H. Zell. Own work. Licensed under CC BY-SA 3.0 via Wikimedia Commons.

Sources

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In May 2014, a traveler from the Kingdom of Saudi Arabia was the first person identified with Middle East respiratory syndrome coronavirus (MERS-CoV) infection in the United States. To evaluate transmission risk, we determined the type, duration, and frequency of patient contact among health care personnel (HCP), household, and community contacts by using standard questionnaires and, for HCP, global positioning system (GPS) tracer tag logs. Respiratory and serum samples from all contacts were tested for MERS-CoV. Of 61 identified contacts, 56 were interviewed.

HCP exposures occurred most frequently in the emergency department (69%) and among nurses (47%); some HCP had contact with respiratory secretions. Household and community contacts had brief contact (e.g., hugging). All laboratory test results were negative for MERS-CoV. This contact investigation found no secondary cases, despite case-patient contact by 61 persons, and provides useful information about MERS-CoV transmission risk. Compared with GPS tracer tag recordings, self-reported contact may not be as accurate.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a lineage C betacoronavirus that was first reported in September 2012 in a patient from the Kingdom of Saudi Arabia (1). By September 8, 2014, a total of 837 laboratory-confirmed cases and 292 associated deaths had been reported by the World Health Organization. All reported case-patients have resided in or had recent travel to the Arabian Peninsula and neighboring countries (2).

Clusters of MERS-CoV infection have occurred within extended families, households, and healthcare settings (3–6). Contact investigations around imported cases in the United Kingdom, France, and Tunisia identified cases among household and healthcare contacts, suggesting person-to-person transmission (7–9). However, these investigations found limited onward transmission: a maximum of 3 second-generation cases were found among investigations with total contacts ranging from 7–163 persons (7–9). Other contact investigations of imported cases outside of the Middle East have found no secondary transmission (10–13).

On April 29, 2014, the Indiana State Department of Health (ISDH) informed the Centers for Disease Control and Prevention (CDC) of a patient under investigation for MERS-CoV infection. A clinical specimen from the patient was confirmed positive by CDC on May 2, 2014 (5); this infection was identified as the first imported MERS case in the United States. The case-patient, a physician and resident of Saudi Arabia, traveled by airplane to Chicago, Illinois, USA, via London, United Kingdom, then by bus to Indiana, USA. He stayed with his family in Indiana for 4 days, during which time he twice met with a business
associate in Illinois before seeking medical care at an Indiana hospital; multiple healthcare personnel (HCP) at the hospital were exposed to the patient (14). Given the uncertainty around how MERS-CoV is transmitted, we conducted a comprehensive contact investigation of this case to characterize exposures in household, community, and hospital settings and to quantify the risk of transmission. We also compared contact reported by HCP during standardized interviews with those in global positioning system (GPS) tracer tag recordings.

Methods

Ethical Review
This investigation was part of a public health response, so it was determined by CDC to be a nonresearch investigation and not subject to review by the CDC Institutional Review Board. All participants provided verbal consent before interview; parental permission and assent from from minors were obtained as appropriate.

Definitions and Identification of Contacts
For the purpose of this investigation, we defined contacts as all persons who had potential exposure to the case-patient before airborne and contact precautions were instituted. More specifically, we defined HCP contacts as all persons who had a face-to-face (within 1 meter) interaction with the case-patient or who entered the case-patient’s room without appropriate personal protective equipment (PPE; i.e., gloves, N95 respirator, gown, and eye protection) before airborne and contact precautions were instituted. HCP contacts were identified by reviewing GPS tracer tag logs, the case-patient’s medical chart, and emergency department (ED) security video footage or through the hospital hotline, on which personnel could self-identify. GPS tracer tags were worn routinely by registered nurses (RNs) and certified nursing assistants (CNAs). The tags track the date and time that staff enter and exit a patient’s room. We reviewed hospital GPS records to determine the exposure time and number of patient visits for attending RNs and CNAs.

Hospital visitor contacts were defined as all persons who visited the case-patient at the hospital before airborne and contact isolation precautions were instituted. Household contacts were defined as all persons who stayed overnight in the same household as the case-patient between his arrival in the United States and his admission to the hospital. Community contacts were defined as all persons, other than household or HCP contacts, who had face-to-face exposure to the case-patient. Hospital visitor, household, and community contacts were identified from interviews with the case-patient, family members, and hospital staff.

Duration of Exposure, Infection Monitoring, and Quarantine
Duration of exposure was determined by asking contacts how much time they had spent with the case-patient. Duration of exposure was also calculated from GPS records.

Following confirmation (on May 2, 2014) that the patient was infected with MERS-CoV, HCP and household contacts checked their body temperature twice daily and self-monitored for respiratory or gastrointestinal symptoms for a total of 14 days after their last exposure to the case-patient. HCP also reported to the hospital’s Employee Health Services each day. In addition, nonphysician HCP contacts were requested to self-quarantine at home or wear surgical masks in the community, and physician HCP contacts were requested to wear surgical masks at work.

Interviews
The case-patient was asked to report his medical and exposure history, health care–seeking behaviors, job-related activities, and social activities during the 14 days before illness onset. HCP, household, and community contacts answered standard questionnaires covering basic demographic information; infection control practices when in contact with the case-patient; type, length, and frequency of contacts with the case-patient; chronic medical conditions; and symptoms since first exposure to the patient.

Biologic Specimen Collection
Serum, nasopharyngeal swab, oropharyngeal swab, stool, and urine samples were collected from the case-patient on various dates (15). Two sets of nasopharyngeal and oropharyngeal swab samples and serum samples were collected from all contacts. The initial and follow-up sets of specimens were collected on postexposure days 3–8 and 12–14, respectively. An additional set of specimens was collected within 48 hours from any contacts who became symptomatic.

Nasopharyngeal and oropharyngeal swab samples were tested at the ISDH laboratory, Massachusetts Department of Public Health, Illinois Department of Public Health, or CDC within 72 hours of collection. Stool and urine samples were tested at the ISDH laboratory, and serum samples were tested at CDC.

Laboratory Testing
Nasopharyngeal, oropharyngeal, urine, serum, and stool specimens were tested by using a MERS-CoV real-time reverse transcription PCR (rRT-PCR) developed by CDC, as previously described (15). Serum specimens collected on postexposure days 12–14 were screened for MERS-CoV–specific IgG, IgM, and IgA by using a recombinant nucleocapsid–based ELISA. Positive ELISA results were confirmed by MERS-CoV immunofluorescence assay.
(IFA) and microneutralization assay (14). A specimen positive by ELISA, indeterminate or negative by IFA, and negative by microneutralization was determined to be negative. A positive serologic result required a positive ELISA result and confirmation by IFA or microneutralization assay. On the basis of clinical discretion, a multiplex PCR assay virus panel (Biofire Diagnostics, Salt Lake City, UT, USA) was performed on samples from the case-patient and 3 contacts.

Data Analyses
Basic descriptive analyses were conducted for all contacts. When available, self-reported and GPS-monitored exposure time and number of visits were compared by calculating Pearson correlation coefficients.

Results
Case-Patient
The case-patient worked at a Saudi Arabia hospital where patients infected with MERS-CoV had been treated in April 2014. He did not recall caring for known MERS patients or patients with respiratory symptoms, but he did perform noninvasive procedures, using appropriate PPE, on 3 or 4 intubated patients. None of his colleagues, friends, or household members had respiratory symptoms during April. Beginning on April 18 (i.e., day of illness [DOI] 1), he had low-grade fever, fatigue, and myalgias. On DOI 6, he departed for the United States; on DOI 10, a mild, nonproductive cough and shortness of breath developed. The case-patient was admitted to the hospital on DOI 11 for right lower lobe pneumonia with hypoxia. On DOI 12, he was suspected of having MERS-CoV infection, so airborne precautions (i.e. N95 respirator and patient isolation in an airborne infection isolation room) were instituted. At 11:00 AM on DOI 13, after MERS-CoV infection was confirmed, contact precautions were initiated and the case-patient was moved to another airborne infection isolation room with an anteroom. Test results for sputum, oropharyngeal swab, and plasma samples continued to be positive for MERS-CoV until DOI 16. A detailed report of the case-patient’s clinical course is published elsewhere (14). The case-patient was discharged from the hospital 11 days after admission (DOI 21).

Contact Investigation
HCP Contacts
Fifty-three HCP self-identified as contacts of the case-patient or were identified as contacts from security video footage, GPS tracer tag logs, or the case-patient’s medical record. Two HCP declined to be interviewed, and 3 could not be reached. Of the 48 HCP contacts interviewed within 1 week of exposure, 3 were determined to not to be contacts and were excluded from the analyses. Of the remaining 45 HCP contacts, 23 were exposed to the patient on hospitalization day 1 (13 in the ED and 10 in the patient’s room or the computerized tomography suite), 19 were exposed on hospitalization day 2, and 9 were exposed on hospitalization day 3; several HCP were exposed on multiple days (Figure 1).

Of the 45 HCP contacts, 7 (16%) were men and 38 (84%) were women. The median age was 41.5 years (range 22.0–61.0 years). HCP in several job classifications were exposed to the case-patient, but most (47%) were RNs or CNAs. Most HCP contacts (71%) were assigned to work in the ED (n = 21 [47%]) or the ward in which the case-patient was hospitalized (11 [24%]); however, 12 (27%) HCP contacts worked in multiple departments (Table).

Six HCP contacts were nonclinical staff (administration, housekeeping, or social services) who had direct contact with the case-patient’s surroundings but never touched the case-patient. Thirty-three HCP contacts (physicians, RNs, CNAs, phlebotomists, and radiology technicians) touched the case-patient while performing activities such as recording vital signs, listening to his lungs, and drawing blood. RNs and CNAs had the most frequent exposures; the median number of self-reported visits for each RN and CNA were 7 and 2, respectively (Table). Six respiratory therapists touched the case-patient and administered nebulizer treatments or spirometry tests.

Figure 1. Number and type of contacts exposed to a Middle East respiratory syndrome coronavirus case-patient per day after his arrival in the United States on April 24, 2014. The same persons could be counted on multiple days of exposure. CT, computed tomography department; ED, emergency department; UNK, unknown; ward, patient’s hospital floor.
Because airborne precautions began ≥24 hours after admission, most HCP contacts (39 [86.7%]) did not use a respirator or surgical mask while attending to the case-patient. Four HCP contacts had underlying medical conditions (current pregnancy, diabetes, or chronic steroid use), which might increase their risk for MERS-CoV infection or disease. Most HCP contacts (26 [58%]) were exposed to the case-patient 1 time; 18 were exposed >2 times, and 4 were exposed ≥10 times (Table). Overall, the median total self-reported exposure time was 11 minutes 30 seconds (range 15 s to 69 min 45 s). Two HCP contacts were excluded from length and frequency of exposure analyses because they could not remember their exposure to the case-patient.

The following symptoms most commonly developed in 9 HCP contacts: rhinorrhea (33%), odynophagia (22%), or headache (22%) within postexposure day 14; more than 1 symptom developed in some contacts. Fever did not develop in any of these contacts.

**Hospital Visitor Contacts**

Three family members were identified as hospital visitor contacts: 2 were also household contacts, and the other was an out-of-town family member who had not been exposed in the household. Two of these contacts were exposed on hospitalization days 1 and 2 without wearing any PPE, and all 3 were exposed on hospital day 3 while wearing N95 masks but no other PPE (Figure 1).

**Household Contacts**

Of the 7 household contacts, 5 permanently resided in the house where the case-patient stayed in the United States, and 2 were visiting from Massachusetts. One household contact was also an HCP contact and was included in both categories. All household contacts had minimal exposure to the case-patient during DOI 7–10 because he had isolated himself during most of his stay. Three household contacts reported hugging and kissing him on the day he arrived (DOI 7) and spending a few hours in the car with him before hospital admission (DOI 7–10). Coryza, but not fever, developed in 2 household contacts; 1 of these contacts tested positive for rhinovirus.

**Community Contact**

The 1 community contact was a business associate of the case-patient. The contact shook hands with the case-patient and had 2 face-to-face meetings with him on April 25 (2.0 h in length) and April 26 (1.5 h in length). At that time, the case-patient had mild myalgias and fever without any respiratory symptoms. On May 14, the contact had a runny nose and mild cough, but fever did not develop, and the contact had test results positive for rhinovirus.

**Table.** Demographic, employment, and exposure information for health care personnel contacts of patient with the first imported case of Middle East respiratory syndrome into the United States, 2014

<table>
<thead>
<tr>
<th>Occupation</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Housekeeping</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Medical doctor</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Nurse practitioner</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Nursing assistant</td>
<td>10 (22)</td>
</tr>
<tr>
<td>Phlebotomist</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Radiology technician</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Respiratory therapist</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Registered nurse</td>
<td>11 (24)</td>
</tr>
<tr>
<td>Social personnel</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary employment location in hospital</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ward</td>
<td>21 (47)</td>
</tr>
<tr>
<td>Emergency department</td>
<td>11 (24)</td>
</tr>
<tr>
<td>Multiple locations</td>
<td>12 (27)</td>
</tr>
<tr>
<td>Computed tomography suite</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Personal protective equipment worn while in contact with the patient†</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gown</td>
<td>0</td>
</tr>
<tr>
<td>Goggles</td>
<td>2 (5)</td>
</tr>
<tr>
<td>N95 respirator</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Surgical mask</td>
<td>2 (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pre-existing condition‡</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>4 (9)</td>
</tr>
<tr>
<td>No</td>
<td>40 (89)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. self-reported times HCP visited the patient’s room between 6:00 PM April 28 and 11:00 PM April 30</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td>1</td>
<td>26 (58)</td>
</tr>
<tr>
<td>2–5</td>
<td>11 (24)</td>
</tr>
<tr>
<td>6–9</td>
<td>3 (7)</td>
</tr>
<tr>
<td>≥10</td>
<td>4 (9)</td>
</tr>
</tbody>
</table>

†HCP, health care personnel; Ward, patient’s hospital floor.
‡Pre-existing conditions that may increase the risk of infection included current pregnancy, chronic steroid use and diabetes.

**Laboratory Results**

For 60 contacts, both initial and follow-up nasopharyngeal and oropharyngeal swab samples and serum samples were negative for MERS-CoV by rRT-PCR and for MERS-CoV–specific antibodies by serologic testing. For the community contact, MERS-CoV test results for initial and follow-up nasopharyngeal and oropharyngeal swab samples and serum samples were negative by rRT-PCR, low titer–antibody positive by ELISA, indeterminate by IFA, and negative by microneutralization assay. His MERS-CoV antibody status was determined to be negative because the ELISA result could not be confirmed by either IFA or microneutralization assay. Additional nasopharyngeal,
oropharyngeal, and serum samples from 8 symptomatic HCP contacts were negative for MERS-CoV by rRT-PCR and serologic testing.

**Self-Reported Versus Monitored HCP Exposure Duration and Number of Visits**

Of the 45 HCP contacts, 11 (24%; 3 RNs and 8 CNAs) wore GPS tracer tags. Of those 11 contacts, 8 reported a number of visits to the patient’s room similar (±2) to that recorded by the tracer tag; 1 underestimated the number by 22 visits; 1 underestimated the number by 16 visits; and 1 did not recollect the tag-recorded visits (Figure 2, panel A). There was no consistent pattern in the way HCP reported their number of visits: some overestimated and others underestimated the number. The total exposure time was more difficult for HCP to recall. Five estimated their exposure time within 10 minutes of the tracer tag–reported time, and 4 estimated within 20 minutes (Figure 2, panel B). The maximum time difference between cumulative self-reported and tracer tag–recorded time was 39 minutes. No significant correlation was found between self-reported and GPS-measured time (R² = 0.47) and number of visits (R² = 0.45) with the case-patient.

**Discussion**

We describe the contact investigation of the first identified MERS patient in the United States. All 61 identified contacts had negative test results for MERS-CoV even though some had face-to-face interactions with or prolonged exposure to the case-patient or administered nebulizer treatments and spirometry tests to the case-patient.

The absence of transmission to household contacts could be explained by the case-patient’s mild initial respiratory symptoms, his hospital admission <24 hours after respiratory symptom onset (DOI 11), his self-isolation at home, and his lack of need for caregiving assistance before admission, all of which served to limit household members’ exposure. Similarly, the absence of transmission to the community contact may have been due to the case-patient’s lack of respiratory symptoms during the 2 meetings. The absence of transmission to household and community contacts in this investigation is similar to that seen in contact investigations of several other patients with MERS (11–13); however, in other settings, transmission to household members who provided care to persons with MERS-CoV infection have been reported, and household clusters have been documented (3–7,16).

When the case-patient was admitted during the second week of illness, the virus load in his sputum was high (14). However, none of the HCP contacts became infected. Serologic results may become positive >10–14 days after exposure, so we minimized the possibility of missing any asymptomatic infections by combining serologic results with clinical evaluation and PCR results. The absence of transmission to the HCP contacts may have been due to the absence of high-risk procedures (e.g., intubation, respiratory suctioning, and bronchoscopy), the short duration of exposure, and the few HCP contacts with underlying medical conditions. In addition, the hospital implemented strict infection control practices soon after the case-patient was suspected of having MERS-CoV infection, limiting the number and duration of exposures. These findings are similar to those from some previously documented contact investigations (12). However, there have been reports of transmission to HCP contacts in hospitals with multiple MERS patients or delayed implementation of appropriate infection control practices (5,7). The different findings from reported investigations illustrate that the specific activities that lead to an increased risk for MERS-CoV transmission still need to be clearly defined.

Severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in 2002 in China and spread globally in 2003. SARS-CoV shares similar characteristics with MERS-CoV, including likely zoonotic origin and transmission (17–20). Recent research on MERS-CoV has demonstrated plausibility for zoonotic transmission from dromedary camels to humans (21,22). MERS-CoV
seems less able than SARS-CoV to spread from person-to-person (23–26). Reports from the SARS-CoV epidemic showed tertiary transmission to >100 people, and 20% of health care workers become infected from the index patient (24,27). Most documented clusters of MERS-CoV infection show limited spread outside certain hospital settings, and unlike transmission in the SARS-CoV epidemic, there have been no foci of sustained transmission outside of the MERS-CoV infection epicenter in and near the Arabian Peninsula (23). However, as with SARS-CoV, the risk for MERS-CoV transmission may vary by patient, and health care facilities must maintain a high index of suspicion and immediately institute appropriate infection control practices for suspected cases.

This investigation is unique because we had independent documentation of duration of exposure from GPS-based tracer tags for 20% of HCP contacts. Most HCP contacts accurately reported case-patient exposure. However, HCP with the most contact had poorer recall accuracy, and 20 minutes’ difference in exposure may alter the HCP contact risk, given that each visit was generally <3 minutes in duration. These findings have important implications for future contact investigations, and we recommend using objective measures of exposure, such as surveillance footage or GPS tracer tags, when available. In addition, we note that self-reported exposures are not always accurate because the accuracy of recalled time versus actual time spent with case-patients may be less reliable for HCP contacts that see a patient regularly for short periods of time.

This investigation had some limitations. First, risk factors for transmission could not be analyzed because none of the contacts were infected. Second, the use of the GPS tracer tag system to monitor HCP interaction with the case-patient might not always have given accurate results because HCP may not have been wearing their assigned tag when entering the room or, conversely, may have stood close to but not in the room, causing the tracking system to record incorrectly that the HCP had entered the room. Use of the GPS system also does not account for changes in risk to HCP contacts, such as if they entered the room while the case-patient was having a computed tomography scan.

In summary, we conducted a thorough contact investigation of this MERS case, including a detailed characterization of the type, duration, and frequency of exposures among HCP, household, and community contacts and testing of contacts for acute disease and asymptomatic infection. We documented the absence of transmission of MERS-CoV from the first identified imported case-patient in the United States despite his having multiple contacts at home and in the hospital before the implementation of appropriate infection control procedures. In addition, our comparison of GPS-monitored contact with HCP recall of contact calls into question the accuracy of information collected by recall during a contact investigation because not all HCP reported information could be confirmed by the GPS tracer tag logs. Although factors leading to MERS-CoV transmission are likely to be complex, additional information is needed regarding the natural history of the illness, in terms of virus shedding, modes of transmission, the role of asymptomatic infections in transmission, effective infection control practices, and the length and types of exposures that do and do not lead to transmission of the virus.

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To support Liberia’s response to the ongoing Ebola virus (EBOV) disease epidemic in Western Africa, we established in-country advanced genomic capabilities to monitor EBOV evolution. Twenty-five EBOV genomes were sequenced at the Liberian Institute for Biomedical Research, which provided an in-depth view of EBOV diversity in Liberia during September 2014–February 2015. These sequences were consistent with a single virus introduction to Liberia; however, shared ancestry with isolates from Mali indicated at least 1 additional instance of movement into or out of Liberia. The pace of change is generally consistent with previous estimates of mutation rate. We observed 23 nonsynonymous mutations and 1 nonsense mutation. Six of these changes are within known binding sites for sequence-based EBOV medical countermeasures; however, the diagnostic and therapeutic impact of EBOV evolution within Liberia appears to be low.

The outbreak of Ebola virus disease (EVD) in Western Africa that started in November 2013 (1) is the largest recorded filovirus disease outbreak. As the outbreak continues, public health and emerging infectious disease officials have declared a continuing need for real-time monitoring of Ebola virus (EBOV) evolution (2,3). As of March 11, 2015, a total of 41% of reported cases had been fatal (4). By the end of March 2015, the intensity of the outbreak, which throughout its course affected 6 Western Africa countries, appeared to be receding, with near 0 activity in Liberia and no cases in Mali, Nigeria, and Senegal. However, EBOV continues to spread in Guinea and Sierra Leone. The epidemic is still causing more infections per week than have been recorded in previous EVD outbreaks (5). Therefore, public health officials continue to use media to maintain public awareness, to advocate for diligent handwashing and use of other protective measures, and to avoid complacency that could lead to reemergence (5).

Vigilance is of paramount importance because currently used assays for EVD diagnosis, and many medical countermeasures in development, were designed using EBOV reference genome variants from previous outbreaks (6–9). Therefore, monitoring EBOV genomic drift is crucial because genetic changes can affect the efficacy of sequence-based therapeutics and diagnostics.

The size and spread of the current EVD outbreak reinforces the need to build public health infrastructure, including state-of-the-art diagnostic and surveillance capabilities, to implement and maintain effective EVD monitoring, treatment, and prevention platforms. The Liberian Institute for Biomedical Research (LIBR), established in 1975, is located in Charlesville, 50 km southeast of Liberia’s capital, Monrovia. As of April 2, 2015, it is one of the few local facilities within Liberia processing clinical samples from persons suspected to have EVD. A consortium comprising US government and nongovernment agencies has been working with the Liberian government to equip LIBR with...
advanced genomic sequencing capabilities. These capabilities are dedicated primarily to EVD surveillance activities, including genome sequencing of EBOV-positive samples. The new LIBR Genome Center has a Miseq sequencer (Illumina, San Diego, CA, USA) and ancillary supporting capabilities, including electrophoresis for qualification, fluorometry for quantitation, PCR for amplification, and fully functional computational analysis capabilities to perform pathogen discovery and microbial genome characterization. The US Army Medical Research Institute of Infectious Diseases (USAMRIID) Center for Genome Sciences supports LIBR operation and development. Sample preparation procedures under biosafety containment are provided within the same building complex by the Liberian National Reference Laboratories, operated by USAMRIID and the National Institutes of Health Integrated Research Facility Ebola Response Team (Fort Detrick, Frederick, MD, USA). Throughput at the LIBR Genome Center is 10–20 samples (∼10 billion bases of sequence data) per week, with a target turnaround time of 7 days from sample receipt for high-priority samples. To ensure long-term sustainment of surveillance-based sequencing capabilities, local biomedical scientists have been trained and can proficiently perform all daily activities.

Here we demonstrate the utility and capabilities of the LIBR Genome Center. With the immediate goal of continuing the natural history characterization of the EBOV Makona variant (EBOV/Mak [10]) currently circulating in Western Africa and to support ongoing clinical trials to evaluate candidate medical countermeasures, we describe 25 EBOV genome sequences from the first 5 sequencing runs conducted at the LIBR Genome Center. We chose these samples for full-genome characterization from ∼1,700 available samples on the basis of high viral load (cycle threshold [Ct] value <24) and date of collection to ensure up-to-date temporal coverage.

Materials and Methods

Samples

We chose samples from 25 patients from the larger collection (∼1,700 positive cases) on the basis of diagnostic Ct values that indicated a high enough viral load to provide a full genome (Ct<24), beginning with the most recent available at the time of preparation in February 2015. Sampling continued with progressively older samples to describe the lineages most likely to still be circulating at the time. These patients were treated in 7 different Ebola treatment units and had resided in 7 of the 15 counties in Liberia (Table 1; online Technical Appendix 1 Figure 1, http://wwwnc.cdc.gov/EID/article/21/7/15-0522-Techapp1.pdf). Plasma or oral swab samples from which viral RNA was recovered and sequenced were tested at LIBR during September 23, 2014–February 14, 2015. Patients’ ages were as follows: 1 infant (1 year), 6 children (2–15 years), 8 young adults (18–35 years), and 10 middle-aged adults (42–67 years). The male:female ratio was 2:1. However, among ∼1,700 samples at LIBR from persons with EVD, the ratio was close to 1:1 (48%/52%), and viral load did not differ by patient sex, which demonstrates that our higher ratio is a sampling artifact.

Sample Processing

RNA was converted to cDNA and amplified by using sequence-independent single-primer amplification (11). Amplified cDNA was quantified with a Qubit 3.0 fluorometer (Life Technologies, Carlsbad, CA, USA) and used as the starting material for the Illumina Nextera XT DNA library preparation kit (Illumina). Sequencing was performed on an Illumina Miseq by using either V2 or V3 reagent kits (Illumina) with a minimum of 2 × 151 cycles per run.

Genome Assembly

We assembled EBOV genomes by aligning reads to the genome of Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3686.1 (GenBank accession no. KM034562.1) (12). Amplification primers were removed from the sequencing reads by using Cutadapt version 1.21 (13), and low-quality reads/bases were filtered by using Prinseq-lite version 0.20.4 (-min_qual_mean 25 -trim_left 20 -min_len 50) (14). Reads were aligned to the reference genome by using DNAStar Lasergene nGen (DNAStar, Madison, WI, USA), and a new consensus was generated by using a combination of Samtools v0.1.18 (15) and custom scripts. Only bases with Phred quality score ≥20 were used in consensus calling, and a minimum of 3× read-depth coverage, in support of the consensus, was required to make a call; positions lacking this depth of coverage were treated as missing (i.e., called as “N”).

Genetic Analysis

Consensus sequences generated here were aligned with additional publicly available EBOV genomes by using Sequencher version 5.2.3 (Gene Codes, Ann Arbor, MI, USA). SnpEff version 4.1b (build 2015-02-13) was used to annotate all single-nucleotide polymorphisms (SNPs) by using the genome of Ebola virus/H.sapiens-wt/GIN/2014/Makona-C15 (GenBank accession no. KJ660346.2) as a reference (16). All 25 genomes from Liberia were used to identify variable sites. For the rest of the genetic analysis, we used only the 14 sequences with >90% genome coverage. A median-joining haplotype network was constructed in PopART version 1.7.2 (http://popart.otago.ac.nz). Path-O-Gen version 1.4 (17) was used to calculate the root-tip distances by using a maximum-likelihood phylogeny (PhyML version 3.0 (18); general time reversible model)
with rooting based on the EBOV phylogeny published by Gire et al. (12). BEAST version 1.8.2 (17) was used to estimate the mutation rate and the time to the most recent common ancestor for several evolutionary lineages that included Liberia EBOV isolates. For analysis, we divided the alignment into 3 partitions (i.e., first + second codon sites, third codon site, and noncoding sites). The substitution process was modeled independently for each by using the Hasegawa, Kishino, and Yano model with 4 gamma categories. An exponential growth coalescent model was used with a strict clock. The XML input file is available on request from the authors.

Results

From the first 5 sequencing runs, we obtained 25 EBOV genomes with >50% coverage; 6 of these were coding complete (Table 2) (19). These genomes contained 97 new sequence variants: 47 synonymous, 23 nonsynonymous, 1 nonsense, and 26 noncoding mutations (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/21/7/15-0522-Techapp2.xlsx). Multiple distinct evolutionary lineages were detected, but all were consistent with a single introduction of a cluster 2–type (12) virus into Liberia followed by within-country diversification (Figure 1, panel A). Because 19 of the 25 genomes had calls at all 5 positions that discriminate clusters 1, 2, and 3, we have high confidence in cluster attribution.

Molecular dating places the common ancestor to all of the sampled isolates from Liberia during May 2–July 9, 2014 (95% highest posterior density [HPD] interval), which corresponds with the early days of the outbreak in Monrovia (3). However, we cannot rule out ongoing EBOV exchange among EVD-infected countries. In fact, shared ancestry among 3 isolates from Liberia and the 4 available sequences from Mali suggests some level of international movement. We estimated dates associated with 2 nodes along the shared Liberia/Mali EBOV lineage (labeled * and ** in Figure 1, panel A); these estimates ranged from July 6 through September 15, 2014, and from July 26 through September 27, 2014, respectively (95% HPD). Overall, collection dates correlated well with root-to-tip distances within the Western Africa EVD outbreak (Figure 1, panel B). Linear regression analysis (using the lm function in R version 3.1.1; http://www.r-project.org/) estimated an overall rate of change of 9.17 × 10–4 substitutions/site/year (95% HPD). Overall, collection dates correlated well with root-to-tip distances within the Western Africa EVD outbreak (Figure 1, panel B). Linear regression analysis (using the lm function in R version 3.1.1; http://www.r-project.org/) estimated an overall rate of change of 9.17 × 10–4 substitutions/site/year (95% HPD).

We reviewed all publicly available genomic information for EBOV/Mak (122 genome sequences [1,12]) to evaluate the effect of genomic drift on biomedical countermeasures (drugs and diagnostic assays). We assessed the potential impact of intra-outbreak genetic divergence on 13 drugs and 2 diagnostic assays (known to be used in Liberia) with the same approach previously used (6). Two sequence-binding treatment modalities are available for postexposure treatment of EVD: small interfering RNAs (siRNAs) (20) and phosphorodiamidate morpholino oligomers (21) targeting L, VP24, and/or VP35 gene transcripts, and passive immunotherapy based on antibodies or

Table 1. Characteristics of Ebola virus samples from selected patients, Liberia, September 2014–February 2015*

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Patient age, y/sex</th>
<th>County of residence</th>
<th>Test date</th>
<th>Sample type</th>
<th>Average C value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIBR10054</td>
<td>53/M</td>
<td>Bomi</td>
<td>2014 Sep 23</td>
<td>Plasma</td>
<td>20.5</td>
</tr>
<tr>
<td>LIBR10053</td>
<td>42/NA</td>
<td>Not Available</td>
<td>2014 Oct 1</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>LIBR0058</td>
<td>67/M</td>
<td>Rivercess</td>
<td>2014 Nov 5</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>LIBR0059</td>
<td>27/M</td>
<td>Rivercess</td>
<td>2014 Nov 5</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>LIBR0073</td>
<td>27/M</td>
<td>Grand Bassa</td>
<td>2014 Nov 6</td>
<td>Plasma</td>
<td>18.5</td>
</tr>
<tr>
<td>LIBR0067</td>
<td>26/NA</td>
<td>Bomi</td>
<td>2014 Nov 6</td>
<td>Plasma</td>
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</tr>
<tr>
<td>LIBR0063</td>
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<td>Montesserrado</td>
<td>2014 Nov 6</td>
<td>Oral swab</td>
<td>17.5</td>
</tr>
<tr>
<td>LIBR0093</td>
<td>47/M</td>
<td>Montesserrado</td>
<td>2014 Nov 6</td>
<td>Plasma</td>
<td>15.5</td>
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<tr>
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<td>18/F</td>
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<tr>
<td>LIBR0090</td>
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<td>Margibi</td>
<td>2014 Nov 8</td>
<td>Plasma</td>
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<tr>
<td>LIBR0116</td>
<td>4/F</td>
<td>Grand Bassa</td>
<td>2014 Nov 10</td>
<td>Plasma</td>
<td>19</td>
</tr>
<tr>
<td>LIBR0168</td>
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<td>2014 Nov 13</td>
<td>Plasma</td>
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</tr>
<tr>
<td>LIBR0176</td>
<td>42/M</td>
<td>Montesserrado</td>
<td>2014 Nov 14</td>
<td>Oral swab</td>
<td>22.5</td>
</tr>
<tr>
<td>LIBR0173</td>
<td>64/M</td>
<td>Montesserrado</td>
<td>2014 Nov 14</td>
<td>Oral swab</td>
<td>22</td>
</tr>
<tr>
<td>LIBR0286</td>
<td>9/F</td>
<td>Grand Cape Mount</td>
<td>2014 Nov 22</td>
<td>Plasma</td>
<td>22</td>
</tr>
<tr>
<td>LIBR0333</td>
<td>35/F</td>
<td>Grand Cape Mount</td>
<td>2014 Nov 25</td>
<td>Plasma</td>
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<td>2014 Dec 3</td>
<td>Plasma</td>
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<tr>
<td>LIBR0430</td>
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<td>2014 Dec 3</td>
<td>Oral swab</td>
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<td>2014 Dec 10</td>
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<tr>
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<tr>
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<td>Oral swab</td>
<td>23</td>
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<tr>
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<td>Montesserrado</td>
<td>2015 Feb 14</td>
<td>Plasma</td>
<td>22.5</td>
</tr>
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</table>

* C value used as indicator of viral load obtained from 2 diagnostic assays performed on all samples (Kulesh-TM and Kulesh-MGB [9]).
†Average C value used as indicator of viral load obtained from 2 diagnostic assays performed on all samples (Kulesh-TM and Kulesh-MGB [9]).
AMRID has suggested that the changes will be tolerated (online Technical Appendix 1 Figure 2, online Technical Appendix 1 Table). We observed 25 changes, of which 6 were reported previously (12). Each SNP has the potential to affect the efficacy of available therapeutic drugs (original and updated versions) or diagnostic assays (Table 3; Figure 2; online Technical Appendix 1 Figure 2, online Technical Appendix 1 Table). We observed 25 changes, of which 6 were reported previously (12). Each SNP has the potential to affect the efficacy of available therapeutic drugs (original and updated versions) or diagnostic assays (Table 3; Figure 2; online Technical Appendix 1 Figure 2, online Technical Appendix 1 Table; nucleotide positions are reported relative to EBOV/Kik-9510621, for consistency [6]).

Several of the 27 previously identified changes (green in Figure 2) already have been demonstrated to be tolerated while maintaining efficacy (24,30,32–34), thus minimizing their potential effect (6). Six of these 33 SNPs (EBOV-LIB <100%; orange in Figure 2) appeared during the surveillance period of this study (September 23, 2014–February 14, 2015) in samples obtained in Liberia (12). None of these changes have been previously associated with EBOV resistance to any therapeutic drug. Five of the new changes might affect 1 of the components of the ZMapp antibody cocktail (mAb 13C6). However, the conformational target site for this antibody (positions 1–295, soluble glycoprotein) is broader in length and more poorly defined than the other sequence-based countermeasure targets considered in our risk assessment. The sixth mutation might affect the
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binding site of the siRNA viral protein (VP) 35 target (for that particular sample, the mutation appears in an area of low sequencing coverage depth). Thus, when these new changes are combined with the changes observed previously (yellow in Figure 2), we can conclude that retesting several therapeutic drugs against isolates currently circulating might be necessary to determine whether any of these mutations impact their efficacy. In particular, it is important to reevaluate drugs that include mAb 13C6 (part of the ZMapp, ZMAb, and MB-003 antibody cocktails), mAb 13F6 (part of MB-003), mAb 1H3 (part of ZMAb), and the siRNA VP35 targets (Table 3, Figure 2) (6).

Discussion

Our study details the establishment of a genomic sequencing and analysis center within Liberia for real-time monitoring of viral evolution. The initial sequences generated at this facility have provided a first glimpse into EBOV/Mak evolution from the end of 2014 to the beginning of 2015. Although genetically diverse, the viruses circulating in Liberia during this period are consistent with a single introduction event followed by diversification within Liberia. The cluster 2 haplotype from which all the sampled Liberia sequences radiate is thought to have been circulating in Guinea and Sierra Leone during late May 2014 (12). Moreover, it was the second most common sequence detected in Sierra Leone during late May through mid-June (12). Introduction of this haplotype from either of these neighboring countries could have resulted in the sampled diversity; however, we cannot rule out the possibility of multiple introductions. Additional spatial and temporal sampling within Liberia, Guinea, and Sierra Leone will help to differentiate these 2 scenarios.

The 25 Liberia EBOV/Mak genomes included 23 nonsynonymous mutations and 1 nonsense mutation that have not previously been seen in Western Africa (although some of these mutations have been observed in EBOV isolates from previous EVD outbreaks). A nonsense mutation, which is present within 2 of the 25 sequences, is predicted to result in premature truncation (6 aa) of VP30. VP30 is an essential protein for viral transcription; it is needed for the RNA-dependent RNA polymerase (L) to read beyond a cis-RNA element in the nucleoprotein mRNA 5′ untranslated region (35) and is required to reinitiate transcription at gene junctions (36). Moreover, VP30 phosphorylation modulates the composition and function of the RNA synthesis machinery (37). To our knowledge, no functional domains have been described in the truncated region. Further characterization is needed to determine whether this or any of the other detected mutations impacted the relative fitness of the affected EBOV isolates. Within Liberia, geography showed little correlation with phylogeny; most

Figure 1. A) Median-joining haplotype network constructed from a full-genome alignment of 122 clinical Ebola virus Makona (EBOV/Mak) isolates (list of isolates in online Technical Appendix 3, http://wwwnc.cdc.gov/EID/article/21/7/15-0522-Techapp3.xlsx). Each colored vertex represents a sampled viral haplotype, with the numbered vertices representing the centers of the 3 clusters described in (12). All sampled isolates from Liberia originated from cluster 2. The size of each vertex is relative to the number of sampled isolates, and the colors indicate country of origin. Hatch marks indicate the number of mutations along each edge. Because of missing data, 2,764 sites (14.6% of total genome) were excluded from the analysis, including 26 sites with variability among isolates (16.7% of all variable sites). B) Root-to-tip distance correlates well with test date and estimates a rate of evolution equal to 9.17 × 10−4 substitutions/site/year. This analysis comprises 110 clinical EBOV/Mak isolates collected during March 17, 2014–January 20, 2015 (online Technical Appendix 3, isolates with dates).
EBOV lineages within Liberia appear to be geographically widespread within the sampled regions.

Previous analysis of EBOV/Mak genomes from Sierra Leone and Guinea suggests that the evolutionary rate within the current EVD outbreak might be higher than the rate between outbreaks (12). After incorporation of sequences from Liberia, which were collected later in the outbreak, our estimates of substitution rate fell between the previous estimates for EBOV/Mak only and for all EBOV (12,38). As more sequence data become available, it will be interesting to see whether a significant change in the evolutionary rate can be detected within the current EVD outbreak.

Our ability to quantify international EBOV exchange is limited because few isolates from other countries were available during the sampled timeframe. However, shared ancestry between isolates from Mali and 3 isolates from Liberia suggests at least 1 transmission event across national borders (3). All EVD cases in Mali have been attributable to movement of infected persons into Mali from Guinea (39). With the current dataset, it is impossible to say whether the shared Liberia/Mali lineage originated in Liberia and was then transported to Mali through Guinea or whether the lineage emerged in Guinea and later moved independently to Liberia and Mali. Active EBOV outbreaks were occurring in both Liberia and Guinea during the period estimated for the emergence of this shared lineage (July–September 2014).

The genomic changes observed for EBOV/Mak during its circulation in Liberia append 5 additional mutations to the list of changes that might affect the binding of the 13C6 mAb, a component of ZMapp. All of these changes, however, were present at relatively low frequency (<12%) in our current sample, and none of the sampled lineages have accumulated >1 change per therapeutic drug type. We observed no significant changes (i.e., likely to affect efficacy) in the binding sites for the therapeutic drug type. We observed no significant changes (i.e., likely to affect efficacy) in the binding sites for the therapeutic drug type. We observed no significant changes (i.e., likely to affect efficacy) in the binding sites for the therapeutic drug type. We observed no significant changes (i.e., likely to affect efficacy) in the binding sites for the therapeutic drug type. We observed no significant changes (i.e., likely to affect efficacy) in the binding sites for the therapeutic drug type. We observed no significant changes (i.e., likely to affect efficacy) in the binding sites for the therapeutic drug type.

Table 3. Mutation analysis of candidate therapeutic drug and diagnostic binding sites for EBOV*

<table>
<thead>
<tr>
<th>Reference position</th>
<th>Reference base</th>
<th>Called base</th>
<th>EBOV-WA, %</th>
<th>EBOV-LIB, %</th>
<th>Codon</th>
<th>Feature name</th>
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<td>100</td>
<td>100</td>
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<tr>
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<td>SNP</td>
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<td>G</td>
<td>100</td>
<td>100</td>
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<tr>
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<td>SNP</td>
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<td>SNP</td>
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<td>100</td>
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<td>P:CCA @ 683 → P:CCg</td>
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*EBOV, Ebola virus; GP, glycoprotein; L, RNA-dependent RNA polymerase; LIB, Liberia; NP, nucleoprotein; SNP, single-nucleotide polymorphism; WA, Western Africa.
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need for global sequencing capabilities to be part of the first response during future virus outbreaks.

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Dr. Kugelman is a biodefense research scientist, computational biologist, and head of Bioinformatics at the Center for Genome Sciences at USAMRIID. His research interests include the genomic study of filovirus and orthopoxvirus infections.

Figure 2. Mutation analysis of candidate therapeutic drug and diagnostic binding sites used in outbreak of Ebola virus (EBOV) disease, Western Africa. A single-nucleotide polymorphism (SNP) table is combined with a heat map based on 2 categories: 1) mutations tolerated by the therapeutic drug or diagnostic target (highlighted in green); 2) mutations within the binding region of a therapeutic drug or diagnostic assay that have not yet been tested (highlighted in yellow/orange) (20–24,27,30,31). Changes previously described are highlighted in yellow; changes that appeared during circulation in Liberia are highlighted in orange. The reference nucleotide positions reported here are in relation to EBOV/Kik-9510621 (GenBank accession no. AY354458), which is one of the primary isolates used as reference for developing these therapeutic drugs and diagnostic assays. A summary of the changes to the probes is available in online Technical Appendix 1 Table (http://wwwnc.cdc.gov/EID/article/21/7/15-0522-Techapp1.pdf). PMO, phosphorodiaminate morpholino oligomer; mAB, monoclonal antibody; siRNA, small interfering RNA; Ref pos, reference positive; VP, viral protein.
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Parechovirus Genotype 3 Outbreak among Infants, New South Wales, Australia, 2013–2014

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From October 2013 through February 2014, human parechovirus genotype 3 infection was identified in 183 infants in New South Wales, Australia. Of those infants, 57% were male and 95% required hospitalization. Common signs and symptoms were fever >38°C (86%), irritability (80%), tachycardia (68%), and rash (62%). Compared with affected infants in the Northern Hemisphere, infants in New South Wales were slightly older, both sexes were affected more equally, and rash occurred with considerably higher frequency. The New South Wales syndromic surveillance system, which uses near real-time emergency department and ambulance data, was useful for monitoring the outbreak. An alert distributed to clinicians reduced unnecessary hospitalization for patients with suspected sepsis.

The clinical manifestations of infection with human parechoviruses (HPeVs), members of the family Picornaviridae, are often indistinguishable from those caused by human enterovirus infections. Over the past decade, outbreaks of human parechovirus genotype 3 (HPeV3) have been reported from the Northern Hemisphere and are particularly well documented in Japan (where the virus was discovered), Canada, the United Kingdom, Denmark, and the Netherlands (1–4). Of the 16 HPeV genotypes, HPeV3 is the most aggressive and causes a sepsis-like syndrome in neonates (5). HPeV infection seems to follow a seasonal pattern; incidence is higher in summer and autumn (2,3). It can be spread by the fecal–oral and respiratory routes (4).

On November 22, 2013, Health Protection New South Wales (NSW), Australia, was notified of a possible cluster of HPeV cases at The Children’s Hospital at Westmead in Sydney. At that time, 7 neonates had experienced rapid onset of acute sepsis-like illness with fever >38°C and a combination of irritability/pain, diarrhea, confluent erythematous rash, tachycardia, tachypnea, encephalitis, myoclonic jerks, and hepatitis. Inquiries revealed that neonates described as “red, hot, angry” had also been admitted to other tertiary children’s hospitals in NSW (6). An expert advisory group comprising staff from the NSW Ministry of Health, Health Protection NSW, public health units, and the Sydney Children’s Hospital Network was convened to coordinate the investigation.

On November 25, 2013, PCR detection of HPeV RNA confirmed HPeV infection in 2 of the children. The NSW public health network and clinicians agreed that a surveillance program should be initiated to gather information on the epidemiologic and clinical characteristics and outcomes of children with HPeV infection.

In addition to the public health response, Health Protection NSW issued a media release to alert members of the public to the outbreak. On November 29, 2013, HPeV3 information including a case definition, instructions for accessing diagnostic testing, and recommended clinical management was distributed to all emergency departments, pediatricians, and early childhood health services in NSW. During the outbreak, the expert advisory group met regularly via teleconference to discuss and address any emerging issues. HPeV3 active surveillance activities were concluded on January 31, 2014, while other forms of surveillance continued into February 2014. We describe the epidemiology of the outbreak as observed through several surveillance mechanisms.

Methods
HPeV infection is not a notifiable disease under the Public Health Act 2010 (NSW). This HPeV3 outbreak was detected and reported by clinicians alert to unusual clusters and patterns of disease. Other forms of surveillance were developed as a result of this alert. Surveillance consisted of 3 components: 1) active surveillance (case finding at the sentinel sites); 2) passive surveillance (laboratory reporting of all positive HPeV specimens from sentinel sites and elsewhere in NSW to Health Protection NSW); and 3) syndromic surveillance (reporting of infants seen in emergency departments by the NSW syndromic surveillance system that uses near real-time emergency department and ambulance data [7]) (Figure 1). The sentinel sites were 3 tertiary children’s hospitals in NSW: The Children’s Hospital at Westmead, The Sydney Children’s Hospital Randwick, and John Hunter Children’s Hospital Newcastle. Passive and syndromic surveillance continued into February 2014. In addition to surveillance, public health communication in the form of an HPeV information sheet for clinicians was distributed on November 29, 2013, alerting emergency department staff, pediatricians, and early childhood health service staff of current HPeV activity in NSW, providing a description of HPeV infection, and recommending management options (i.e., early laboratory testing and provision of supportive care after receipt of confirmation of HPeV infection).

Active Surveillance
Active surveillance activities commenced at the 3 hospitals (sentinel sites) on November 25, 2013, and continued through January 19, 2014; however, some retrospective case finding was included for cases with onset dating back to October 1, 2013, when the outbreak was thought to have started. A patient with a suspected case of HPeV was defined as a neonate or young infant <3 months of age with sepsis-like illness and fever >38.0°C and ≥2 of the following: irritability/pain, rash, diarrhea, tachycardia, tachypnea, encephalitis, myoclonic jerks, or hepatitis. A patient with a confirmed case of HPeV was a suspected case-patient for whom PCR was positive for HPeV. Clinicians at the sentinel sites collected case information by using an HPeV case investigation form and PCR testing of patient stool, cerebrospinal fluid (CSF), nasopharyngeal aspirate, throat
swab, rectal swab, or whole blood samples for HPeV; stool and CSF samples were preferred. (3,8,9). These data were entered into the NSW Notifiable Conditions Information Management System. Weekly reports informed the NSW public health network of outbreak progression.

**Passive Surveillance**

All positive HPeV test results from NSW residents referred to the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Melbourne, Victoria (the only laboratory in the region testing for HPeV), from October 1, 2013, through February 2, 2014, were reported to NSW Health. Specimen date; estimated illness onset date; sample type; and patient date of birth, sex, and postcode were recorded in the NSW Notifiable Conditions Information Management System.

**Syndromic Surveillance**

The NSW emergency department syndromic surveillance system monitored the number of infants <1 year of age for whom a provisional diagnosis of fever/unspecifed infection was made in the emergency department and the number of patients who required hospital admission, including admission to critical care wards. A diagnosis of fever/unspecifed infection can include fever symptoms, unspecified viral infection, unspecified viremia, unspecified bacteremia, unspecified bacterial infection, or unspecified infection. Weekly reports compared recent data with historical data from the previous 5 years.

**Laboratory Methods**

From all clinical samples, nucleic acid was extracted by using QIAGEN DX reagents (QIAGEN, Hilden, Germany) on a QIAxtractor NA extraction robot (QIAGEN). cDNA was synthesized by using a method previously described (10) and was tested in an HPeV real-time PCR selective for the 5′ untranslated region, which was developed at VIDRL (10). (The primer and probe sequence details for this assay can be supplied upon request to G.C.)

Molecular analysis to obtain the HPeV genotype was performed on selected samples that had been positive by real-time PCR. Specimens from 41 patients were selected for genotyping on the basis of ensuring representation of infants’ geographic locations, ages, sex, illness onset dates, specimen types, and sex. Identification of specific HPeV genotypes was obtained through amplification of the viral protein 1 gene by use of a gel-based seminested PCR (11). The generated PCR products were sequenced and compared with reference sequences by using the primers and methods described elsewhere (12).

**Statistical Analyses**

Descriptive analysis of epidemiologic variables and patient demographic characteristics were performed. Characteristics of infants <3 months of age, such as length of stay (LOS), were compared by using t-tests to determine the effects of public health messaging. Analyses was performed by using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA).

**Results**

**Laboratory Findings**

From November 1, 2013, through February 28, 2014, a total of 420 specimens were submitted for HPeV PCR testing; for some patients, >1 specimen was submitted. PCR results were HPeV positive for 289 (69%) specimens from 198 patients (Table 1). In addition to confirming HPeV RNA
in samples from 198 patients in NSW, HPeV type 3 was identified from all 41 (21%) positive samples for which molecular analysis was subsequently performed. The phylogenetic tree demonstrating all HPeV3 isolates genotyped at VIDRL during the outbreak is reported elsewhere (6).

Because of the algorithms used in the testing, enterovirus results were also available for all samples submitted (Table 1). A total of 194 patients had HPeV infection only, 4 had dual infections (HPeV and enterovirus), and 14 had enterovirus infection only (Figure 2). Results for the rest of the patients were negative. Focusing on CSF and fecal samples, 123 (73%) of 168 CSF samples were HPeV positive by PCR (mean cycle threshold [Ct] detection value 31.6), and 114 (73%) of 156 fecal samples were positive (mean Ct 27.2) (Table 2). PCR was run for 45 cycles; therefore, Ct values >45 were considered negative.

### Table 1. Results of PCR testing of specimens from patients from New South Wales received at the VIDRL, Melbourne, Victoria, Australia, November 1, 2013–February 28, 2014 *

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimens, no. (%)</th>
<th>Patients, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no.</td>
<td>420 (100)</td>
<td>308 (100)</td>
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<tr>
<td>PCR+ for HPeV</td>
<td>289 (69)</td>
<td>198 (64)</td>
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<tr>
<td>PCR+ for HPeV only</td>
<td>285 (68)</td>
<td>194 (63)</td>
</tr>
<tr>
<td>PCR+ for HPeV and EV†</td>
<td>4 (1)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>PCR+ for EV only</td>
<td>15 (4)</td>
<td>14 (5)</td>
</tr>
<tr>
<td>PCR+ for EV</td>
<td>19 (5)</td>
<td>18 (6)</td>
</tr>
<tr>
<td>PCR – for HPeV</td>
<td>131 (31)</td>
<td>110 (36)</td>
</tr>
<tr>
<td>PCR – for HPeV and EV</td>
<td>116 (28)</td>
<td>96 (31)</td>
</tr>
</tbody>
</table>

*EV, enterovirus; HPeV, human parechovirus; VIDRL, Victorian Infectious Disease Reference Laboratory: +, positive; –, negative.
†Because of how the testing algorithm is set up at VIDRL, all samples tested for HPeV are also tested for EV. EV results are shown to demonstrate the small amount of EV detected in this HPeV outbreak period, with even fewer co-infections.

**Active and Passive Surveillance Findings**

Active surveillance identified 94 infants whose illness met the definition of a confirmed case (patient <3 months of age and HPeV-positive laboratory results, originating from sentinel sites). Passive surveillance spanning specimen collection dates from October 1, 2013, through February 2, 2014, identified another 89 laboratory-confirmed cases in NSW in patients 0–17 months of age. The outbreak peaked during the first 2 weeks of December 2013 (Figure 3).

More cases (105 [57%]) were in male than female patients; median patient age was 1.51 months (or median 46 days, range 0–537 days) (Figure 4; Table 3). Intrafamily HPeV3 transmission was identified in twins, 2 parent–child pairs, and a set of cousins. A descriptive case series of the infants infected with HPeV3 during this outbreak, containing further clinical details on select cases, is reported elsewhere (6).

Analysis of case investigation forms from the sentinel sites reporting HPeV signs and symptoms showed that the most commonly reported signs for infants <3 months of age were fever (86%), irritability/pain (80%), tachycardia (68%), and rash (62%) (Table 3). Similar signs were displayed by those >3 months of age; however, 20% fewer in this age group had fever and tachycardia (Table 3). As described previously, all infants at the sentinel sites were well at the time of hospital discharge, and further longitudinal follow-up studies are examining the long-term outcomes of these infections having occurred in early life (6).

Of the 183 confirmed cases, 108 (59%) were captured by the 3 sentinel surveillance sites, and another 75 (41%) were diagnosed at other hospitals. Most (57%) patients resided in the Sydney metropolitan area, and the remaining 43% were from regional or rural areas of NSW. This finding compares with 64% and 36% of the NSW population residing in metropolitan Sydney and regional/rural areas, respectively (13).

Analysis of case investigation forms for the 108 patients at the sentinel sites also showed that 103 (95%) patients were admitted to hospital and had an average LOS of 4.4 (1–13) days (Table 3). Mean LOS was greater for infants <3 months of age (4.5, range 1–13 days) than for...
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Table 2. HPeV testing of specimens from patients from New South Wales, received at the VIDRL, Melbourne, Victoria, Australia, November 1, 2013–February 28, 2014, by specimen type

<table>
<thead>
<tr>
<th>Sample and result</th>
<th>Specimens, no. (%)</th>
<th>Patients, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>420 (100)</td>
<td>308 (100)</td>
</tr>
<tr>
<td><strong>CSF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total samples tested</td>
<td>168 (40)</td>
<td>161 (52)</td>
</tr>
<tr>
<td>HPeV+ (mean C, 31.6)†</td>
<td>123 (73)</td>
<td>116 (72)</td>
</tr>
<tr>
<td>PCR+ for HPeV and EV</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>Stool</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total samples tested</td>
<td>156 (37)</td>
<td>147 (48)</td>
</tr>
<tr>
<td>HPeV+ (mean C, 27.2)</td>
<td>114 (73)</td>
<td>106 (72)</td>
</tr>
<tr>
<td>PCR+ for HPeV and EV</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total samples tested</td>
<td>96 (23)</td>
<td>8 (28)</td>
</tr>
<tr>
<td>HPeV+</td>
<td>52 (54)</td>
<td>45 (52)</td>
</tr>
<tr>
<td>PCR+ for HPeV and EV</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>Sequenced: HPeV3</strong></td>
<td>41 (14)</td>
<td>41 (21)</td>
</tr>
</tbody>
</table>

*CSF, cerebrospinal fluid; C, cycle threshold; EV, enterovirus; HPeV, human parechovirus; VIDRL, Victorian Infectious Disease Reference Laboratory; +, positive.
†At VIDRL, PCRs are run for 45 cycles, so any C value greater than 45 is considered negative.

those ≥3 months of age (3.7, range 1–11 days). The rate of admission to an intensive care unit was lower for older infants (14%) than for those <3 months of age (30%) (Table 3).

The trend statistic on the distribution of LOS in infants <3 months of age showed that LOS was significantly reduced among infants <3 months of age who became ill after the HPeV alert was sent on November 29, 2013, to emergency departments and pediatricians. Mean LOS at the sentinel sites was 5.7 days before and 4.0 days after sending of the alert (Satterthwaite t-test, p<0.05) (Figure 5).

Syndromic Surveillance Findings

The number of infants <1 year of age with a provisional emergency department diagnosis of fever/unspecified requiring hospital admission began to rise sharply in mid-November 2013 and peaked during the first week of December (Figure 6, panel A). At the peak, the number of admissions was 83, compared with an average of 52 for the same week in previous years. Admissions remained elevated until mid-January 2014, when they returned to background levels. Admissions to critical care wards spiked during the second week of December, when 9 patients were admitted, well above the average of 1 admission per week for the same week in previous years (Figure 6, panel B). During the surveillance period, most admissions were to the 2 children’s hospitals in metropolitan Sydney.

Discussion

This outbreak is probably one of the first large parechovirus outbreaks to be reported in Australia. We observed a large number of cases over a relatively short period (≈4 months), peaking around late spring/early summer, which is earlier than documented seasonality for parechovirus in the Northern Hemisphere (3). Although sequencing in this series was incomplete (21% of patients), this parechovirus outbreak was determined to have been caused by HPeV3 for the following reasons: all sequenced HPeV-positive samples were genotype 3; the epidemiology and spectrum of illness seen by clinicians at the sentinel sites was relatively

![Figure 3](image-url). Number of laboratory-confirmed human parechovirus (HPeV) cases identified by active and passive surveillance, by week of symptom onset, in New South Wales (NSW), Australia, during the October 2013–early February 2014 outbreak (total = 183 cases). Source: NSW Notifiable Conditions Information Management System data (http://www.health.nsw.au/epidemiology/Pages/Notifiable-diseases.aspx), February 18, 2014.

![Figure 5](image-url). Number of laboratory-confirmed HPeV cases identified by active and passive surveillance, by week of symptom onset, in New South Wales (NSW), Australia, during the October 2013–early February 2014 outbreak (total = 183 cases). Source: NSW Notifiable Conditions Information Management System data (http://www.health.nsw.au/epidemiology/Pages/Notifiable-diseases.aspx), February 18, 2014.
homogenous and in keeping with other reports of HPeV3 infections in infants (3,14); and PCR was enterovirus positive for only 18 patients but HPeV positive for 198 patients. Infected infants in this outbreak were older than those reported elsewhere. The median age of 46 days was higher than that reported in Denmark (39 days) and the Netherlands (40 days) (3,14). The occurrence of a substantial proportion (17%) of HPeV3 infections in infants ≥3 months of age was consistent with results from a US study reporting 18% of cases in infants >60 days of age but contrary to other data indicating that HPeV3 infection occurs almost exclusively in infants <3 months of age (2,4).

The age cutoff in the case definition for active surveil-
lance at the sentinel sites may have introduced a bias to-
ward HPeV infection being more frequently suspected in
infants <3 months of age; thus, these infants might have
undergone more HPeV testing than older infants, thereby
underestimating the true mean age of infants affected in
this outbreak. Had the age cutoff in the case definition been
set at 12 months (all infants), bias toward younger infants

Table 3. Characteristics of patients with laboratory-confirmed HPeV detected by active surveillance, New South Wales, Australia, October 1, 2013–February 2, 2014*  

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Passive surveillance for all laboratory-confirmed HPeV case-patients† no. (%)</th>
<th>Active surveillance, enhanced data for all HPeV case-patients from sentinel sites,‡ no. (%), n = 108</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>183 (100)</td>
<td>94 (87)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>14 (13)</td>
</tr>
<tr>
<td>F</td>
<td>78 (43)</td>
<td>45 (48)</td>
</tr>
<tr>
<td>M</td>
<td>105 (57)</td>
<td>6 (57)</td>
</tr>
<tr>
<td>Admitted to ward</td>
<td></td>
<td>103 (95)</td>
</tr>
<tr>
<td>Length of stay, days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>Maximum</td>
<td>NA</td>
<td>11</td>
</tr>
<tr>
<td>Mean</td>
<td>NA</td>
<td>13</td>
</tr>
<tr>
<td>Admitted to ICU</td>
<td></td>
<td>30 (28)</td>
</tr>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>NA</td>
<td>90 (83)</td>
</tr>
<tr>
<td>Irritability/pain</td>
<td></td>
<td>86 (80)</td>
</tr>
<tr>
<td>Diarrhea/loose stool</td>
<td></td>
<td>31 (29)</td>
</tr>
<tr>
<td>Tachypnea</td>
<td>NA</td>
<td>29 (27)</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>NA</td>
<td>71 (66)</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>NA</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Rash</td>
<td>NA</td>
<td>67 (62)</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>NA</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Myoclonic jerks</td>
<td></td>
<td>5 (5)</td>
</tr>
</tbody>
</table>

*Table source: NSW Notifiable Conditions Information Management System data (http://www.health.nsw.au/epidemiology/Pages/Notifiable-diseases.aspx), February 18, 2014. HPeV, human parechovirus; ICU, intensive care unit; NA, not available; NSW, New South Wales; VIDRL, Victorian Infectious Disease Reference Laboratory, Melbourne, Victoria, Australia.
†Passive laboratory surveillance ceased at NSW Health on February 2, 2014, leaving a total of 183 laboratory-confirmed HPeV cases. This number differs from the total of 198 confirmed HPeV cases reported by VIDRL surveillance, which continued through to February 28, 2014, and identified another 15 cases.
‡Sentinel surveillance sites collected additional demographic, hospital, and clinical data onto the HPeV case investigation forms, which were entered into the NSW Notifiable Conditions Information Management System. No information on hospital stay and clinical features was available for cases identified by laboratory-only surveillance. Surveillance at sentinel sites is a subset (59%) of the outbreak total of 183 laboratory-confirmed cases.
would have been avoided, and the median age might have been older than that reported here.

After the November 29, 2013, release of the HPeV alert to emergency department staff and pediatricians, mean LOS among infants <3 months of age decreased by 30%, from ≈5.7 days at the start of the outbreak to 4.0 days (p = 0.0250). This statistically significant finding reflects a degree of effectiveness of public health messaging in raising clinician awareness of HPeV. During the latter part of the outbreak, after Health Protection NSW issued the alert, informed clinicians may have felt more comfortable discontinuing treatment and discharging infants sooner if their illness met the criteria of the HPeV case definition. The alert seemed to result in improved clinical management of cases, avoidance of unnecessary prolonged exposure to empirically prescribed antimicrobial drugs, provision of appropriate supportive treatment, and earlier discharge from hospital. The mean LOS during the NSW outbreak (4.4 days) was lower than that reported in the Netherlands (7 days), according to an analysis of retrospective diagnoses when no public health intervention would have taken place (8). Another factor that could contribute to reduced LOS in the latter part of an outbreak is the increasing age of young infants becoming ill with HPeV infection in the second half of the outbreak, although this factor was not statistically significant in this dataset (15).

The male:female split in this outbreak was less pronounced than that reported for other studies, although more cases consistently occurred in boys. Others have reported a higher preponderance of infection in boys,


![Figure 6. Total weekly counts of visits to the emergency department for fever or unspecified infection for which patients were (A) admitted to ward and (B) admitted to critical care for 2013, compared with each of the 5 previous years, children <1 year of age, for 59 hospitals in New South Wales, Australia. Source: Emergency department syndromic surveillance report produced on June 3, 2014.]

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ranging from 70% to 90%, compared with our finding of 57% (2,5,8,14).

Clinical signs reported in the literature for HPeV3-infected infants were consistent with our findings of fever, irritability, and encephalitis (2,6,8). However, rash occurred with much higher frequency (62% vs. 17%) in the NSW outbreak than in other outbreaks (8).

Syndromic surveillance that used emergency department data proved to be a useful and timely way to monitor emergency department hospital admissions temporally associated with the HPeV3 outbreak. Increased presentation of infants <1 year of age with a provisional emergency department diagnosis of fever/unspecified infection requiring hospital admission, in particular admission to critical care, were associated with increased detection of HPeV3 at the clinical level. Emergency department syndromic surveillance reports also helped confirm an overall decline in admissions from emergency departments in early 2014, supporting the eventual withdrawal of active surveillance. Emergency department syndromic surveillance in NSW does not routinely monitor age-specific or admission-specific (hospital ward or critical care unit) aberrations; that is, all children <5 years of age are monitored as a group. In the future, data generated through the emergency department syndromic surveillance system may continue to be useful for monitoring the evolution of an HPeV or similar outbreak. The cost of maintaining emergency department syndromic surveillance like that used during this outbreak (fever/unspecified infection among children <1 year of age and admission to hospital) would need to be considered. Costs of doing so include personnel time for checking reports and investigating signals, infrastructure costs, and opportunity costs; choosing to monitor HPeV3-related signals indirectly means that signals for diseases that are not prioritized are not monitored. In addition, the sensitivity of this grouping will need to be tested in future outbreaks before it can be considered a reliable proxy indicator of a seasonal outbreak.

We initiated sentinel surveillance on the assumption that nearly all neonates with severe HPeV3 disease would be referred to 1 of the 3 tertiary children's hospitals in NSW. Through passive surveillance we identified an additional 41% of HPeV patients who had been seen at other health facilities. Enhanced data were not collected for these presumably milder cases, which has limited our capacity to conduct more extensive significance testing across the observed differences in clinical features. Recording more information on potential exposures (e.g., infants’ daycare attendance, existence of older siblings, and occurrence of family illness in weeks preceding infants’ admission) would have further aided our understanding of HPeV3 transmission in the community.

Conclusion
The objectives of HPeV surveillance were achieved: document the outbreak, describe the clinical features of cases, help inform clinicians and the public, monitor the evolution of the outbreak, and add to the knowledge base. The HPeV3 infection outbreak in NSW, Australia, differed slightly from that documented in the Northern Hemisphere; the NSW outbreak apparently affected slightly older infants (as well as neonates and young infants), cases were more evenly split between boys and girls, and rash occurred at a considerably higher frequency. The value of awareness-raising communication strategies was demonstrated by the statistically significant 30% reduction in LOS during the outbreak immediately after release of the alert to emergency department staff and pediatricians. This alert helped to minimize unneeded use of antimicrobial drugs and reduce unnecessary hospitalization. Although active surveillance is resource intensive, it has helped to define HPeV3 infection in NSW and link it with a syndromic surveillance indicator in the emergency department syndromic surveillance system. Syndromic surveillance is a potentially useful proxy indicator that should be considered for future detection and surveillance of seasonal outbreaks of viral infections.

Acknowledgments
We express our gratitude to clinicians at the sentinel sites and laboratory staff at South Eastern Areas Laboratory Services, Children’s Hospital Westmead Pathology, and Hunter Area Pathology Service. Particular thanks go to Stephanie Francis and Emma Goeman, Murray Webber, and Karin Timmers for their assistance with data collection and reporting to Public Health. We also thank the staff at the public health units for managing case information and laboratory results in the NSW Notifiable Conditions Information Management System, particularly Margaret Osbourn, Julie K. Kohlhaagen, and Gareth Hockey. We thank the Ministry of Health staff, particularly Tracie Reitzen, for statistical analysis contributions, and David Muscatello and Tina Navin-Cristina for continued weekly syndromic surveillance of HPeV infection in infants in NSW. We are very grateful for the knowledge, expertise, and advice received from our Expert Panel members, particularly Sheena Adamson, Stephen Corbett, David Durrheim, Alison Kessom, Tony Merritt, and Murray Webber. We appreciate the vital support received from VIDRL, particularly Julian Druce, for an excellent speedy turnaround of HPeV testing and genotyping, which was not available in NSW at the time.

This work was completed while G.C. was employed as a trainee in the NSW Public Health Officer Training Program funded by the NSW Ministry of Health.

Ms. Cumming is a public health specialist working with the NSW health system. Her research areas of interest include population health surveillance and evaluations and public health response preparedness.
References


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To assess the temporal dynamics of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in dromedary camels, specimens were collected at 1–2 month intervals from 2 independent groups of animals during April 2013–May 2014 in Al-Ahsa Province, Saudi Arabia, and tested for MERS-CoV RNA by reverse transcription PCR. Of 96 live camels, 28 (29.2%) nasal swab samples were positive; of 91 camel carcasses, 56 (61.5%) lung tissue samples were positive. Positive samples were more commonly found among young animals (<4 years of age) than adults (>4 years of age). The proportions of positive samples varied by month for both groups; detection peaked during November 2013 and January 2014 and declined in March and May 2014. These findings further our understanding of MERS-CoV infection in dromedary camels and may help inform intervention strategies to reduce zoonotic infections.

Materials and Methods

Sample Collection
This study was approved by the Institutional Review Board of the Camel Research Center, King Faisal University, Al-Ahsa, Saudi Arabia. Respiratory specimens were collected from 2 independent groups of mixed-age dromedary camels (Camelus dromedarius). The first collection was obtained during April 2013–May 2014 at the Al Omran Abattoir, Al Omran City, in Al-Ahsa Province in the eastern region of Saudi Arabia. Livestock slaughtered at this abattoir include cattle, goats, sheep, and camels originating from Al-Ahsa and neighboring provinces. Animals selected for slaughter were mainly from the livestock market and from herds located around Al-Ahsa Province. At the livestock market in Al-Ahsa, dromedary camels are housed in small groups (10–15 animals), where they may stay for no more than 4 days. They are then transported in vehicles to the abattoir, where they are kept for no more than 24 hours before slaughter.

Samples were taken from slaughtered dromedary camels on 8 occasions (every 1–2 months). On each particular collection date, tissue specimens were collected from the lungs of all slaughtered dromedary camels. A total of 91 animal carcasses were sampled; 28 had been young animals (<4 years of age) and 63 had been adults (≥4 years of age). Lung lobes that showed pulmonary lesions were sampled; if both lobes showed lesions or if no lesions were visible, milk collected from camels in Qatar (9,13), Oman (14), Saudi Arabia (5,15,16), and Egypt (17).

The few published studies that looked for MERS-CoV in the respiratory tract of naturally infected dromedary camels examined nasal or ocular swab samples but not samples from the lower respiratory tract. Moreover, several studies relied on only a few specimens or collected specimens at only 1 time point (9,13–15). To address these limitations and to clarify the dynamics of MERS-CoV infection in these animals, we conducted a year-round study in which we collected a large number of specimens from the upper respiratory tracts of live dromedary camels and from the lungs of dromedary camel carcasses.
the left lobe was sampled because of its close proximity to the person collecting the sample. The tissue samples (≤1–2 g) were collected aseptically from inside the lung lobes by using sterile surgical instruments (scalpsels, forceps, and scissors). To avoid cross-contamination, lungs were moved to a clean room adjacent to the slaughtering hall and examined on a freshly disinfected table by a person wearing a newly donned gown, face mask, and sterile gloves and using a new set of sterile surgical instruments. Collected tissue samples were immediately deposited in labeled sterile plastic bags and placed in a cooler containing ice packs for transport to the laboratory.

A second sample was collected from age-matched animals over the same period and consisted of 96 nasal swab specimens (36 young animals and 60 adults), 94 from visually healthy dromedary camels and 2 from camels with nasal and lachrymal discharge. Nasal swabs were collected from animals at 3 locations in Al Ahsa Province (Al Omran abattoir, Al Ahsa livestock market, and the veterinary hospital of King Faisal University). For this procedure, a long sterile flexible swab was inserted into 1 nostril until slight resistance was felt; the swab was then rotated, held in place for 5 seconds, withdrawn, and placed in 1 mL of cold viral transport medium containing antibiotics (this medium was chosen to enable future attempts to isolate the virus).

Both swab and lung specimens were transported on ice to the laboratory within 1–2 hours of collection and stored at −80°C until testing. Collection dates and numbers of samples are listed in Table 1.

Sample Processing and RNA Extraction
Swab specimens in transport media were mixed and then clarified by centrifugation at 350 × g for 10 minutes; the supernatants were recovered for extraction. Lung samples were thawed and homogenized by using a TissueRuptor homogenizer (QIAGEN, Hilden, Germany), and 20% suspensions were prepared in 5 mL of transport medium. The resulting homogenates were subjected to centrifugation as above, and the supernatants were recovered for extraction. Total RNA was extracted from 140 μL of each nasal swab or lung sample by using the QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer’s instructions.

Reverse Transcription PCR
Extracted RNA was tested by using a gel-based pan-coronavirus reverse transcription PCR (RT-PCR) assay according to the protocol of Vijgen et al. (18). Real-time RT-PCR (rRT-PCR) was performed by using an assay kit provided by the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA). This assay panel targets the MERS-CoV nucleocapsid protein gene (19) and a region upstream of the envelop protein gene described by Corman et al. (20). All samples were screened by using gel-based RT-PCR and 2 rRT-PCR assays and were considered positive for MERS-CoV if a positive result was obtained with at least 2 of the 3 tests following World Health Organization recommendations (http://www.who.int/csr/disease/coronavirus_infections/WHO_interim_recommendations_lab_detection_MERS-CoV_092014.pdf). All RT-PCRs included no-template negative controls and quantified MERS-CoV transcript as positive control. cDNA was prepared from 20 positive samples and shipped to CDC for independent confirmation and sequencing.

Nucleotide Sequencing and Phylogenetic Analyses
To assess the genetic variability of MERS-CoV, we sequenced the spike protein gene coding region (4,062 nt) on the 20 positive samples. Sequencing was performed on an Applied Biosystems 3130xl Genetic Analyzer (Thermo Fisher Scientific, Grand Island, NY, USA) by using Sequencher version 4.8 software (Gene Codes, Ann Arbor, MI, USA) for sequence assembly and editing. Sequence alignments were performed by using ClustalX version 1.83 implemented in BioEdit version 7.2.5 (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html). Phylogenetic analyses were performed by using MEGA version 6.06 (http://www.megasoftware.net). The neighbor-joining method (tree algorithm inferred with the Kimura 2-parameter substitution model of sequence evolution) was used to construct
phylogenetic trees, and bootstrap resampling analyses were performed (1,000 replicates) to test tree reliability.

Results
During the study, a total of 91 lung tissue samples and 96 nasal swabs were obtained from the 2 groups of camels (Table 1). Overall, 84 (44.9%) of 187 animals were MERS-CoV positive by RT-PCR. The proportion of MERS-CoV–positive animals sampled varied by month and year. For months when specimens were available from both groups, the proportion of positive samples from both groups was highest during the cool months (November 2013–January 2014), then steadily declined, reaching the lowest point during the warm month of May 2014.

MERS-CoV RNA was detected by RT-PCR in a high proportion (56 [61.5%] of 91) of lung tissue samples from animal carcasses. In contrast, MERS-CoV RNA was detected in 28 (29.2%) nasal swab samples collected from the 96 live animals (Table 1).

All animals from both groups appeared healthy on visual inspection except for 2. These 8-month-old dromedary camel calves, located outside of the Al Omran abattoir, exhibited purulent nasal and lacrimal discharge; MERS-CoV RNA was detected in nasal swab specimens from these 2 calves (Figure 1). MERS-CoV RNA was more often detected in the lung and nasal cavity of young camels than adult camels (Table 2).

cDNA prepared from 20 samples positive for MERS-CoV by RT-PCR were shipped to CDC for independent confirmation. All 20 samples were confirmed MERS-CoV positive by multiple rRT-PCRs selective for independent regions of the MERS-CoV genome. However, attempts to amplify larger regions of the genome for sequencing were less successful. Despite repeated attempts, only 4 samples had cDNA of sufficient quality for successful sequencing. Sequences of the full MERS-CoV spike gene coding region were obtained from nasal swabs collected from 3 live animals in December 2013 (camels C8, C9) and May 2014 (camel C23) and from a lung sample collected from 1 animal carcass (camel C7) in November 2013 (GenBank accession nos. KP405225 [camel C8], KP405226 [camel C7], KP405227 [camel C9], KP966104 [camel C23]). The spike sequences differed from each other and clustered with published MERS-CoV sequences from humans and dromedary camels with no clear correlation in time or location. Sequences from the sample from camel C7 most closely matched sequences obtained from a human in Hafar Al-Batin in 2013; sequences from camel C9 most closely matched sequences obtained from a human in Riyadh in 2014; and the sequence from camel C23 was identical to a sequence obtained from a dromedary camel in an unidentified region of Saudi Arabia in 2014 (Figure 2). No coding differences from consensus were identified in the spike protein receptor binding domain region (residues 484–567) that directly interacts with the dipeptidyl peptidase-4 receptor (21).

Discussion
Our results confirm previous reports documenting wide circulation of MERS-CoV in dromedary camel populations in the Middle East. In other studies, RT-PCR detection of MERS-CoV in nasal swab specimens from these animals has ranged from 1.6% to 41.7%. Studies conducted in Qatar detected MERS-CoV in 4 (35.7%) of 14 (13) and 5 (41.7%) of 12 (9) animals tested; in Saudi Arabia, 9 (22%) of 41 (16) and 51 (25%) of 202 (5); in Oman, 5 (6.6%) of 76 (14); and in Egypt, 4 (3.6%) of 110 (17). A recent large study of 7,803 dromedary camels in the United Arab Emirates identified MERS-CoV RNA in only 1.6% of animals (22). Of note, these authors found proportionately more positive animals near the border with Saudi Arabia and detected >5-fold more among animals sampled from slaughter houses.

Overall, we detected MERS-CoV in the upper respiratory tract of a higher proportion of animals tested in Al-Ahsa, but this proportion was within the upper range previously reported. In contrast, Alagaili et al. (5), in a comprehensive survey conducted in November and December 2013, sampled 5 regions of Saudi Arabia (Gizan in the south, Taif in the west, Tabuk in the north, Uniza in the center, and Hofuf [Al-Ahsa] in the east) and reported 66% positivity by rRT-PCR in animals from Taif versus only 5% from Al-Ahsa, despite seroprevalence of 92% in the latter. During the same period and in the same region, we detected MERS-CoV in 38.5% of nasal swab samples. This difference may be because of differences in the numbers and ages of animals sampled, time

Figure 1. Mucopurulent nasal discharge and lacrimation in 8-month-old dromedary camel naturally infected with Middle East respiratory syndrome coronavirus, Ahsa, Saudi Arabia, December 2013.
possibility that sample contamination occurred cannot be eliminated. Further studies that include immunohistologic examination and virus isolation from the lower respiratory tract of naturally infected dromedary camels will be needed to substantiate these findings.

Our detection of MERS-CoV RNA in 2 camel calves with purulent nasal discharge was consistent with those of Hemida et al. (16), who also observed mild clinical signs characterized by nasal discharge in some naturally infected young dromedary camels, and of Adney et al. (23), who documented appearance of purulent nasal discharge in the 3 experimentally infected adult dromedary camels. We also detected MERS-CoV RNA in a higher proportion of specimens from younger than from older adult dromedary camels, consistent with findings of previous studies that MERS-CoV infection is more common among young camels (5,16).

Our study also investigated temporal variation in MERS-CoV infection in dromedary camels. Although data interpretation was complicated by discontinuity in the months sampled and sampling from only 1 animal group in some months, a temporal pattern in MERS-CoV prevalence was apparent. For both animal groups, peak detection occurred during November 2013–January 2014, followed by a steady decline, reaching the lowest point in May 2014. Although we observed no clear temporal differences in the geographic origins or ages of dromedary camels brought to slaughter, which might bias these results, our data are nevertheless limited and should not be used to imply a general pattern of MERS-CoV circulation in dromedary camels in Saudi Arabia. Nevertheless, these findings would not be unexpected. Increased circulation of MERS-CoV among dromedary camels during the cool season is consistent with the prevailing cooler ambient temperatures, which have been shown to enhance coronavirus survivability outside the host (26,27), and the cool season is the period of peak circulation of other respiratory viral pathogens of humans in Saudi Arabia (28–30). This period also corresponds with the peak calving season for dromedary camels in Saudi Arabia (16); higher rates of MERS-CoV infections among a greater proportion of young animals with higher virus loads may increase opportunities for virus spread (5,16).

Whereas the link between dromedary camels and MERS-CoV infection of humans is well established (15,31), the overall contribution of zoonotic infections to community-acquired MERS-CoV remains unclear. Serologic studies of animal handlers in Saudi Arabia who work...
MERS-CoV in Respiratory Tract of Camels

in close proximity to dromedary camels have shown limited evidence of MERS-CoV infection (32–34). Alghamdi et al. (35), who examined patterns of MERS-CoV infections among humans in Saudi Arabia between June 2013 and May 2014, did not find a concomitant temporal increase in human infections that corresponded with our findings in dromedary camels. Those authors observed a slight, temporary increase in cases among humans in June and September 2013 and few cases from October through February, after which cases and deaths sharply increased beginning in April 2014. The authors concluded that lower relative humidity and higher temperatures during these months might have contributed to the dramatic surge in reported cases. However, more recent data from the World Health Organization (36) show a sharp decline in MERS-CoV cases among humans in May 2014; low numbers of cases were reported from June through August 2014, when mean temperature was highest and relative humidity was lowest in Saudi Arabia (34). Moreover, a recent increase in numbers of MERS-CoV cases in humans from September 2014 through February 2015 corresponds more closely with the temporal pattern we found in dromedary camels the preceding year. Further studies conducted over multiple years are needed to better understand the ecology of MERS-CoV, which might help inform intervention strategies to reduce zoonotic infections.

Acknowledgments
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References

Figure 2. Midpoint-rooted phylogenetic tree of Middle East respiratory syndrome coronavirus spike gene open reading frame sequences of this virus obtained from camels and select humans (sequences available from GenBank). The estimated neighbor-joining tree was constructed from nucleotide alignments by using MEGA version 6.06 (http://www.megasoftware.net). Sequence names are derived from GenBank accession number | virus strain name | month-year of collection. Numbers in parentheses denote number of additional available identical spike gene sequences obtained from same identified region of the representative strains. Bootstrap support values (1,000 replicates) ≥70% are plotted at the indicated internal branch nodes. Scale bars indicate number of nucleotide substitutions per site. Sequences obtained from camels are designated by an icon; sequences obtained from camels in Al-Ahsa Province, Saudi Arabia, 2013–2014, are designated by an asterisk (*).


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Assessment of Arbovirus Surveillance 13 Years after Introduction of West Nile Virus, United States

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Before 1999, the United States had no appropriated funding for arboviral surveillance, and many states conducted no such surveillance. After emergence of West Nile virus (WNV), federal funding was distributed to state and selected local health departments to build WNV surveillance systems. The Council of State and Territorial Epidemiologists conducted assessments of surveillance capacity of resulting systems in 2004 and in 2012; the assessment in 2012 was conducted after a 61% decrease in federal funding. In 2004, nearly all states and assessed local health departments had well-developed animal, mosquito, and human surveillance systems to monitor WNV activity and anticipate outbreaks. In 2012, many health departments had decreased mosquito surveillance and laboratory testing capacity and had no systematic disease-based surveillance for other arboviruses. Arboviral surveillance in many states might no longer be sufficient to rapidly detect and provide information needed to fully respond to WNV outbreaks and other arboviral threats (e.g., dengue, chikungunya).

B

In 1999, there was no appropriated funding in the United States for arboviral surveillance, and many states had no arboviral surveillance systems (2). After the emergence of West Nile virus (WNV) in New York, New York, in 1999 (3), Congress appropriated annual funding to support WNV surveillance activities in affected states and large cities; funds were awarded to these areas through epidemiology and laboratory capacity (ELC) cooperative agreements from the Centers for Disease Control and Prevention. CDC collaborated with state, local health, and academic partners to develop WNV detection, monitoring, and prevention guidance (4,5). By 2004, WNV had spread across the continental United States (6), and transmission to humans had been documented by multiple routes, including blood transfusions and organ transplantation (7–10). That year, CDC distributed nearly $24 million to all states and 6 large city/county health departments for WNV surveillance and prevention.

In 2000, CDC established ArboNET, a comprehensive national surveillance data capture platform to monitor WNV patterns. In 2003, CDC expanded ArboNET to include other arboviral diseases. ArboNET relies on a distributed surveillance system, whereby ELC-supported state and local health departments report data weekly on detection of arboviruses in humans, animals, and mosquitoes. CDC posts all data on the Internet with weekly updates (11). In 2004, the Council of State and Territorial Epidemiologists (CSTE) conducted a WNV surveillance capacity assessment and found that WNV surveillance programs were in place and well developed in jurisdictions receiving WNV surveillance funding (12). CSTE attributed the success of capacity development primarily to availability of federal funds and technical guidance from CDC.

Annual funding for WNV and other arbovirus surveillance distributed through the ELC cooperative agreements has steadily decreased since 2006 to 39% of its 2004 zenith, reaching lows of $9.3 million in 2012 and in 2013 (R.S. Nasci, unpub. data). Concomitantly in 2012, the nation experienced the highest incidence of confirmed WNV neuroinvasive disease since 2003 and the highest number of confirmed deaths (286) for any year thus far (13). In addition to the continued challenge of WNV to financially stressed arbovirus surveillance systems, there is the growing threat of other arboviral diseases, such as dengue (14), chikungunya (15–17), and Powassan virus encephalitis (18).

In August 2013, CSTE conducted another assessment of state and selected local health departments (LHDs) to...
measure their current surveillance and staffing capacity for WNV and other arboviruses and compare findings with those from the 2004 assessment (19). Its objectives were to describe 1) national capacities for surveillance for WNV and other arboviruses in the 50 states and 6 ELC-funded LHDs in 2012 and changes since 2004; 2) surveillance capacities of LHDs with historically high WNV burdens but no direct federal funding and how they compare with those in ELC-supported LHDs; and 3) the outstanding needs to bring US arbovirus surveillance to full capacity.

Methods
The assessment tool was developed by a working group that included representatives from CSTE, the Association of State and Territorial Health Officials, the National Association of County and City Health Officials, the Association of Public Health Laboratories, the CDC Division of Vector-Borne Diseases, and Emory University. The working group developed the 2013 survey by modifying the 2004 assessment tool and adding unique questions that reflected new WNV surveillance, prevention, and control guidance (20) and assessed specific staffing needs, presence of Aedes aegypti mosquitoes, and effect of federal WNV surveillance funding reductions on WNV surveillance activities over the past 5 years.

After pilot studies in 7 states and 4 LHDs, CSTE emailed the final state survey to the 50 state health departments and instructed key respondents to obtain relevant information from laboratory and mosquito surveillance and control staff, and complete the assessment online. The Epi Info Web Survey System was used to collect responses (21). CSTE used a similar process for distributing the assessment to 30 large city/county health departments that met at least 1 of 3 criteria: 1) receive supplemental WNV surveillance funding through the ELC grant (n = 6 [Washington, DC; New York, NY; Los Angeles County, CA; Chicago, IL; Houston, TX; and Philadelphia, PA); 2) had at least 100 cumulative reported cases of WNV neuroinvasive disease during 1999–2012 (n = 22, excluding 4 of the ELC-funded LHDs); or 3) had recent local dengue transmission (n = 2).

The 2 assessments were analyzed separately. Frequencies of response to each question were examined in aggregate and by groupings of state health departments on the basis of whether they reported a need for additional staff. LHDs were grouped by whether they received federal WNV surveillance funding, which was referred to as ELC-supported. Additional need to achieve full capacity was based on response to the question, “How many additional FTE (full-time equivalent) staff-persons are needed at the state level in your state to achieve full epidemiology and laboratory capacity to conduct WNV and other mosquito-borne disease surveillance?” Full capacity was defined as 1) ability to complete a standard case report form on every suspected/confirmed mosquito-borne arboviral disease case and report it to ArboNET; 2) ability to test for IgM for all relevant arboviruses (including dengue) on any cerebrospinal fluid (CSF) or serum specimen submitted to the state or city/county laboratory for a suspected case of arboviral disease; and 3) having an environmental surveillance system that includes mosquito surveillance to “routinely monitor arboviral activity in all parts of the jurisdiction in which there is the potential for human outbreaks of arboviral disease based on past experience.”

For staffing-related questions, nonresponses were coded as no staff needed. For all other questions, nonresponses were assumed to be missing responses. Differences of $\geq 10\%$ between groups being compared were deemed functionally useful and are highlighted in the results. Data analysis was performed by using Microsoft Excel (Microsoft, Redmond, WA, USA) and Epi Info version 7 (CDC, Atlanta, GA, USA).

Results
All 50 states (100%), all 6 ELC-supported LHDs (100%), and 15 LHDs without ELC support (62.5%) responded. In 2012, nearly all states (98%) conducted surveillance for human WNV disease; fewer conducted WNV-related surveillance for equine disease (90%), mosquitoes (80%), and avian deaths (39%) (Table 1). Less than 60% of jurisdictions contacted medical specialists (neurologists, critical care, infectious disease) to encourage reporting of suspected WNV cases, and less than one third had an active surveillance component for human surveillance. Although only 80% of states conducted mosquito surveillance, 90% collected information about mosquito surveillance from LHDs in their state, including 86% by mosquito species. Overall, 46 (94%) states had at least some information on mosquito populations, either by collecting it themselves or from LHDs. It took a median of 6 days (range 1.5–17 days) from the date a WNV-positive human specimen was collected for data to be reported to the WNV surveillance program, and a median of 16.5 days (range 4–45 days) from date of onset to date reported to ArboNET.

Fewer jurisdictions in 2012 than in 2004 conducted WNV-related surveillance activities, particularly avian deaths (26 states, −59%), active human surveillance (9 states –18%), contact with infectious disease specialists (12 states, −24%), and state-level mosquito surveillance (8 states, −16%). In addition, the percentage of states responding that most LHDs in their state conducted adult mosquito surveillance decreased from 48% to 34% (Table 1). There was a slight improvement in timeliness of reporting, from a median of 7 days to 6 days.
In 2012, 92% of states had some public health laboratory capacity for WNV testing to support human surveillance and 84% to support mosquito surveillance (Table 2). Most (93%) states tested human specimens for IgM and mosquito specimens by using PCR (72%) or culture (13%). Relatively few states tested human specimens by using PCR (13%) or culture (2%). When compared with 2004, many fewer laboratories conducted IgG, PCR and culture tests on human specimens in 2012. Testing methods for mosquitoes did not change greatly.

We also assessed state public health laboratory capacity to test for 10 arboviruses, in addition to WNV, in human serum or CSF specimens. St. Louis encephalitis (SLE) virus testing capacity was most common (34 laboratories), followed by testing for eastern equine encephalitis (EEE) (24), western equine encephalitis (WEE) (16), LaCrosse (16), dengue (9), Powassan (4), chikungunya (2), Colorado tick fever (2), yellow fever (2), and Japanese encephalitis (1) viruses. These laboratories reported performing 41,159 tests for arboviruses in 2012, of which 19,180 (46.6%) were for WNV. Of these tests, the highest percentage of positive test results was for dengue virus (137/328, 41.8%), followed by WNV (2,953/19,178, 15.4%), Powassan virus (62/1,257, 4.9%), LaCrosse virus (121/3,372, 3.6%), SLE virus (164/8,216, 2.0%), Colorado tick fever virus (2/139, 1.4%), and WEE virus (12/3,888, 0.3%).

Although many laboratories had the capability to test for arboviruses other than WNV, not all routinely did so. Overall, 26 (60%) of 43 responding laboratories reported routinely testing human CSF specimens submitted for WNV for at least 1 other arbovirus. Of these 26 laboratories, 24 routinely tested for SLE virus, 12 for EEE virus, 6 for WEE virus, 5 for LaCrosse virus, and 2 for Powassan virus. Among laboratories serving the 45 states that either test mosquitoes or use another laboratory, 24 reported routinely testing mosquito pools for SLE virus, 22 for EEE virus, and 13 for the California serogroup. To manage federal WNV surveillance funding reductions over the past 5 years, 57% of states reported eliminating avian death surveillance, 58% decreased mosquito trapping, 68% decreased mosquito testing, and 46% decreased the number of human specimens tested for WNV.

The responses from the 6 LHDs with ELC WNV surveillance support to each surveillance capacity were similar to those from the 50 states in 2004 and 2012, except for laboratory capacity. Currently, only 4 ELC-supported LHDs do some of their own WNV testing.

### Table 1. States conducting selected West Nile virus surveillance activities, United States, 2004 and 2012*

<table>
<thead>
<tr>
<th>Surveillance activity</th>
<th>No. responding states (% with activity)</th>
<th>% Difference from 2004 to 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal surveillance system</td>
<td>50 (98)</td>
<td>49 (100)</td>
</tr>
<tr>
<td>Active surveillance component</td>
<td>49 (29)</td>
<td>49 (47)</td>
</tr>
<tr>
<td>Use official case definition</td>
<td>50 (88)</td>
<td>49 (88)</td>
</tr>
<tr>
<td>Require reporting of encephalitis of unknown etiology</td>
<td>50 (48)</td>
<td>49 (63)</td>
</tr>
<tr>
<td>To encourage reporting and to suggest a high index of suspicion, did you contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurologists</td>
<td>48 (50)</td>
<td>48 (60)</td>
</tr>
<tr>
<td>Critical care specialists</td>
<td>48 (48)</td>
<td>49 (57)</td>
</tr>
<tr>
<td>Infectious disease specialists</td>
<td>48 (58)</td>
<td>49 (82)</td>
</tr>
<tr>
<td>Equine surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal surveillance system</td>
<td>49 (90)</td>
<td>49 (94)</td>
</tr>
<tr>
<td>Active surveillance component</td>
<td>44 (5)</td>
<td>46 (24)</td>
</tr>
<tr>
<td>Designated public health veterinarian within the agency?</td>
<td>Yes</td>
<td>50 (76)</td>
</tr>
<tr>
<td>Avian surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal avian death surveillance</td>
<td>49 (39)</td>
<td>49 (98)</td>
</tr>
<tr>
<td>Active component</td>
<td>19 (10)</td>
<td>48 (44)</td>
</tr>
<tr>
<td>Sentinel chicken surveillance</td>
<td>50 (10)</td>
<td>-</td>
</tr>
<tr>
<td>Adequate access to wildlife expertise within agency</td>
<td>50 (76)</td>
<td>49 (92)</td>
</tr>
<tr>
<td>Mosquito surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal surveillance system</td>
<td>49 (80)</td>
<td>49 (96)</td>
</tr>
<tr>
<td>Collect information about mosquito surveillance from LHDs in state? (states only)</td>
<td>Yes</td>
<td>49 (90)</td>
</tr>
<tr>
<td></td>
<td>By species?</td>
<td>43 (86)</td>
</tr>
<tr>
<td>Do most LHDs in your state conduct surveillance for (states only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult mosquitoes</td>
<td>44 (34)</td>
<td>44 (48)</td>
</tr>
<tr>
<td>Larval mosquitoes</td>
<td>44 (18)</td>
<td>44 (30)</td>
</tr>
<tr>
<td>Adequate access to entomologist in agency or by contract</td>
<td>50 (64)</td>
<td>49 (71)</td>
</tr>
</tbody>
</table>

*–, not asked; NA, not applicable; asked; LHDs, local health departments.

### Arboviral Surveillance in LHDs without Federal WNV Surveillance Support

The 15 LHDs without ELC grants included 13 with high WNV burden and 2 with recent dengue transmission. These LHDs were generally less likely to take an active role in...
surveillance for human disease or avian deaths than the 6 LHDs with ELC WNV surveillance support (Table 3). Furthermore, they were less likely to conduct their own mosquito surveillance (67% vs. 100%); 5 LHDs did not conduct any mosquito surveillance. LHDs that conducted mosquito surveillance tended to more consistently conduct larval surveillance and identify trapped mosquitoes to species.

Few of these 15 LHDs had their own laboratory capacity to support either testing of human specimens (n = 1) or mosquitoes (n = 3) for WNV. Most were dependent on their state health department for this function.

### Staffing Levels and Need for Additional Staffing

A total of 503 persons worked on arbovirus surveillance in state health departments in 2012. Of these, 206 worked at least half-time on it and 297 worked less than half-time. Overall, 40% of those working at least half-time were CDC funded. When converted to FTEs, there were 208.9 FTEs working on arbovirus surveillance in state health departments in 2012; 17% were epidemiologists, 31% laboratory workers, 27% mosquito surveillance staff, and 25% support staff (Table 4).

In the 21 LHDs, 187 persons worked on arbovirus surveillance in 2012; a total of 104 worked at least half-time

### Table 2. States with laboratory capacity to support WNV and other arboviral surveillance activities, United States, 2004 and 2012*

<table>
<thead>
<tr>
<th>Laboratory capacity</th>
<th>No. responding states (% with activity)</th>
<th>% Difference between 2004 and 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have some in-state capacity for WNV testing</td>
<td>50 (92)</td>
<td>NA</td>
</tr>
<tr>
<td>Human surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for IgG</td>
<td>46 (48)</td>
<td>47 (72)</td>
</tr>
<tr>
<td>Test for IgM</td>
<td>46 (93)</td>
<td>47 (100)</td>
</tr>
<tr>
<td>Test by culture</td>
<td>46 (2)</td>
<td>47 (19)</td>
</tr>
<tr>
<td>Test by PCR</td>
<td>46 (13)</td>
<td>47 (49)</td>
</tr>
<tr>
<td>Test by PRNT</td>
<td>46 (22)</td>
<td>47 (21)</td>
</tr>
<tr>
<td>Test all CSF specimens submitted for WNV for ≥1 other arbovirus</td>
<td>43 (60)</td>
<td>–</td>
</tr>
<tr>
<td>Avian surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test by culture</td>
<td>46 (4)</td>
<td>47 (13)</td>
</tr>
<tr>
<td>Test by PCR</td>
<td>46 (39)</td>
<td>47 (77)</td>
</tr>
<tr>
<td>Test IgG or IgM</td>
<td>46 (11)</td>
<td>47 (9)</td>
</tr>
<tr>
<td>Test by any of above methods</td>
<td>46 (43)</td>
<td>47 (77)</td>
</tr>
<tr>
<td>Mosquito surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-state capacity to test mosquitoes (state or local level)</td>
<td>50 (84)</td>
<td>–</td>
</tr>
<tr>
<td>Testing for &gt;1 other arbovirus</td>
<td>42 (81)</td>
<td>–</td>
</tr>
<tr>
<td>Culture or PCR</td>
<td>42 (81)</td>
<td>47 (81)</td>
</tr>
<tr>
<td>Vec Test or RAMP</td>
<td>42 (19)</td>
<td>47 (21)</td>
</tr>
</tbody>
</table>

*WNV, West Nile virus; –, not asked; NA, not applicable; PRNT, plaque reduction neutralization test; CSF, cerebrospinal fluid; Vec Test, vector test; RAMP, rapid analyte measurement platform.

### Table 3. Local health departments conducting selected WNV surveillance activities, by whether they received federal WNV surveillance funding (ELC) support, United States, 2012*

<table>
<thead>
<tr>
<th>Surveillance activity</th>
<th>No. responding LHDs (% with activity)</th>
<th>% Difference between no ELC support and ELC support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal local-level surveillance system</td>
<td>15 (0)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>To encourage reporting and suggest a high index of suspicion, did you contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurologists</td>
<td>15 (33)</td>
<td>6 (83)</td>
</tr>
<tr>
<td>Critical care specialists</td>
<td>15 (47)</td>
<td>6 (83)</td>
</tr>
<tr>
<td>Infectious disease specialists</td>
<td>15 (47)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Emergency departments</td>
<td>15 (53)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Equine surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal surveillance system</td>
<td>15 (33)</td>
<td>55 (39)</td>
</tr>
<tr>
<td>Designated public health veterinarian within the agency?</td>
<td>Yes</td>
<td>15 (33)</td>
</tr>
<tr>
<td>Avian surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal avian death surveillance</td>
<td>15 (20)</td>
<td>6 (67)</td>
</tr>
<tr>
<td>Mosquito surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal surveillance system</td>
<td>15 (67)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>For larval mosquitoes?</td>
<td>10 (90)</td>
<td>3 (67)</td>
</tr>
<tr>
<td>For adult mosquitoes?</td>
<td>10 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Identify trapped mosquitoes to species?</td>
<td>10 (90)</td>
<td>6 (83)</td>
</tr>
<tr>
<td>Calculate minimal mosquito infection rates?</td>
<td>10 (50)</td>
<td>6 (83)</td>
</tr>
<tr>
<td>Adequate access to entomologist in agency or by contract</td>
<td>14 (31)</td>
<td>6 (50)</td>
</tr>
</tbody>
</table>

*WNV, West Nile virus; ELC, epidemiology and laboratory capacity (received specific WNV surveillance funding through the Epidemiology and Laboratory Capacity Cooperative Agreement); LHDs, local health departments.
and 83 worked less than half-time on it. Similar to state health departments, only 35% of the at least half-time time staff were CDC funded (either directly or through the state). These persons accounted for 168.9 FTEs: 19% were epidemiologists, 4% laboratory workers, 56% mosquito surveillance staff, and 21% support staff (Table 4). LHDs had the same proportions of FTEs involved in mosquito surveillance (56%), regardless of whether they were ELC-supported.

### Staffing Changes from 2004 to 2012 and Additional Needs

In states and the 6 LHDs with ELC grants for WNV surveillance, the overall numbers of persons working in arbovirus surveillance and the numbers of those working at least half-time on it decreased from 2004 to 2012. In states, the decreases were 28% (from 702 to 503) and 41% (from 348 to 206), respectively (Figure 1). In LHDs, these decreases were 18% (from 228 to 187) and 5% (from 109 to 104), respectively.

Regarding staffing needs, 40 (80%) states reported needing 122.6 additional FTEs, a 59% increase over current capacity: 27 needed epidemiologists, 30 laboratory staff, 28 mosquito surveillance staff, and 19 support staff. Of the 122.6 needed FTEs, the single largest category was mosquito surveillance staff, which accounted for 44% of additional need, followed by laboratorians (22%). For LHDs, 64.2 additional FTEs were needed, a 38% increase, and most (57%) needed positions in mosquito surveillance staff (Table 4).

### Association of Staffing Needs with Level of Arbovirus Surveillance

States needing more staff were less likely to conduct WNV and other arbovirus surveillance activities than those with no need. States needing more epidemiologists were less likely to have conducted outreach to encourage medical specialists to report WNV cases (Figure 2, panel A). These states were also less likely to have performed year-end catch-up surveillance by contacting hospital or commercial laboratories (0% vs. 16%). States reporting a need for laboratorians were less likely to have at least some WNV testing capacity, perform testing on mosquito pools in 2012, and test WNV-positive specimens for other mosquito-borne viruses and were more likely to report a reduction in mosquito pool testing capacity since 2008 (Figure 2, panel B). States needing additional mosquito surveillance staff were less likely to test mosquito pools and to have identified any *Ae. aegypti* mosquitoes in the past 5 years and were more likely to have decreased the numbers of mosquito trap-nights and mosquito pools tested and report that their mosquito testing capacity had decreased since 2008 (Figure 2, panel C).

### Discussion

There are several critical objectives of arbovirus-related surveillance at each level of government: 1) monitor for and detect early signs of an outbreak threat to enable a timely response and prevent human illness and death; 2) monitor for arboviruses of human health concern and their vector populations; 3) detect changes in arbovirus disease burden over time and space; and 4) inform the public of the risks and how they can decrease them. Several findings from this assessment highlight the current capacity to meet these objectives and help to inform federal, state, and local public health and preparedness officials interested in evaluating their current arbovirus surveillance capacity.

First, current surveillance capacities at the national and state levels are far greater now than those in 1999, before the introduction of WNV. Almost all states are conducting surveillance for human WNV disease, and most are monitoring mosquito populations for WNV and have some

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**Table 4. FTE positions for arbovirus surveillance in 2012 and additional FTEs needed by functional job category, 50 states and 21 local health departments, United States**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FTE epidemiologists (%, All)</th>
<th>FTE laboratory staff (%, All)</th>
<th>FTE mosquito surveillance staff (%, All)</th>
<th>FTE support and administrative staff (%, All)</th>
<th>Total FTEs (%, All)</th>
</tr>
</thead>
<tbody>
<tr>
<td>State</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>34.6 (16.6)</td>
<td>64.6 (30.9)</td>
<td>57.2 (27.4)</td>
<td>52.5 (25.1)</td>
<td>208.9</td>
</tr>
<tr>
<td>No. needed</td>
<td>25.1 (20.5)</td>
<td>26.4 (21.5)</td>
<td>53.6 (43.7)</td>
<td>17.5 (14.3)</td>
<td>122.6</td>
</tr>
<tr>
<td>Total</td>
<td>59.7</td>
<td>91.0</td>
<td>110.8</td>
<td>70.0</td>
<td>331.5</td>
</tr>
<tr>
<td>Local</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>32.8 (19.3)</td>
<td>7.4 (4.4)</td>
<td>93.9 (55.6)</td>
<td>34.8 (20.6)</td>
<td>168.9</td>
</tr>
<tr>
<td>No. needed</td>
<td>6.2 (9.7)</td>
<td>7.5 (11.7)</td>
<td>36.3 (56.5)</td>
<td>14.2 (22.1)</td>
<td>64.2</td>
</tr>
<tr>
<td>Total</td>
<td>39.0</td>
<td>14.9</td>
<td>130.2</td>
<td>49.0</td>
<td>233.1</td>
</tr>
</tbody>
</table>

*Values are no. (%). FTE, full-time equivalent.*

---

**Figure 1.** Total and at least 50% time staff performing West Nile virus surveillance in state health departments, United States, 2004 and 2012.
WNV testing capacity. All are reporting WNV and other arboviruses to ArboNET.

Second, the ability to detect the early signs of an outbreak of WNV and other arboviruses that can threaten large human populations has been compromised since 2004. Endemic arboviruses that have caused outbreaks of severe illness and death in densely populated areas continue to pose annual threats, and emerging diseases, such as dengue and chikungunya, continue to pose annual threats, and emerging diseases, such as dengue and chikungunya, pose new ones (14,15–17,22). Knowledge of local vector mosquito populations and early detection of arbovirus activity in these vectors, animals, and humans are essential to guide public health action ranging from health advisories to mosquito control. Many fewer states now conduct any form of active surveillance that enables rapid detection of the first sentinel human cases of arbovirus disease. Most states have cut back on support for mosquito surveillance. Some states and large metropolitan areas, including some with previously large WNV outbreaks, lack the necessary mosquito surveillance information to anticipate a surge in WNV infection. Most lack the resources to map the distribution and size of either *Ae. aegypti* or *Ae. albopictus* mosquito populations to enable risk evaluation or to mount an effective response to identification of local transmission of dengue or chikungunya viruses.

Third, in addition to the decreased ability to monitor vector mosquito populations, testing for arboviruses other than WNV, SLE virus, and EEE virus is patchy and inadequate to detect or monitor their presence in many states. Some endemic arboviruses that cause either encephalitis or acute systemic or febrile disease (e.g., Powassan, LaCrosse, Colorado tick fever, and Heartland viruses) have not been included in systematic public health surveillance, and their ecology and epidemiology might be changing.

For example, Powassan virus spreads to humans from animal reservoirs by the same tick genus (*Ixodes*) that transmits Lyme disease and babesiosis. Although Lyme disease and babesiosis have increased dramatically in incidence and geographic distribution in the United States in the past decades, there is still a poor understanding of Powassan virus epidemiology >50 years after its discovery. Most state health department laboratories do not test for Powassan virus when they test for WNV or other arboviruses, and few clinicians order commercial tests specifically for this virus. Powassan, LaCrosse, and Colorado tick fever viruses were tested for in only 8%, 32%, and 4% of state laboratories, respectively, in 2012. However, a higher percentage of specimens were positive for Powassan and LaCrosse virus infections than for SLE and for Colorado tick fever than EEE or WEE. These results support surveillance for these viruses in jurisdictions with relevant vectors when routinely testing for WNV. If their epidemiology were better understood, estimates of their disease burden could be improved, and the public could be better informed of the risk for infection.

Fourth, state laboratory capacity is essential to enable LHDs to monitor virus activity through mosquito, avian death, or sentinel-chicken surveillance. The ability of ArboNET to synthesize and report useful surveillance information is possible only because of efforts made at each state and local health department to conduct the nationally recommended level of surveillance to meet surveillance objectives. This assessment documents, that as resources have decreased, LHDs dependent on state laboratories to conduct testing for them have reduced or eliminated mosquito-based surveillance to the point where 15% of states no longer provide support for LHDs and one third of responding LHDs in areas with a high incidence of WNV no longer conduct mosquito-based surveillance.

In 2004, all states approached full capacity for 2 of the 3 criteria for full arbovirus surveillance capacity used in this report: ability to complete a standard case report
form on every suspected/confirmed mosquito-borne arboviral disease case and report it to ArboNET and having an environmental surveillance system that includes mosquito surveillance “to routinely monitor arboviral activity in all parts of the jurisdiction in which there is the potential for human outbreaks of arboviral disease based on past experience.” In 2012, although the first criterion continued to be met, the second criteria was no longer met. Although the 2004 assessment did not measure the ability to test for IgM for all relevant arboviruses (including dengue viruses) on any CSF or serum specimen submitted to the state or city/county laboratory on a suspected case of arboviral disease, this assessment found that many states are not meeting this remaining criterion.

This assessment has several major limitations. First, not all jurisdictions answered all questions. Second, additional personnel needs were based on state and local health department self-assessment and are subjective. In addition, because of the way the assessment was worded and responded to, we assumed states not specifying a need for additional personnel had no need. Thus, results showing that states that identified a need also performed far fewer surveillance activities than states with no reported additional need are subject to possible inaccuracies in this assumption. Third, the relative role of different surveillance methods shifted between 2004 and 2012. Whereas needs for human surveillance and laboratory testing capability and capacity are largely unchanged, the need for avian death and equine surveillance data in many jurisdictions has decreased, but the need for mosquito surveillance data has increased. Jurisdictions have adjusted resources to accommodate these changes. This adjustment may explain, in part, the generally high US WNV surveillance capacity, despite federal funding cuts of more than 50%. Fourth, measures of workload and staffing need may be difficult to compare among years because they depend, in part, on levels of WNV activity. The human WNV burden in 2012 was more than double that in 2004, which may have influenced estimates of need. Finally, the 2012 assessment did not solicit information on funding or unmet needs for anything other than staff. For example, limited fiscal resources might preclude purchase of updated laboratory equipment and testing reagents, thereby limiting laboratory testing of mosquito pools and testing of human and nonhuman specimens for arboviruses other than WNV. Unmet non-personnel needs might have contributed to loss of arbovirus surveillance capacity and would need to be addressed in any effort to maintain or improve it.

In summary, WNV emergence in the United States stimulated building of a robust national arbovirus surveillance system with human and vector early detection components and laboratory services. This system, although still highly functional, has become less robust and might be near a large-scale tipping point, especially in areas of vector surveillance and laboratory support for human diagnostic and mosquito testing. Already, arbovirus surveillance is inadequate in many states to rapidly detect and control outbreaks and to give the public the critical information it needs for prevention.

Acknowledgments
We thank the workgroup for developing the assessment tool and providing valuable insights on interpretation of findings. In addition to the authors, workgroup members are Jane Getchell and Kelly Wroblewski (Association of Public Health Laboratories, James Blumenstock and Abraham Kulungara (Association of State and Territorial Health Officials), and Alfred DeMaria, Catherine Brown, Carina Blackmore, and Jennifer Lemmings (CSTE). We also thank Kimberly Miller, Rebecca Rutledge and Jessica Wurster for assistance with data analysis.

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References
April 2015: Emerging Viruses

Including:

- Reappearance of Chikungunya, Formerly Called Dengue, in the Americas
- Hantavirus Pulmonary Syndrome, Southern Chile, 1995–2012
- Evolution of Ebola Virus Disease from Exotic Infection to Global Health Priority, Liberia, Mid-2014
- Population Structure and Antimicrobial Resistance of Invasive Serotype IV Group B Streptococcus, Toronto, Ontario, Canada
- Norovirus Genotype Profiles Associated with Foodborne Transmission, 1999–2012
- Deaths Associated with Respiratory Syncytial and Influenza Viruses among Persons >5 Years of Age in HIV-Prevalent Area, South Africa
- Sequence Variability and Geographic Distribution of Lassa Virus, Sierra Leone
- Influenza A(H7N9) Virus Transmission between Finches and Poultry
- Highly Pathogenic Avian Influenza A(H5N1) Virus Infection among Workers at Live Bird Markets, Bangladesh, 2009–2010
- Increased Risk for Group B Streptococcus Sepsis in Young Infants Exposed to HIV, Soweto, South Africa, 2004–2008

In 2002, the National Legionella Outbreak Detection Program was implemented in the Netherlands to detect and eliminate potential sources of organisms that cause Legionnaires’ disease (LD). During 2002–2012, a total of 1,991 patients with LD were reported, and 1,484 source investigations were performed. Of those sources investigated, 24.7% were positive for Legionella spp. For 266 patients with LD, 105 cluster locations were identified. A genotype match was made between a strain detected in 41 patients and a strain from a source location. Despite the systematic approach used by the program, most sources of LD infections during 2002–2012 remained undiscovered. Explorative studies are needed to identify yet undiscovered reservoirs and transmission routes for Legionella bacteria, and improved laboratory techniques are needed to detect Legionella spp. in samples with a high background of microbial flora such as soil.

Legionnaires’ disease (LD) is an acute pneumonia characterized by clinical symptoms and signs (e.g., cough, fever, lung infiltration observed on a chest radiograph) similar to those of pneumonias resulting from other pathogens. LD is caused by infection with Legionella spp. bacteria, which are most often transmitted to persons through inhalation of bacteria disseminated into the air as an aerosol from natural or man-made sources of water (1). The incubation period is 2–14 days. LD is thought to account for 2%–20% of all community-acquired pneumonias (2) and is fatal in ≈6%–11% of cases (3,4).

After a large outbreak of LD at a flower show in Bovenkarspel, the Netherlands, in 1999 (5), prevention and control of Legionella spp. infections became a national concern in the Netherlands, and legislation to prevent Legionella spp. in drinking water systems was introduced (6,7). This legislation obligated owners of aerosol-producing devices (e.g., shower heads and whirlpools), if third parties may be exposed to them, to conduct a risk analysis, develop a control plan, keep logs of control measures, and perform regular sampling for Legionella spp. contamination. In addition, in 2002, a National Legionella Outbreak Detection Program (NLODP) was implemented (8) on the basis of a report that LD outbreaks are often preceded and followed by small clusters of solitary cases (9). The aims of NLODP are early detection of small clusters of cases, identification of sources of infection, and implementation of early control measures to prevent additional LD cases or an outbreak. For evaluation of transmission pathways, infection sources are sampled, and genotypes of Legionella strains found in these samples are compared with those of clinical isolate(s) from the patient(s) associated with that source. To evaluate the findings of the NLODP during 2002–2012, we analyzed data to determine whether extensive investigation efforts could detect Legionella spp. in collected samples and conclusively identify environmental sources.

Methods

Patients

LD has been notifiable in the Netherlands since 1987. A case of LD is defined as laboratory-confirmed infection in a person having symptoms compatible with pneumonia or radiologic signs of infiltration. Laboratory evidence may be ≥1 of the following: isolation of Legionella spp. from respiratory secretions or lung tissue, detection of L. pneumophila antigen in urine, seroconversion or a ≥4-fold rise in antibody titers to L. pneumophila in paired acute- and convalescent-phase serum samples, a high antibody titer to L. pneumophila in a single serum sample, and direct fluorescent antibody staining of the organism or detection of Legionella DNA by PCR in respiratory secretions or lung tissue. In the Netherlands, microbiologic laboratories involved in the diagnosis and treatment of patients with pneumonia are requested to send available clinical isolates of Legionella spp. to the Legionella Source Identification Unit (LSIU), a part of the NLODP. LD cases in persons who had been outside the country for ≥5 of 9 days before disease onset were defined as nondomestic cases and excluded from

Author affiliations: Regional Public Health Laboratory Kennemerland, Haarlem, the Netherlands (J.W. Den Boer, S.M. Euser, L. Reijnen, J.P. Bruin); National Institute for Public Health and the Environment, Bilthoven, the Netherlands (P. Brandsema)

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the analyses. Cases in persons who stayed in a hospital or other health care setting (e.g., nursing home or rehabilitation center) for ≥1 day during the 2–14 days before symptom onset were defined as nosocomial cases.

Source Identification and Cluster Detection
Potential sources of infection were identified by Municipal Health Services (MHS) public health physicians and nurses, who used a standardized questionnaire to interview patients or relatives (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/21/7/14-1130-Techapp1.pdf). The interview focused on tracking each patient’s exposure to potential sources of infection during the 2–14 days before symptom onset. All potential sources of infection were recorded in a database by the LSIU and used to identify clusters of LD cases by location and date. Each new LD case in this database was examined to determine if reported potential sources were linked to other LD cases. Because outbreaks of Legionnaires’ disease are often preceded and followed by small clusters of solitary cases (9), an arbitrary cluster definition was constructed that defined 2 types of clusters: location and geographic. A location cluster, which may represent a local contamination, was defined as cases reported within 2 years of each other in ≥2 persons who were reported to have been exposed to the same potential source of infection during the 2–14 days before symptom onset. A geographic cluster was defined as cases in >3 persons who lived <1 km apart and whose infections were reported within 6 months of each other. The concept of a geographic cluster was constructed to identify sources that patients were exposed to but unaware of (e.g., cooling towers). Patients could belong to >1 cluster. Data from the location cluster of the LD outbreak in Amsterdam in 2006 were excluded from our analyses.

Sampling Procedure
As part of the NLODP, the LSIU is available to each MHS to collect samples from potential sources of Legionella infection for reported domestic LD cases. During 2002–2006, all identified potential sources of infection were investigated. However, because of budgetary reasons, after June 1, 2006, potential sources were investigated only if ≥1 of 4 sampling criteria was met: 1) a patient-derived isolate of Legionella spp. (from respiratory secretions or lung tissue) was available; 2) a location cluster was identified; 3) a geographic cluster was identified; or 4) the patient had stayed in a hospital or other health care setting during the incubation period. For geographic clusters, efforts were focused on identifying yet undiscovered potential sources (e.g., cooling towers near patients’ residences). If ≥1 of the 4 sampling criteria was met, trained LSIU laboratory staff collected water and swab samples from identified potential sources when possible. For each location, sampling points were selected by LSIU staff in cooperation with the facility’s technical team (when a team is available), and a comprehensive collection of water and swab samples was obtained from that location for further analysis.

Laboratory Investigations
Samples collected during the source investigation were analyzed for the presence of Legionella spp. (for an extensive description, see online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/21/7/14-1130-Techapp2.pdf). All L. pneumophila serogroup 1 (SG1) strains (clinical and environmental) were subsequently genotyped by sequence-based typing, as recommended by the ESCMID Study Group for Legionella Infections (10–12), and further determined by using the Dresden panel of monoclonal antibodies (13). The sequence-based typing profiles of the patient isolates were compared with those of the environmental strains found in samples of potential sources.

Statistical Analyses
Comparisons were made by using independent samples t-tests, nonparametric Mann-Whitney U-test, 2-tailed χ² tests (proportions), and linear regression analyses (trends over time). All analyses were performed with PASW Statistics 8.0 (SPSS Inc., Chicago, IL, USA).

Results

Patients
During August 2002–August 2012, a total of 2,796 LD cases were reported in the Netherlands, 805 (28.8%) of which were nondomestic (Figure). These travel-associated cases were excluded from the analyses, resulting in 1,991 reported possible domestic LD cases (mean of 193 [SD 76] cases annually); 119 (6.0%) of these were characterized as nosocomial cases. Most patients (72%) for this period were male (Table 1). The median age of reported case-patients

Figure. Legionnaires’ disease cases reported in the Netherlands, August 1, 2002–August 1, 2012. A total of 2,796 cases were reported; LD cases in persons who had been outside the country for ≥5 of 9 days before disease onset were defined as nondomestic cases and excluded from analyses. All other cases were classified as domestic.
increased from 55.3 (range 26.4–78.3) years in 2002 to 62.5 (range 27.0–91.6) years in 2012 (Table 1; linear regression, p trend <0.001).

Diagnostic Tests
The 1,991 LD cases were ascertained by 2,541 diagnostic tests (Table 2). Most cases were diagnosed by using urinary antigen tests (83.2%) or cultures (23.1%). Nosocomial LD cases (n = 119) were more often diagnosed by culture compared with community-acquired cases (37.0% vs. 22.2%; Pearson χ² test, p<0.001). Nosocomial cases were more evenly distributed among male and female patients than were community-acquired cases (52.9% vs. 73.1% of cases in male patients, respectively; Pearson χ² test, p<0.001).

Source Investigation
A total of 3,035 potential sources were identified for the 1,991 reported LD cases (mean of 1.5 [SD 1.0] potential sources per patient). Online Technical Appendix 2 Table 1 shows the distribution of the different types of reported sources. Using the NLODP sampling criteria, the LSRII sampled 1,418 unique potential sources (47% of 3,035 reported sources). Some sources were sampled >1 time, resulting in 1,484 source investigations performed during the study period. In 367 (24.7%) of these investigations, Legionella spp. were identified in ≥1 sample, but large variations were seen among the different source types (Table 3). In 30 investigations, >1 Legionella spp. was found, identified as L. pneumophila SG1 or L. pneumophila non-SG1 if no L. pneumophila SG1 was found (Table 3). The proportions before and after introduction of the 4 criteria for sampling on June 1, 2006, were similar: 24.6% vs. 25.2%, respectively.

L. pneumophila SG1 was found in 97 (6.5%) investigations, L. pneumophila non-SG1 in 76 (5.1%), and Legionella spp. other than L. pneumophila in 194 (13.1%) (Table 3). The proportion of investigations in which L. pneumophila SG1 was found showed large variations among source types (Table 3). For instance, L. pneumophila SG1 was often detected in wellness centers (i.e., facilities offering spas, saunas, fitness equipment, massages, etc.) (40.5%); hospitals and health care settings (25.6%); and cooling towers (20.9%). However, L. pneumophila SG1 was not detected in investigated camp sites, car wash or gasoline stations, or decorative water fountains and was detected in only a small proportion of investigated garden centers (1.2%). Residences were the most frequently sampled sources (51.3% of investigations); L. pneumophila SG1 was found in 21 (2.8%) of the 762 investigated residences (Table 3). Exclusion of source investigation data for the 119 nosocomial cases did not markedly change these results (online Technical Appendix 2 Table 2).

Clusters
The cluster definition used by NLODP resulted in 105 identified clusters, of which 98 (93.3%) were location clusters and 7 (6.7%) were geographic clusters. These clusters involved 266 patients with LD (Table 4; online Technical Appendix 2 Figure). An average of 2.9 (range 2–11) patients with LD were associated with each cluster (some patients were part of multiple clusters). In 50 clusters (47.6%), patients from ≥1 MHS were involved. Garden centers were the most frequently identified cluster site (27 [25.7%] clusters), followed by hospitals and health care settings (17 [16.2%]).
clusters), residences (10 [9.5%] clusters), wellness centers (9 [8.6%] clusters), and hotels (7 [6.7%] clusters) (Table 5). For the 98 location clusters, 142 source investigations were performed (23 cluster locations were investigated ≥1 time during the study period). *Legionella* spp. were found in 56 (39.4%) of investigations. *L. pneumophila* SG1 was found in 28 (19.7%) investigations, *L. pneumophila* non-SG1 in 6 (4.2%), and *Legionella* spp. other than *L. pneumophila* in 22 (15.5%).

### Strain Characteristics

For the 1,991 reported patients with LD, 392 clinical isolates of *Legionella* spp. (85% of 460 reported patients diagnosed by culture) were sent to LSIIU by the participating microbiologic laboratories in the Netherlands. All *L. pneumophila* SG1 clinical isolates and environmental strains were genotyped by using sequence-based typing (10–12), and monoclonal antibody determination was performed (13) (online Technical Appendix 2 Tables 3, 4).

### Matches

For the 392 patients with LD for whom a clinical isolate was available, 704 unique potential sources of investigation were identified (mean 1.8 [SD 1.2] sources per patient). For these sources, 478 investigations were performed, and *Legionella* spp. were found in a sample from 120 (25.1%) investigations.

Environmental strains were compared with the clinical isolate(s) from the patients associated with the sampled potential sources. During August 2002–August 2012, a total of 38 genotype matches were found for 41 patients with LD (3 matches involved 2 clinical isolates, and 35 matches involved 1 clinical isolate). For each patient with an isolate that was part of a genotype match, a mean of 1.9 (SD 1.6) potential sources of infection was identified. This mean was significantly higher than the mean 1.5 (SD 1.0) sources identified for patients whose clinical isolate could not be matched with an environmental strain (independent samples t-test, p<0.01). Table 6 shows the different types of sources from which the matching environmental strains were isolated. Most matches (15 [39%]) were with strains from hospitals or other health care settings, followed by those from residences (7 [18%]). A genotype match was found for 38 (31.7%) of 120 available clinical isolates that could be compared with an environmental strain (online Technical Appendix Table 5). For the 266 patients who were part of a cluster, 24 had clinical isolates that could be genotypically compared with environmental strains, and a genotype match occurred for 19 (79.2%) of these 24 patients.

### Table 3. Sampling results (N = 1,484) by potential sources of infection for patients with Legionnaires’ disease, the Netherlands, 2002–2012*

<table>
<thead>
<tr>
<th>Source type (no. samples)</th>
<th>Total (1,484)</th>
<th>L. pneumophila non-SG1 (976)</th>
<th>L. pneumophila SG1 (508)</th>
<th>L. non-pneumophila (360)</th>
<th>Samples negative for <em>Legionella</em> spp., no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wellness center (37)†‡</td>
<td>27 (73.0)</td>
<td>4 (10.8)</td>
<td>15 (40.5)</td>
<td>8 (21.6)</td>
<td>10 (27.0)</td>
</tr>
<tr>
<td>Hospital/health care setting (90)</td>
<td>46 (51.1)</td>
<td>5 (5.6)</td>
<td>23 (25.6)</td>
<td>18 (20.0)</td>
<td>44 (48.9)</td>
</tr>
<tr>
<td>Cooling tower (43)</td>
<td>19 (44.2)</td>
<td>6 (16.8)</td>
<td>9 (20.9)</td>
<td>2 (4.7)</td>
<td>24 (55.8)</td>
</tr>
<tr>
<td>Sports facility (29)</td>
<td>10 (34.5)</td>
<td>2 (6.9)</td>
<td>5 (17.2)</td>
<td>3 (10.3)</td>
<td>19 (65.5)</td>
</tr>
<tr>
<td>Swimming pool (40)</td>
<td>13 (32.5)</td>
<td>2 (5.0)</td>
<td>6 (15.0)</td>
<td>5 (12.5)</td>
<td>27 (67.5)</td>
</tr>
<tr>
<td>Hotel (20)</td>
<td>8 (40.0)</td>
<td>3 (15.0)</td>
<td>3 (15.0)</td>
<td>2 (10.0)</td>
<td>12 (60.0)</td>
</tr>
<tr>
<td>Holiday park (23)</td>
<td>5 (21.7)</td>
<td>1 (4.3)</td>
<td>2 (8.7)</td>
<td>2 (8.7)</td>
<td>18 (78.3)</td>
</tr>
<tr>
<td>Residence (762)</td>
<td>155 (20.3)</td>
<td>30 (3.9)</td>
<td>21 (2.8)</td>
<td>104 (13.6)</td>
<td>607 (79.7)</td>
</tr>
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<td>Workplace (92)</td>
<td>19 (20.7)</td>
<td>8 (8.7)</td>
<td>2 (2.2)</td>
<td>9 (9.8)</td>
<td>73 (79.3)</td>
</tr>
<tr>
<td>Car wash/gasoline station (44)</td>
<td>6 (13.6)</td>
<td>1 (2.3)</td>
<td>NA</td>
<td>5 (11.4)</td>
<td>38 (86.4)</td>
</tr>
<tr>
<td>Garden center (86)</td>
<td>8 (9.3)</td>
<td>1 (1.2)</td>
<td>1 (1.2)</td>
<td>6 (7.0)</td>
<td>78 (90.7)</td>
</tr>
<tr>
<td>Campsite (28)</td>
<td>2 (7.1)</td>
<td>1 (3.6)</td>
<td>NA</td>
<td>1 (3.6)</td>
<td>26 (92.9)</td>
</tr>
<tr>
<td>Decorative fountain (23)</td>
<td>1 (4.3)</td>
<td>NA</td>
<td>NA</td>
<td>1 (4.3)</td>
<td>22 (95.7)</td>
</tr>
<tr>
<td>Other (167)</td>
<td>48 (28.7)</td>
<td>10 (6.7)</td>
<td>10 (6.0)</td>
<td>28 (16.8)</td>
<td>119 (71.3)</td>
</tr>
<tr>
<td>Total (1,484)</td>
<td>367 (24.7)</td>
<td>76 (5.1)</td>
<td>97 (6.5)</td>
<td>194 (13.1)</td>
<td>1,117 (75.2)</td>
</tr>
</tbody>
</table>

*Study period was August 1, 2002–August 1, 2012. **SG1, serogroup 1; NA, not possible to calculate. †Recreational facility offering spas, saunas, fitness equipment, massages, etc.

### Table 4. Characteristics of 105 clusters reported for patients with Legionnaires’ disease (n = 266), the Netherlands, 2002–2012*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location clusters (%)†</td>
<td>98 (93.3)</td>
</tr>
<tr>
<td>Geographic clusters (%)‡</td>
<td>7 (6.7)</td>
</tr>
<tr>
<td>Mean no. patients per cluster (range)</td>
<td>2.9 (2–11)</td>
</tr>
<tr>
<td>No. multiple municipal health services involved (%)</td>
<td>50 (47.6)</td>
</tr>
<tr>
<td>Mean no. municipal health services involved (range)</td>
<td>1.7 (1–5)</td>
</tr>
</tbody>
</table>

*Study period was August 1, 2002–August 1, 2012.
†A location cluster is defined as a group of cases reported within 2 years of each other in >2 persons who were reported to have been exposed to the same potential source of infection during the 2–14 days before symptom onset.
‡A geographic cluster is defined as cases in >3 persons who lived <1 km apart and whose infections were reported within 6 months of each other.
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Discussion

During 2002–2012, a total of 1,991 patients with LD were reported in the Netherlands, and 1,484 source investigations were performed; 367 (24.7%) of the sources investigated were positive for *Legionella* spp. A total of 105 clusters were identified among 266 patients with LD. For 41 patients, a genotype match was found between the patient isolate and an environmental strain.

More than half of all source investigations were performed in residences, but only 20% of these investigations were positive for *Legionella* spp.; residences ranked tenth on the list of source types. A total of 43 cooling towers were investigated, ranking them third on the list of source types; >40% of those investigations were positive for *Legionella* spp. This well-known source of LD outbreaks should be considered often during source identification and investigation efforts performed by the MHS and LSIU.

For each patient, a mean of 1.5 potential sources of infection were reported, and about half of the reported sources were sampled. Although several attributes are being used by the MHS to improve source investigation (e.g., an elaborate questionnaire and a geographic information system implemented in 2009 [https://lpgis.geoxplore.nl/webify/?app=lpgis_ggd]), the number of sources being used by the MHS to improve source investigation (e.g., an elaborate questionnaire and a geographic information system implemented in 2009 [https://lpgis.geoxplore.nl/webify/?app=lpgis_ggd]), the number of sources being investigated could be increased. When the genotypic matches were analyzed, the mean number of sources identified and investigated for the patients involved was considerably higher (1.9 sources per patient), suggesting that identification and investigation of more potential sources of infection by the MHS may increase the proportion of patients with LD for whom a likely source of infection can be established.

Garden centers ranked third (after residences and workplaces) on the list of the most frequently reported potential sources of LD infection; 26% of identified clusters were associated with a garden center, indicating that this source type is often visited by patients with LD during the 2–14 days before symptom onset. However, only 8 of 86 investigated garden centers were found positive for *Legionella* spp. during source investigations. Several studies have shown the

### Table 5. Cluster locations reported for 266 Legionnaires’ disease patients, the Netherlands, 2002–2012*

<table>
<thead>
<tr>
<th>Location</th>
<th>No. (%) clusters</th>
<th>Location†</th>
<th>Geographic‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garden center</td>
<td>27 (25.7)</td>
<td>27 (27.6)</td>
<td>0</td>
</tr>
<tr>
<td>Hospital/health care setting</td>
<td>17 (16.2)</td>
<td>17 (17.3)</td>
<td>0</td>
</tr>
<tr>
<td>Residence</td>
<td>10 (9.5)</td>
<td>4 (4.1)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td>Wellness center§</td>
<td>9 (8.6)</td>
<td>9 (9.2)</td>
<td>0</td>
</tr>
<tr>
<td>Hotel</td>
<td>7 (6.7)</td>
<td>7 (7.1)</td>
<td>0</td>
</tr>
<tr>
<td>Cooling tower</td>
<td>5 (4.8)</td>
<td>5 (5.1)</td>
<td>0</td>
</tr>
<tr>
<td>Holiday park</td>
<td>5 (4.8)</td>
<td>5 (5.1)</td>
<td>0</td>
</tr>
<tr>
<td>Swimming pool</td>
<td>4 (3.8)</td>
<td>4 (4.1)</td>
<td>0</td>
</tr>
<tr>
<td>Industrial complex</td>
<td>3 (2.9)</td>
<td>2 (2.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Car wash installation</td>
<td>3 (2.9)</td>
<td>3 (3.0)</td>
<td>0</td>
</tr>
<tr>
<td>Sports facility</td>
<td>2 (1.9)</td>
<td>2 (2.0)</td>
<td>0</td>
</tr>
<tr>
<td>Cruise ship</td>
<td>2 (1.9)</td>
<td>2 (2.0)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>11 (10.5)</td>
<td>11 (11.2)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>105 (100.0)</td>
<td>98 (100.0)</td>
<td>7 (100.0)</td>
</tr>
</tbody>
</table>

*Study period was August 1, 2002–August 1, 2012.
†A location cluster is defined as cases reported within 2 years of each other in ≥2 persons who were reported to have been exposed to the same potential source of infection during the 2–14 days before symptom onset.
‡A geographic cluster is defined as cases reported within 2 years of each other in ≥3 persons who lived ≤1 km apart and whose infections were reported within 6 months of each other.
§Recreational facility offering spas, saunas, fitness equipment, massages, etc.

### Table 6. Genotypic matches (n = 38) from available isolates (n = 41) by source type reported for patients with Legionnaires’ disease, the Netherlands, 2002–2012*

<table>
<thead>
<tr>
<th>Source type</th>
<th>No. (%) matches</th>
<th>No. (%) available isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital/health care setting</td>
<td>19 (39.5)</td>
<td>17 (41.5)</td>
</tr>
<tr>
<td>Residence</td>
<td>7 (18.4)</td>
<td>8 (19.5)</td>
</tr>
<tr>
<td>Industrial complex</td>
<td>3 (7.9)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Swimming pool</td>
<td>2 (5.3)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Wellness center†</td>
<td>3 (7.9)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Hotel</td>
<td>2 (5.3)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Travel trailer</td>
<td>1 (2.6)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Whirlpool</td>
<td>2 (5.3)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Sports facility‡</td>
<td>1 (2.6)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Potting soil</td>
<td>1 (2.6)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Car wash installation</td>
<td>1 (2.6)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Total</td>
<td>38 (100.0)</td>
<td>41 (100.0)</td>
</tr>
</tbody>
</table>

*Study period was August 1, 2002–August 1, 2012. Data from the LD outbreak in Amsterdam in 2008 (7) are excluded from these data.
†Recreational facility offering spas, saunas, fitness equipment, massages, etc.
‡This genotypic match was made with a clinical isolate collected during 2000 and an environmental strain collected in 2005.
presence of Legionella spp. in potting soil samples (14–16),
and the use of amebal coculture techniques has shown prom-
ing results in recovering L. pneumophila SG1 sequence
type (ST) 46 (the third most frequently found ST in clinical
isolates) from samples with a high likelihood of microbial
flora (17). At this time, potting soil samples collected by
NLODP are not regularly being investigated by the amebal
coculture technique. These findings suggest that potting soil
samples from garden centers identified as potential sources
of infection for patients with LD should be examined closely.

Notwithstanding the extensive efforts by NLODP col-
laborators, the number of L. pneumophila SG1 strains that
could be derived from investigated potential sources was
relatively low (114 strains over 10 years). Despite system-
atic methods of source identification by using a standardized
questionnaire covering >20 source types, a source could not
be confirmed in most cases. Although the questionnaire is
regularly evaluated and adjusted on the basis of new insights
concerning reported sources of infection, it primarily cov-
ers sources identified from the literature, possibly explaining
the low success rate; actual sources of infection may not be
captured in the questionnaire. This hypothesis is supported
by the differences in genotype variation between clinical
isolates and environmental strains: one third of all culture-
positive patients with LD were infected by L. pneumophila
SG1 ST47, a rare finding in environmental samples.

The experiences of NLODP show the importance of or-
organizing a multidisciplinary collaboration in which MHSs,
treating physicians, and microbiologic laboratories are re-
presented and aware of the importance of different aspects of
surveillance and source investigation for patients with LD.
Our findings show the necessity of increasing awareness
among various groups: physicians for diagnosis of LD,
MHSs for extensive source identification, and laboratories
for performance of adequate diagnostics and collection of
clinical and environmental isolates. During 2002–2012,
the number of reported patients with LD and the number of
identified clusters of patients did not change dramati-
cally, which may suggest the limited effects of NLODP.
However, one could argue that this relatively stable number
of patients with LD could have resulted from the program.
Despite the rational, systematic approach used by NLODP
during this decade, most sources of LD infections went
undiagnosed, stressing the need for evaluating other, yet
unknown, potential sources of infection. Also, a need exists
for further investment in improving laboratory techniques
detection of Legionella spp. in clinical samples with a
high background of microbial flora such as soil.

Acknowledgments
We thank the public health physicians and nurses of the MHSs
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sults; the treating physicians and microbiologists for making
patient isolates available for genotyping; and Jacqueline De
Vries and Wim Houtenbos for their support with source investi-
gations.

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control of infectious diseases.

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Seroprevalence for Hepatitis E and Other Viral Hepatitides among Diverse Populations, Malawi

Taha E. Taha, Laura K. Rusie, Alain Labrique, Mulinda Nyirenda, Dean Soko, Melvin Kamanga, Johnstone Kumwenda, Homayoon Farazadegan, Kenrad Nelson, Newton Kumwenda

Data on prevalence of hepatitis E virus (HEV) in Malawi is limited. We tested blood samples from HIV-uninfected and -infected populations of women and men enrolled in research studies in Malawi during 1989–2008 to determine the seroprevalence of HEV, hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Samples were tested for IgG against HEV, total antibodies against HAV and HCV, and presence of HBV surface antigens. Of 800 samples tested, 16.5% were positive for HEV IgG, 99.6% were positive for HAV antibodies, 7.5% were positive for HBV surface antigen, and 7.1% were positive for HCV antibodies. No clear trends over time were observed in the seroprevalence of HEV, and HIV status was not associated with hepatitis seroprevalence. These preliminary data suggest that the seroprevalence of HEV is high in Malawi; the clinical effects may be unrecognized or routinely misclassified.

Hepatitis E virus (HEV) is primarily a waterborne virus that is transmitted by the fecal–oral route. First recognized in the early 1980s, it is now acknowledged to be the primary cause of enterically transmitted non-A, non-B hepatitis (1). HEV has 1 serotype and 4 genotypes (2). Clinical characterization of HEV infection is similar to that of other viral hepatitis infections, ranging from asymptomatic infection to fulminant hepatitis (2). Although illness caused by HEV most often tends to be mild and self-limiting, high rates of illness and death among pregnant women is a unique complication and key epidemiologic feature of HEV infection. Additionally, chronic infection leading to fibrosis and cirrhosis of the liver can occur in the immunosuppressed (3).

Currently, no data on HEV seroprevalence are available for Malawi. However, outbreaks of HEV infection have been documented in several countries in the southern and eastern regions of Africa. In Zambia, the overall seroprevalence of HEV was 42% among 106 adults who participated in a community study in 1999; among children who were included in a prospective study of the same community in 2011, the seroprevalence of HEV was 8% in age group 1–4 years (n = 96), 16% in age group 5–9 years (n = 62), and 36% in age group 10–14 years (n = 36) (4). In northern Uganda, surveillance of health care facilities during 2010–2012 showed that 42% of 347 persons with reported acute jaundice syndrome cases had hepatitis E, 14% had hepatitis B, and 5% had hepatitis C (5). During 2012 in a refugee camp in eastern Kenya, 77.1% of 170 samples from persons with acute jaundice syndrome were positive for HEV IgM, RNA, or both (6). Data from earlier studies in Tanzania suggested either lack of exposure or low levels of HEV among women (7,8). A review of the epidemiology of HEV in Africa by Kim et al. (9) provides a listing of seroprevalence of HEV antibodies in various African countries.

Similar to HEV, HAV is transmitted by the fecal–oral route, although the epidemiology of the viruses is substantially different. Infection with HAV is considered a childhood disease in developing countries; nearly all children are infected at an early age. Disease tends to be mild in children and does not result in chronic infection (10). Unlike HAV and HEV, hepatitis B and C viruses (HBV and HCV) are transmitted through contact with infectious body fluids and can cause acute or chronic infection. Acute infection with HBV or HCV can manifest with a wide range of mild to severe symptoms. Chronic HBV and HCV infection can lead to serious outcomes such as cirrhosis, cancer, and failure of the liver (11,12). High HBV and HCV prevalence have been reported in southern Africa, where HIV prevalence is also high (13). HCV prevalence in Africa varies by country; estimates range from 1% to 10% (14). However, it is unclear whether HCV seroprevalence on the basis of antibody testing alone represents a true estimate because a high number of false-reactive results (compared to those for HCV RNA) have been reported in several HIV-prevalent populations.

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in Africa (15,16). The rate of chronic HBV carriers in sub-Saharan Africa is estimated to be >8% (17).

Previous studies have shown that HBV and HCV are prevalent in Malawi. Among patients in hospitals in Malawi, 17.5% tested positive for hepatitis B surface antigen (HBsAg), and samples from 4.5% were HCV antibody-positive (18). Among male sugar estate workers in Malawi, 14.9% tested positive for HBsAg, and samples from 10.6% were HCV antibody-positive (19).

Co-infection of HIV and HBV or HCV leads to accelerated progression of liver disease (13). The interaction of HEV with HIV has not yet been confirmed but is conceivable, and a strong association has been reported among adults in a retrospective study in Zambia in which 28% of HIV-seronegative and 71% of HIV-seropositive adults were found to be HEV seropositive (4). Superinfection with >1 type of hepatitis has been shown to cause severe disease. Children with simultaneous infection of HAV and HEV may experience accelerated disease progression. A study among persons who chronically carried the HBV surface antigen showed rapid clinical deterioration when co-infected with HEV (20). The high prevalence of HIV in Malawi, combined with the severe implications of co-infections with hepatitis viruses, necessitate clarification of the levels of multiple hepatitis virus infections within the same population. The primary aim of this study, conducted in 2012, was to determine the seroprevalence of HEV, as well as HAV, HBV, and HCV, in samples collected during 1989–2008 from diverse adult populations in Malawi.

Methods

Study Design and Populations

In a cross-sectional study, we analyzed serum and plasma samples collected during 6 studies in Malawi (Table 1) (21–26). These studies were conducted during 1989–2008 and represent diverse populations of HIV-infected and HIV-uninfected adult men and women from rural and urban settings in Malawi. Almost all HIV-infected persons in these studies were antiretroviral naive. Selection of samples stored at −80°C for laboratory testing was aimed to include approximately equal numbers of HIV-negative and HIV-positive samples from each year, on the basis of availability of sufficient sample volume, to test for all hepatitides. Inclusion of the samples was not based on previous knowledge of demographic factors of the study populations. In addition to collecting samples, we used structured case report forms to document demographic and clinical data.

All participants signed written informed consent and agreed to provide study samples. All studies were approved by appropriate institutional review boards in the United States and Malawi.

Laboratory Tests for Hepatitis Seroprevalence

HEV Testing

Serum or plasma samples were tested for the presence of antibodies against HEV by using Wantai HEV-IgG ELISA kits (Wantai Biologic Pharmacy Enterprise Co., Ltd., Beijing, China). The Wantai assay uses a recombinant capsid protein (E2) encoded by the conserved region of open reading frame 2 of HEV. Compared with the Genelabs HEV IgG EIA (Genelabs, Inc., Singapore), the Wantai assay has been shown to be more sensitive (27). The HEV assays were performed manually following the manufacturer’s instructions in the serology laboratory of the Department of Epidemiology at Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, USA.

Testing for Other Hepatitides

We used the same samples tested for HEV to additionally test for total antibodies (IgG and IgM) against HAV and HCV and for the presence of HBsAg by using commercially available kits from Bio-Rad Laboratories (Hercules, CA, USA). The HAV kits detect both acute and past infection; the HCV assays detect acute, past, and chronic infections; and the HBsAg assay detects acute and chronic infection with HBV. These tests were performed by using the automated EVOLIS microplate system from Bio-Rad at the Johns Hopkins Bloomberg School of Public Health in Baltimore. The Johns Hopkins School of Public Health serology laboratory is Clinical Laboratory Improvement Amendments–certified to perform virologic and serologic testing.

Because of operational constraints, all samples were tested once for each type of hepatitis and no duplicate testing was done. Quality control was performed with the inclusion of positive and negative controls in each assay. Results were considered positive when the optical density of a well was equal to or greater than the cutoff value calculated for each run of each assay as recommended by the manufacturer.

Statistical Analysis

Overall seropositivity for each virus was estimated and stratified by study year, HIV status, sex, and age category. Associations of the 4 hepatitides with selected exposure variables were assessed by using logistic regression. The covariates considered were education level, rural or urban residence, marital status, employment, running water in house, electricity in house, parity, multiple sexual partners, reported history of sexually transmitted infections, and condom use. Variables with a statistically significant association with a hepatitis seroprevalence in univariate analysis as well as variables considered epidemiologically important were included in the multivariate logistic.
regression to estimate adjusted odds ratios. Because of differences in follow-up periods and inconsistencies in availability of comparable data, we used baseline data for all covariates, regardless of whether an enrollment or follow-up sample was used for the hepatitis testing. A p value of \( \leq 0.05 \) was considered statistically significant. Analysis was performed by using Stata version 11.2 (StataCorp LLC, College Station, TX, USA).

Previous data on the prevalence of hepatitis E in Malawi was not available. To estimate an appropriate sample size for this analysis, a conservative prediction of 2% prevalence was used. A sample size of 800 was determined to enable detection of a prevalence of 2% with a precision of 1% (28).

Results
Table 2 shows the distribution of hepatitis seropositivity overall and across main exposure variables. Overall, 132 (16.5%) of 800 samples tested positive for HEV antibodies. The overall seroprevalence of HAV antibodies, HBsAg and HCV antibodies in samples collected from different populations in Malawi was 99.6%, 7.5%, and 7.1%, respectively (Table 2). HEV seroprevalence was higher among HIV-infected (20.2%) than among HIV-infected (12.9%) persons, and HCV seroprevalence was higher among male (10.2%) than female (5.6%) (p<0.05 by Fisher exact test) participants. Seroprevalence varied by study year and age group; seroprevalence of enteric HEV was 20.8% for a male occupational cohort in the 1994–1999 Sugar Company of Mulawi (SUCOMA) study (23), and 26.4% for an female urban cohort in the 2003–2005 metronidazole gel (METRO) study (25) in which efficacy of intravaginal metronidazole gel in reducing bacterial vaginosis was assessed. These overall differences by study year were statistically significant (p<0.001 by \( \chi^2 \) test).

Unlike HEV seroprevalence, the seroprevalence of the other enterically transmitted infection (HAV) did not show variation by year of study and was \( \approx 100\% \) in all studies. The prevalence of HBsAg and HCV antibodies was low (0% HBsAg; 2.9% HCV antibodies) in the 1989–1995 cohort of urban pregnant and postpartum women of the International Collaborations on AIDS Research (ICAR) study (21,22). In subsequent years, the reported prevalence of HBsAg and HCV antibodies increased and was approximately comparable in all studies reviewed for this study; exceptions are the high seroprevalence of both hepatitis in the men of the occupational cohort of SUCOMA and the high seroprevalence of HBsAg among women in the postexposure prophylaxis of infants (PEPI) cohort (2004–2009) (26). Stratification by age group (excluding those 60–69 years of age because of limited numbers) showed comparable HEV seroprevalence among persons <50 years of age (or trend of decrease with increase in age) and a seroprevalence of 33% among persons 50–59 years of age. The seroprevalence of HAV, however, was uniformly high and was \( \approx 100\% \) in all age groups. Both HBsAg and HCV seroprevalence were stable and comparable in age groups <50 (no. persons in older age groups was small). Among 757 persons with laboratory results available for both HBsAg and HCV, 7 were dually seropositive for HBsAg and HCV (0.9%; 95% CI 0.2–1.6; p = 0.11 by \( \chi^2 \) test); all 7 were HIV-uninfected.

Table 3 shows univariate (crude) and multivariate (adjusted) odds ratios (OR) of being seropositive for HEV. In the univariate analysis, participants in the SUCOMA (1994–1999) and METRO (2003–2005) studies were
significantly more likely to be positive for HEV antibodies compared with participants in the ICAR earlier study (1989–1994) (OR 2.80 and 3.82, p = 0.03 and 0.004, respectively). In the multivariate analysis, only the METRO study year (2003–2005) remained statistically significant after adjusting for other variables included in Table 3 (p = 0.01 for METRO study). Although not statistically significant in the multivariate analysis, participants who tested HIV-positive and those who reported having running water in the house were less likely to be seropositive for HEV antibodies in the univariate analyses (HIV-positive OR 0.59, p = 0.01; have running water OR 0.61, p = 0.06).

HAV seroprevalence was high, and the distribution was comparable across all variables and strata of each covariate; therefore, no associations of HAV with covariates included in this study were observed in both univariate and multivariate analyses. Table 4 shows the univariate, unadjusted ORs for HBsAg and HCV. Participants in the Nevirapine-Zidovudine (NVAZ) study were less likely to be positive for HBsAg than participants in the ICAR study (OR 0.30, p = 0.01 [21,22]). Residents of urban areas also had lower odds for testing positive for HBsAg compared with residents of rural areas (OR 0.57, p = 0.04), and female participants were less likely to test positive than male participants (OR 0.59, p = 0.05). A statistically nonsignificant trend of increasing odds of HBsAg over time on the basis of enrollment calendar year in the parent study is also shown in Table 4 (OR 1.07, p = 0.06). The univariate analysis of HCV association with the risk factors listed in Table 3 showed that female participants had statistically significantly lower odds of being seropositive for HCV compared with male

Table 2. Prevalence of anti-HEV IgG, total anti-HAV Ig, HBsAg, and total anti-HCV Ig in Malawi*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. positive/no. tested</th>
<th>% Positive (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cohort</strong></td>
<td>132/800</td>
<td>16.5 (13.9–19.1)</td>
</tr>
<tr>
<td><strong>HAV</strong></td>
<td>777/780</td>
<td>99.6 (99.2–100.0)</td>
</tr>
<tr>
<td><strong>HBsAg</strong></td>
<td>58/773</td>
<td>7.5 (5.6–9.4)</td>
</tr>
<tr>
<td><strong>HCV</strong></td>
<td>55/779</td>
<td>7.1 (5.3–8.9)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>HIV status</th>
<th>No. positive/no. tested</th>
<th>% Positive (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>80/397</td>
<td>20.2 (16.2–24.1)</td>
</tr>
<tr>
<td>Positive</td>
<td>52/403</td>
<td>12.9 (9.6–16.2)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. positive/no. tested</th>
<th>% Positive (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>45/246</td>
<td>18.3 (13.4–23.2)</td>
</tr>
<tr>
<td>F</td>
<td>87/554</td>
<td>15.7 (12.7–18.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study, period</th>
<th>No. positive/no. tested</th>
<th>% Positive (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAR, 1989–1995</td>
<td>6/70</td>
<td>8.6 (1.8–15.3)</td>
</tr>
<tr>
<td>NVAZ, 2000–2003</td>
<td>19/165</td>
<td>11.5 (6.6–16.4)</td>
</tr>
<tr>
<td>Mwanza, 2001–2005</td>
<td>13/100</td>
<td>13.0 (6.3–19.7)</td>
</tr>
<tr>
<td>Pepli, 2004–2009</td>
<td>18/139</td>
<td>12.9 (7.3–18.6)</td>
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<table>
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<tr>
<th>Age range, y</th>
<th>No. positive/no. tested</th>
<th>% Positive (95% CI)</th>
</tr>
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<td>15–19</td>
<td>15/77</td>
<td>19.5 (10.4–28.5)</td>
</tr>
<tr>
<td>20–29</td>
<td>72/439</td>
<td>16.4 (13.0–19.9)</td>
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<tr>
<td>30–39</td>
<td>28/191</td>
<td>14.7 (9.6–19.7)</td>
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<td>40–49</td>
<td>7/60</td>
<td>11.7 (3.3–20.0)</td>
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<tr>
<td>50–59</td>
<td>6/18</td>
<td>33.3 (9.2–57.5)</td>
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<tr>
<td>60–69</td>
<td>2/4</td>
<td>50.0 (–41.9 to 141.9)</td>
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</table>

<table>
<thead>
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<th>% Positive (95% CI)</th>
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<tr>
<td><strong>Total cohort</strong></td>
<td>132/800</td>
<td>16.5 (13.9–19.1)</td>
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<tr>
<td><strong>HAV</strong></td>
<td>777/780</td>
<td>99.6 (99.2–100.0)</td>
</tr>
<tr>
<td><strong>HBsAg</strong></td>
<td>58/773</td>
<td>7.5 (5.6–9.4)</td>
</tr>
<tr>
<td><strong>HCV</strong></td>
<td>55/779</td>
<td>7.1 (5.3–8.9)</td>
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</tbody>
</table>

*HEV, hepatitis E virus; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; ICAR, International Collaborations on AIDS Research; SUCOMA, a study of HIV prevalence among male workers for the Sugar Company of Malawi; NVAZ, a study of efficacy of nevirapine/zidovudine postexposure prophylaxis to prevent mother-to-child transmission of HIV; Mwanza, a study of risk factors associated with prevalent HIV infection in the rural town of Malawi; Metro, a study in which efficacy of intravaginal metronidazole gel in reducing bacterial vaginosis was assessed; Pepli, a study of antiretroviral postexposure prophylaxis to prevent mother to child transmission of HIV.
participants, and urban residents were less likely to test positive than rural residents (OR 0.52 and 0.51, p = 0.02 and 0.02, respectively). Although not statistically significant (p = 0.06), the ORs for participation in the SUCOMA study and having electricity in the house (an indicator of higher socioeconomic status) were 4.06 (95% CI 0.92–17.93) and 0.46 (95% CI 0.20–1.03), respectively. In the multivariate logistic regression analyses for the association of HBsAg and HCV with various covariates (study enrollment, HIV status, age, sex, whether there were multiple lifetime sexual partners, history of sexually transmitted diseases, and history of condom use), only enrollment in the NVAZ study (2000–2003) remained significantly associated with lower likelihood to test positive for HBsAg (adjusted OR 0.24, p = 0.006). No other variables were found to be associated with HBV or HCV seroprevalence.

**Discussion**

This study provides preliminary estimates of HEV seroprevalence in Malawi, which has a population of ≈12 million, is mostly rural, and has a limited safe water supply and constrained health care services (29). Overall, 16.5% (95% CI 13.9%–19.1%) of the samples were positive for HEV IgG. In addition to examining seroprevalence of HEV in Malawi, this study has several other notable features: 1) samples from 6 epidemiologic studies conducted by the same research team among adult men and women in urban and rural Malawi during 1989–2008 were included, representing diverse

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Crude OR</th>
<th>p value</th>
<th>Adjusted OR (95% CI)</th>
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<td>Study, period</td>
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<tr>
<td>ICAR, 1989–1995</td>
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<td>Reference</td>
</tr>
<tr>
<td>SUCOMA, 1994–1999</td>
<td></td>
<td></td>
<td>2.80 0.03 3.54 (0.75–16.76)</td>
</tr>
<tr>
<td>NVAZ, 2000–2003</td>
<td>1.39</td>
<td>0.51</td>
<td>1.59 (0.55–4.62)</td>
</tr>
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<td>MWANZA, 2001</td>
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<td>0.37</td>
<td>1.85 (0.51–6.70)</td>
</tr>
<tr>
<td>METRO, 2003–2005</td>
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<td></td>
<td>3.82 0.004 3.44 (1.37–8.67)</td>
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<tr>
<td>PEPI, 2004–2009</td>
<td>1.59</td>
<td>0.35</td>
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<td>Enrollment year†</td>
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<tr>
<td>HIV-negative</td>
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<td>HIV-positive</td>
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<td>0.01</td>
<td>0.72 (0.38–1.36)</td>
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<td></td>
<td></td>
<td>1.01 0.56 1.00 (0.97–1.02)</td>
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<tr>
<td>M</td>
<td></td>
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</tr>
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<td>F</td>
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<td>Running water in house</td>
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<tr>
<td>Employed</td>
<td>1.18</td>
<td>0.41</td>
<td>NA</td>
</tr>
<tr>
<td>Parity†</td>
<td>0.90</td>
<td>0.61</td>
<td>NA</td>
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</table>

*OR; odds ratio; ICAR, International Collaborations on AIDS Research; SUCOMA, a study of HIV prevalence among male workers for the Sugar Company of Malawi; NVAZ, a study of efficacy of nevirapine/zidovudine post-exposure prophylaxis to prevent mother-to-child transmission of HIV; MWANZA, a study of risk factors associated with prevalent HIV infection in the rural town of Malawi; METRO, a study in which efficacy of intravaginal metronidazole gel in reducing bacterial vaginosis was assessed; PEPI, a study of antiretroviral postexposure prophylaxis to prevent mother-to-child transmission of HIV; NA: not applicable.

†Continuous variable.
Seroprevalence of Hepatitis, Malawi

To understand the seroprevalence of hepatitis in Malawi populations and time periods; 2) analyses of viral hepatitis seroprevalence included both HIV-negative and HIV-positive persons (the prevalence of HIV remains high in southern Africa); and 3) these samples were also tested for total antibodies (IgG and IgM) against HAV and HCV and for the presence of HBsAg, leading to a comprehensive seroprevalence profile of all viral hepatitides in these populations.

Although some statistically significant differences were observed in univariate analyses for the association of selected risk factors with seroprevalence of HEV, none of these remained significant in the multivariate logistic regression analyses, including HIV status, age category, sex, or having access to running water in the household. Recent studies have also found no differences in infection

Table 4. Crude odds ratios for the association of HBV and HCV with selected exposures in studies of hepatitis prevalence, Malawi, 1989–2009*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HBsAg</th>
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<th>CVC</th>
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<td>OR</td>
<td>p value</td>
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<tr>
<td>ICAR, 1989–1995</td>
<td>Reference</td>
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<td>Reference</td>
<td>NA</td>
</tr>
<tr>
<td>SUCOMA, 1994–1999</td>
<td>0.99</td>
<td>0.97</td>
<td>4.06</td>
<td>0.06</td>
</tr>
<tr>
<td>NVAZ, 2000–2003</td>
<td>0.50</td>
<td>0.01</td>
<td>1.94</td>
<td>0.41</td>
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<td>MWANZA, 2001</td>
<td>0.57</td>
<td>0.23</td>
<td>3.40</td>
<td>0.13</td>
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<td>METRO, 2003–2005</td>
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<td>0.08</td>
<td>2.23</td>
<td>0.31</td>
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<td>PEPI, 2004–2009</td>
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<td>ND</td>
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<td>0.36</td>
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<tr>
<td>Enrollment year, continuous</td>
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<td>0.06</td>
<td>1.00</td>
<td>0.96</td>
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<td>0.43</td>
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<td>1.00</td>
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<td>1.19</td>
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<td>Ever</td>
<td>0.75</td>
<td>0.38</td>
<td>0.95</td>
<td>0.88</td>
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</tbody>
</table>

*HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; OR, odds ratio; ICAR, International Collaborations on AIDS Research; SUCOMA, a study of HIV prevalence among male workers for the Sugar Company of Malawi; NVAZ, a study of efficacy of nevirapine/zidovudine post-exposure prophylaxis to prevent mother-to-child transmission of HIV; MWANZA, a study of risk factors associated with prevalent HIV infection in the rural town of Malawi; METRO, a study in which efficacy of intravaginal metronidazole gel in reducing bacterial vaginosis was assessed; PEPI, a study of antiretroviral postexposure prophylaxis to prevent mother to child transmission of HIV; NA, not applicable; ND, no data; STI, sexually transmitted infection.
rates by gender or HIV status (1,30). The current study did not include children, which likely explains why previously identified trends of increasing infection levels with age were not detected. We also did not find a trend of seroprevalence over time. The only risk factor substantially associated with HEV seroprevalence was the METRO study period, 2003–2005, as compared to the ICAR study, which was conducted during 1989–1995. Both studies were conducted among women in Blantyre, Malawi.

It is unclear what may have caused the METRO study participants to have higher levels of HEV seroprevalence. We did not collect data on seasonality and source of water and food; over the years Malawi had fluctuating bouts of drought and food scarcity. We tested diverse study populations from rural and urban settings recruited over a period of nearly 20 years, but no clear major trends were observed.

As expected, HAV seroprevalence was nearly universal in this sample of adults, with an overall prevalence of ≈100%. In low-resource settings such as Malawi, HAV is a childhood infection, and nearly all persons are infected within the first few years of life. Though children were not included in our study, it should be considered that they can be exposed to HEV because of the high prevalence among adults we observed. Although HAV and HEV infection tend to cause mild disease independently, concurrent infections with these pathogens in children may lead to accelerated disease progression (20). The changing epidemiology of HAV in some settings related to rapid industrialization and urban migration in developing countries may result in some children not being exposed to HAV (10).

An overall prevalence of 7.5% (95% CI 5.6%–9.4%) was found for HBsAg and 7.1% (95% CI 5.3%–8.9%) for HCV. The results of the HBsAg and HCV seroprevalence tests are consistent with previously published data from sub-Saharan Africa. Specifically in Malawi, a study published in 2002 reported a prevalence of 14.9% for HBsAg and 10.6% for HCV among the SUCOMA participants (23). In our study, we found a prevalence of 11.8% (95% CI 7.0%–16.6%) and 10.7% (95% CI 6.1%–15.3%) for HBsAg and HCV, respectively, in the SUCOMA study samples. Women who enrolled in NVAZ had lower odds of testing positive for HBsAg compared to those in ICAR. The only distinct difference between these studies was the HIV status of the women (some women in the ICAR study were HIV negative), but because this was included in the multivariate logistic regression, results should not be confounded by the HIV status. No covariates were found to be associated with HCV seroprevalence in this study.

HEV epidemiology is evolving, and circulating genotypes and modes of transmission appear to be complex in both developing and industrialized countries (7). We do not know in Malawi if only waterborne HEV genotypes are the source of potential infections or if other less virulent zoonotic HEV genotypes coexist. High seroprevalence of HEV antibodies does not imply clinical infection or increased association with clinical complications. Nonetheless, some misclassification of acute viral hepatitis is likely in Malawi where screening for HEV antibodies is not performed. Because the seroprevalence of HEV in this study was twice that of HBV or HCV, cases of acute hepatitis may frequently be caused by HEV as opposed to HBV or HCV. Notably, 15.7% (95% CI 12.7%–18.7%) of women in this study cohort had antibodies against HEV, which can pose serious health risks for pregnant women (3).

Although no association was found that HIV-positive persons are at higher risk for anti-HEV than are HIV-negative persons, the finding that 12.9% (95% CI 9.6%–16.2%) of samples from HIV-positive persons were HEV positive is of concern. Malawi and other countries in sub-Saharan Africa have high levels of HIV infection, and co-infection with HEV may lead to chronic HEV infection and accelerated disease progression.

The findings from this study should be regarded as preliminary and require confirmation. Therefore, additional epidemiologic and virological studies should be conducted in this region. As with all cross-sectional data, inferences regarding associations should be interpreted with caution. A related limitation is that the samples used for serologic testing in this study included both enrollment and follow-up samples (in cohort studies) to maximize availability of samples, whereas the covariate data used for all participants was baseline enrollment data. Despite these limitations, we suspect that many of the covariates used, such as having running water in the house, did not substantially change over time. Very few risk factors associated with HEV, HBV, or HCV seroprevalence were found. This result may be because of differences in population characteristics or definitions used in multiple studies. For example, participants in the SUCOMA study were all men working in a rural sugar estate occupational setting and may be considered to be at high risk (23). We also did not have data on some behaviors associated with HCV seroprevalence, such as intravenous drug use, although the practice is very rare in Malawi. The lack of association between viral hepatitis and various risk factors conventionally collected in these studies suggests that better data collection tools to evaluate potential risk factors and different study designs targeting at risk populations may need to be considered in future studies.

Confirmation of the hepatitis testing results, particularly HEV seroprevalence, reported in this study will be critical in subsequent studies because each sample was tested once. Retesting of a subset of positive and negative samples should ideally be done with the same Wantai assay used in this study as well as with other assays. The hepatitis
A, B, and C assays used were commercially available in the United States and well validated, but it is recommended that samples be tested in duplicate for these assays. The consistency of our results with findings from previous seroprevalence studies in Malawi and other countries suggests misclassification may be minimal (taking into account the controversy regarding HCV testing). Molecular analysis to determine what genotypes of HEV are prevalent in Malawi may also be useful and would provide further insight into the epidemiology of this virus.

Acknowledgments
We thank the Wantai Biological Pharmacy Enterprise Co., Ltd., for donating the HEV IgG ELISA kits for this research. We also thank the technologists in H.F.’s laboratory at the Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA, for assistance in performing the hepatitis assays.

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References
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Chronic Q Fever Diagnosis—Consensus Guideline versus Expert Opinion

Linda M. Kampschreur, Marjolijn C.A. Wegdam-Blans, Peter C. Wever, Nicole H.M. Renders, Corine E. Delsing, Tom Sprong, Marjo E.E. van Kasteren, Henk Bijlmer, Daan Notermans, Jan Jelrik Oosterheert, Frans S. Stals, Marrigje H. Naboors-Franssen, Chantal P. Bleecker-Rovers, on behalf of the Dutch Q Fever Consensus Group

Chronic Q fever, caused by Coxiella burnetii, has high mortality and morbidity rates if left untreated. Controversy about the diagnosis of this complex disease has emerged recently. We applied the guideline from the Dutch Q Fever Consensus Group and a set of diagnostic criteria proposed by Didier Raoult to all 284 chronic Q fever patients included in the Dutch National Chronic Q Fever Database during 2006–2012. Of the patients who had proven cases of chronic Q fever by the Dutch guideline, 46 (30.5%) would not have received a diagnosis by the alternative criteria designed by Raoult and, 14 (4.9%) would have been considered to have possible chronic Q fever. Six patients with proven chronic Q fever died of related causes. Until results from future studies are available, by which current guidelines can be modified, we believe that the Dutch literature-based consensus guideline is more sensitive and easier to use in clinical practice.

Coxiella burnetii is the causative agent of Q fever, a zoonosis occurring worldwide (1). Recently, a large epidemic occurred in the Netherlands with >4,000 cases of acute Q fever notified from 2007 through 2010 (2,3). Chronic Q fever develops in an estimated 1%–5% of all infected humans and can become manifest even years after primary infection (1,4). Endocarditis and infection in aneurysms or vascular prostheses are the most common manifestations (1,5,6). Untreated chronic Q fever has a poor prognosis, with a reported mortality rate of up to 60% (1,7). Adequate antibiotic treatment reduces the mortality rate for Q fever endocarditis to <5% (7). Treatment preferably consists of a combination of doxycycline and hydroxychloroquine for at least 18 months (nonprosthetic infection) to 24 months (prosthetic infection) and is recommended to be continued in case of unfavorable clinical or serologic response (7,8). Antibiotic guidelines for vascular chronic Q fever are not yet available, but antibiotic regimes for Q fever endocarditis have been applied to this disease entity as well. Early surgical intervention, with removal of infected material, might improve the prognosis of vascular chronic Q fever (6,9).

In the early course of chronic Q fever, most patients are asymptomatic or experience nonspecific symptoms such as low-grade fever, night sweats, and weight loss (1,4,6,7). Endocarditis, findings on echocardiograph are often nonspecific or absent, which makes the diagnosis of chronic Q fever challenging (7). A PCR positive for C. burnetii or culture of the organism in blood or tissue, in the absence of acute Q fever, is a strong indicator for chronic Q fever. However, sensitivity on blood samples is only 50%–60% for both PCR and culture in patients with chronic Q fever (10,11). Therefore, serologic testing is also valuable for the diagnosis of chronic Q fever. A phase I IgG cutoff titer of 1:800, which is based on an in-house–developed immunofluorescence assay (IFA), has been internationally accepted for the diagnosis of chronic Q fever and is included in the modified Duke criteria for diagnosis of endocarditis (12,13). In the Netherlands, a commercial IFA (Focus Diagnostics, Inc., Cypress, CA, USA) is primarily used, with a proposed IgG cutoff value of 1:1,024 for chronic Q fever (14). Yet, recent studies show that serology results alone are not sufficient for the diagnosis of chronic Q fever, but that they should be combined with clinical data (15).

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POLICY REVIEW

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Dutch Consensus Guideline

Faced with a large Q fever outbreak in the Netherlands and a rising number of (presumed) chronic Q fever patients, we were not able to find answers to all our questions about this complex disease in the literature. Moreover, randomized trials on diagnosis and treatment of this disease were lacking, and available data were not all applicable to the Dutch situation. For example, we found far more vascular localizations of chronic Q fever, with often severe complications, than had been described previously. Therefore, the Dutch Q Fever Consensus Group was initiated in 2010, in which diagnosis and subsequent treatment consequences for suspected chronic Q fever were discussed. We performed a thorough literature review and constructed a new guideline for the diagnosis of chronic Q fever, differentiating between proven, probable, and possible chronic Q fever (Table 1). We added advice for treatment and follow-up regimes for these 3 groups of patients. Antibiotic treatment and, if indicated, surgical treatment are recommended for all patients with proven chronic Q fever. The decision to start antibiotic treatment in patients with probable chronic Q fever depends on clinical characteristics and the condition of the patient, and should be determined by a multidisciplinary team. For possible chronic Q fever patients, antibiotic treatment should not be initiated, but follow-up is indicated.

After the Dutch consensus guideline was reported (14), a reaction by French researcher Didier Raoult was published; he did not agree with this proposed guideline and formulated alternative diagnostic criteria on the basis of his expert opinion (Table 2) (16). Professor Raoult is the undisputed leading authority on Q fever, and his opinion and the scientific publications from his research group should be considered by anyone working in the field of Q fever. Here, we attempt to resolve these differences of opinion by applying both criteria to cases from the Dutch National Chronic Q Fever Database.

Dutch Consensus Guideline versus Expert Opinion Guideline

A critical difference in the diagnostic criteria proposed by Raoult and those of the Dutch Q Fever Consensus Group is the diagnostic value attributed to C. burnetii PCR positivity of blood samples. Because we are unaware of clinical entities, other than acute and chronic Q fever, for which a PCR positive for C. burnetii in blood would be exhibited, we believe that positive blood PCR findings, in the absence of acute Q fever, prove chronic Q fever. The alternative criteria, on the other hand, state that a positive PCR finding in blood should be accompanied by a clear endocarditis focus shown on echocardiograph, a clear vascular focus on imaging studies, or at least 2 or 3 “minor criteria” (Table 2). Moreover, the alternative criteria attribute great value to the phase I IgG titer, proposing a phase I IgG ≥1:6,400 as a major criterion for Q fever endocarditis and Q fever vascular infection, in contrast to a phase I IgG ≥1:800 and <1:6,400 proposed as a minor criterion. This proposal contradicts the internationally accepted modified Duke criteria, which state that a phase I IgG ≥1:800 is a major criterion for infective (Q fever) endocarditis (13).

The alternative criteria also generally oppose the term chronic Q fever but makes a distinction in 2 manifestations: Q fever endocarditis and Q fever vascular infection. More

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### Table 1. Dutch consensus guideline on chronic Q fever diagnostics*

<table>
<thead>
<tr>
<th></th>
<th>Proven chronic Q fever</th>
<th>Probable chronic Q fever</th>
<th>Possible chronic Q fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Positive C. burnetii PCR of blood or tissue†</td>
<td>IFA ≥1:1,024 for C. burnetii phase I IgG‡</td>
<td>IFA ≥1:1,024 for C. burnetii phase I IgG‡ without manifestations meeting the criteria for proven or probable chronic Q fever</td>
</tr>
<tr>
<td>2.</td>
<td>IFA ≥1:800 or 1:1,024 for C. burnetii phase I IgG‡ AND Definite endocarditis according to the modified Duke criteria (13) OR Proven large vessel or prosthetic infection by imaging studies (FDG-PET, CT, MRI, or AUS)</td>
<td>Valvulopathy not meeting the major criteria of the modified Duke criteria (13) Known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by means of TEE/ TTE, FDG-PET, CT, MRI, or AUS Suspected osteomyelitis or hepatitis as manifestation of chronic Q fever Pregnancy Symptoms and signs of chronic infection, such as fever, weight loss and night sweats, hepato-splenomegaly, persistent raised ESR and CRP Granulomatous tissue inflammation, proven by histological examination Immunocompromised state</td>
<td></td>
</tr>
</tbody>
</table>

*Source: (14). IFA, immunofluorescence assay; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography; FDG-PET, fluorodeoxyglucose positron emission tomography; CT, computed tomography; MRI, magnetic resonance imaging; AUS, abdominal ultrasound; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
†In the absence of acute infection.
‡Cut-off depends on the IFA technique used, whether in-house developed or commercial.
Table 2. Diagnostic guideline for chronic Q fever proposed by Raoult*

<table>
<thead>
<tr>
<th>Q fever endocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Definite criteria</strong></td>
</tr>
<tr>
<td>Positive culture, PCR, or immunochemistry of a cardiac valve</td>
</tr>
<tr>
<td><strong>B. Major criteria</strong></td>
</tr>
<tr>
<td>Microbiology: positive culture or PCR of the blood or an emboli or serology with IgG I antibodies ≥6,400</td>
</tr>
<tr>
<td>Evidence of endocardial involvement:</td>
</tr>
<tr>
<td>Echocardiogram positive for IE: oscillating intra-cardiac mass on valve or supporting structure, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; or abscess; or new partial dehiscence of prosthetic valve; or new valvular regurgitation (worsening or changing of pre-existing murmur not sufficient)</td>
</tr>
<tr>
<td>PET scan showing a specific valve fixation and mycotic aneurysm</td>
</tr>
<tr>
<td><strong>C. Minor criteria</strong></td>
</tr>
<tr>
<td>Predisposing heart condition (known or found on echocardiograph)</td>
</tr>
<tr>
<td>Fever, temperature &gt;38°C</td>
</tr>
<tr>
<td>Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm (see at PET scan), intracranial hemorrhage, conjunctival hemorrhages, and Janeway lesions</td>
</tr>
<tr>
<td>Immunologic phenomena: glomerulonephritis, Osler nodes, Roth spots, or rheumatoid factor</td>
</tr>
<tr>
<td>Serologic evidence: IgG I antibodies ≥800 &lt;6,400</td>
</tr>
</tbody>
</table>

**Diagnosis definite**

1. 1A criterion
2. 2B criterion
3. 1B, and 3C criterion

**Diagnosis possible**

1. 1B criterion, 2C criteria (including microbiology evidence, and cardiac predisposition)
2. 3C criteria (including positive serology, and cardiac predisposition)

<table>
<thead>
<tr>
<th>Q fever vascular infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Definite criteria</strong></td>
</tr>
<tr>
<td>Positive culture, PCR, or immunochemistry of an arterial sample (prosthesis or aneurysm) or a periarterial abscess or a spondylodiscitis linked to aorta</td>
</tr>
<tr>
<td><strong>B. Major criteria</strong></td>
</tr>
<tr>
<td>Microbiology: positive culture or PCR of the blood or an emboli or serology with IgG I antibodies ≥6,400</td>
</tr>
<tr>
<td>Evidence of vascular involvement</td>
</tr>
<tr>
<td>CT scan: aneurysm or vascular prosthesis + periarterial abscess, fistula, or spondylodiscitis</td>
</tr>
<tr>
<td>PET scan: specific fixation on an aneurysm or vascular prosthesis</td>
</tr>
<tr>
<td><strong>C. Minor criteria</strong></td>
</tr>
<tr>
<td>Serological IgG I ≥800 &lt;6,400</td>
</tr>
<tr>
<td>Fever, temperature &gt;38°C</td>
</tr>
<tr>
<td>Emboli</td>
</tr>
<tr>
<td>Underlying vascular predisposition (aneurysm or vascular prosthesis)</td>
</tr>
</tbody>
</table>

**Diagnosis definite**

1. 1A criterion
2. 2B criterion
3. 1B and 2C criterion (including microbiology findings and vascular predisposition)

**Diagnosis possible**

Vascular predisposition, serological evidence and fever or emboli

*Source: (16). IE, infective endocarditis; PET, positron emission tomography; IFA, immunofluorescence assay; CT, computed tomography.

Chronic Q Fever Diagnosis

Rare manifestations, such as pericarditis, hepatitis, and osteomyelitis, are left undefined, however.

After the recent outbreak of Q fever in the Netherlands, we initiated the Dutch National Chronic Q Fever Database, a joint effort by multiple hospitals in areas affected by Q fever, to monitor all chronic Q fever cases in the Netherlands. All hospitals with chronic Q fever patients, also outside the notified Q fever–epidemic areas, were actively approached. Design of the database and use of the collected information for analysis and scientific publications were approved by the Medical Research Ethics Committee of the University Medical Center Utrecht in Utrecht, the Netherlands. Part of these data had previously been published in a report discussing serologic profiles of patients with chronic Q fever (15).

Until the end of May 2012, a total of 284 patients had been included in our database (although the epidemic started in 2009, all patients from 2006 on are included): 151 patients (53.2%) had proven chronic Q fever, 64 patients (22.5%) had probable chronic Q fever, and 69 patients (24.3%) had possible chronic Q fever, according to the Dutch consensus guideline. We reevaluated these chronic Q fever cases with the alternative diagnostic criteria (Table 3). Of the case-patients with proven chronic Q fever according to the Dutch guideline, 46 (30.5%) would have been left undiagnosed with the alternative criteria. For cases of probable chronic Q fever, 58 cases (90.6%) would have been undiagnosed, and for possible cases of chronic Q fever, all 69 cases.

The conditions of 8 patients with proven chronic Q fever (based on PCR positivity for *C. burnetii* in blood and suspicion of endocarditis) would have been diagnosed with possible Q fever endocarditis only by the alternative criteria (Table 4). Eighteen patients with proven chronic Q fever would not have been diagnosed with Q fever endocarditis at all, because echocardiography results did not match any major clinical Duke criterion, as is often observed in cases of Q fever endocarditis (7). Of the 8 patients with proven chronic Q fever endocarditis (by Dutch consensus guideline) who had been given a diagnosis of possible endocarditis according to the alternative criteria, 2 patients would have been considered to have definite endocarditis by the modified Duke criteria (13).

Twenty-four patients with a vascular *C. burnetii* infection (Dutch consensus guideline) would not have been diagnosed with chronic Q fever by using the alternative criteria (Table 4). Seventeen of these patients had a positive vascular lesion on fluorodeoxyglucose–positron emission tomography/computed tomography (FDG-PET/CT) with phase I IgG ≥1:800 and <1:6,400. Seven patients had a PCR of blood positive for *C. burnetii*, in combination with an aneurysm or vascular prosthesis but no signs of infection on FDG-PET/CT. According to the Dutch consensus
guideline, there were 5 patients with proven chronic Q fever with no known focus and 2 patients with Q fever with a focus other than endocarditis or vascular infection who would have been missed by using the alternative criteria. Five (repeatedly) had a positive C. burnetii PCR of blood but no clear infectious focus on echocardiograph and FDG-PET/CT scan. One patient had a positive PCR in blood with clinical pericarditis, and 1 patient had a positive PCR in blood during pregnancy with phase I IgG >1:1,024 and a positive PCR of placental tissue.

Notably, 10 patients with cases of proven chronic Q fever that were not diagnosed as definite chronic Q fever by the alternative criteria died (2 with possible chronic Q fever and 8 without chronic Q fever according to the alternative guideline). Six of these patients died due to clear chronic Q fever–related manifestations (2 with possible chronic Q fever and 4 without chronic Q fever according to the alternative guideline). The 2 patients with possible chronic Q fever died of complications caused by endocarditis, one had a double-pathogen endocarditis with Staphylococcus aureus. Two of the 4 patients without chronic Q fever according to the alternative guideline died due to aortoduodenal fistula, both with a phase I IgG >1:1024, but <1:6400, negative PCR on blood, and a clear FDG-positive vascular focus on PET/CT. In 1 of these 2 patients, Q fever vascular infection was confirmed postmortem with a positive PCR of the abdominal aortic aneurysm. No autopsy was performed on the other patient, unfortunately. The third patient, who had a history of a biologic heart valve replacement, an FDG-PET/CT negative aortic aneurysm, and a positive C. burnetii PCR of blood, eventually died of heart failure. Postmortem analysis demonstrated that PCR of the heart valve confirmed C. burnetii infection and thus Q fever endocarditis. Another chronic Q fever patient with positive PCR results of blood and minor valve lesions, according to the Duke criteria, died of gastrointestinal bleeding, probably due to aorto-intestinal fistula.

Conclusions
Several major differences exist between the Dutch consensus guideline for the diagnosis of chronic Q fever and the alternative criteria. These alternative criteria define only Q fever endocarditis and Q fever vascular infection and oppose the term chronic Q fever. However, this distinction

### Table 3. Comparison of chronic Q fever diagnosis according to the Dutch consensus guideline* and the alternative criteria†

<table>
<thead>
<tr>
<th>Alternative criteria</th>
<th>Dutch consensus chronic Q fever guideline</th>
<th>n = 151</th>
<th>Probable, no. (%), n = 64</th>
<th>Possible, no. (%), n = 69</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite Q fever endocarditis</td>
<td>21 (13.9)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Possible Q fever endocarditis</td>
<td>8 (5.3)</td>
<td>4 (6.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Definite Q fever vascular infection</td>
<td>76 (50.3)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Possible Q fever vascular infection</td>
<td>0</td>
<td>2 (3.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No diagnosis of chronic Q fever</td>
<td>46 (30.5)</td>
<td>58 (90.6)</td>
<td>69 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Source: (14).
†Source: (16).

### Table 4. Characteristics and outcome of patients diagnosed with chronic Q fever using the Dutch consensus guideline* but without (definite) chronic Q fever according to alternative criteria†

<table>
<thead>
<tr>
<th>Dutch consensus guideline</th>
<th>Possible Q fever endocarditis or vascular infection, no. (%), n =14</th>
<th>No diagnosis, no. (%), n = 173</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proven Q fever</td>
<td>8 (57.1)</td>
<td>46 (26.8)</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>8 (57.1)</td>
<td>18 (10.4)</td>
</tr>
<tr>
<td>PCR positive for Coxiella burnetii in blood</td>
<td>6 (42.9)</td>
<td>18 (10.4)</td>
</tr>
<tr>
<td>Evidence of endocardial involvement</td>
<td>2 (14.3)</td>
<td>0</td>
</tr>
<tr>
<td>Vascular infection</td>
<td>0</td>
<td>24 (13.9)‡</td>
</tr>
<tr>
<td>PCR positive in blood</td>
<td>0</td>
<td>7 (4.0)</td>
</tr>
<tr>
<td>Vascular focus on imaging</td>
<td>0</td>
<td>17 (9.8)</td>
</tr>
<tr>
<td>Other or no focus§</td>
<td>0</td>
<td>7 (4.1)</td>
</tr>
<tr>
<td>Deceased</td>
<td>2 (14.3)</td>
<td>8 (4.6)</td>
</tr>
<tr>
<td>Death probably due to Q fever</td>
<td>2 (14.3)</td>
<td>4 (2.3)¶</td>
</tr>
<tr>
<td>Probable Q fever</td>
<td>6 (42.9)</td>
<td>58 (33.5)</td>
</tr>
<tr>
<td>Vascular infection</td>
<td>4 (28.6)</td>
<td>22 (12.7)</td>
</tr>
<tr>
<td>Other or no focus</td>
<td>2 (14.3)</td>
<td>16 (9.3)</td>
</tr>
<tr>
<td>Deceased</td>
<td>0</td>
<td>20 (11.6)</td>
</tr>
<tr>
<td>Death probably due to Q fever</td>
<td>2 (14.3)</td>
<td>4 (2.3)</td>
</tr>
<tr>
<td>Possible Q fever</td>
<td>1 (7.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Source: (14).
†Source: (16).
‡In 3 patients with proven chronic Q fever, imaging studies showed that the focus of infection was in both the heart valves and the vascular structures.
§All were PCR positive.
¶For 2 patients, PCR of vascular and heart valve tissue obtained at autopsy was positive for C. burnetii.
is not accompanied by therapeutic consequences for each of these manifestations, which we believe makes these guidelines less practical.

It must be acknowledged that, because all patients included in our study met the Dutch criteria for proven, probable, or possible chronic Q fever, other guidelines can only perform with less accuracy in comparison. Nevertheless, sensitivity of the Dutch guideline is markedly higher than with the alternative criteria: ≈31% of proven chronic Q fever case-patients would have been missed as well as almost all patients with probable and possible cases, including at least 4 patients who eventually died of chronic Q fever related causes. Specificity of the Dutch consensus guideline is probably lower than that of the alternative criteria, but because mortality and morbidity rates are high when chronic Q fever cases are untreated, we believe sensitivity is of greater importance in clinical practice. Our data illustrate that, when proven cases of chronic Q fever are missed, patients are therefore not adequately treated, these patients are at high risk for severe complications and death.

As stated before, the most critical difference between the criteria of the Dutch guideline and those of the alternative guideline is the acknowledgment of a positive C. burnetii PCR as a marker of proven chronic Q fever in the absence of acute Q fever. Of course, this difference should be interpreted with care. In our opinion, patients without endocarditis or vascular infection on imaging studies but with a positive PCR in blood should also be treated for chronic Q fever, as they may suffer from not yet clinically visible endocarditis or vascular infection, which was confirmed by the postmortem results of 2 of our patients described above. A single positive C. burnetii PCR of blood is highly suggestive for chronic Q fever when acute Q fever is excluded. A PCR test will not be performed in patients without symptoms and without any risk factors for chronic Q fever, so cases in whom a positive PCR is the only factor indicating chronic Q fever is a theoretical consideration. We have observed few patients, in the absence of signs of acute Q fever, with elevated phase I IgG titers not fulfilling the serologic criteria of chronic Q fever (phase I IgG ≥1:800 or 1,024) but with a positive C. burnetii PCR of blood or tissue. In these cases, we are convinced that PCR-positivity proves chronic Q fever. No patients in our chronic Q fever database who had a positive PCR on blood or tissue had a phase I IgG titer of ≤1:256.

We agree with the statement that proven chronic Q fever will not develop in some patients with probable chronic Q fever and in most patients with possible chronic Q fever. We therefore do not advocate treating all of these patients with long-term antibacterial drugs. Nevertheless, we do think that these patients should all be examined for a chronic Q fever focus and should continue to be monitored closely, at least until further research offers more clarity regarding the prognosis of these patients. If these patients do not receive a diagnosis of possible or probable chronic Q fever, they might not receive such close follow-up. Moreover, the Dutch consensus guideline is easier to use, adds treatment advice, and also applies to patients with chronic Q fever manifestations that are rarer than endocarditis and vascular infection.

We hope that, with the future results from the Dutch National Chronic Q Fever Database and joint efforts of international researchers and experts in the field of Q fever, these guidelines can be modified to provide definite evidence-based criteria for diagnosis and treatment of this complex disease. In the meantime, the Dutch consensus guideline created on the basis of the scarce available literature is, in our opinion, safer and easier to use in clinical practice than the alternative expert-based criteria.

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We thank all participants in the Dutch National Chronic Q Fever Database in the Netherlands: Monique de Jager-Leclercq (Bernhoven Hospital, Oss), Cornelis A.R. Groot (Bernhoven Hospital), Yvonne Soethoudt (Elkerliek Hospital, Helmond), Sybrandus N. Blank (Maxima Medical Center, Eindhoven/Veldhoven), Marjolijn J.H. Pronk (Catharina Hospital, Eindhoven), Gijis J. Limonard (Diakonessenhuis, Utrecht), Steven F. Thijsen (Diakonessenhuis), Bart J. Vlaminckx (St Antonius Hospital, Nieuwegein), Jacqueline Buijs (Atrium Medical Center, Heerlen), Bas J.M van Kraaij (Atrium Medical Center), Frederika Dijkstra (Centre for Infectious Disease Control, Bilthoven), Clemens Richter (Rijnstate Hospital, Arnhem), E.H. Gisolf (Rijnstate Hospital, Arnhem), Rik Heijligenberg (Gelderse Vallei Hospital, Ede), Ries Schouten (Gelderse Vallei Hospital), Karin Schurink (Erasmus University Medical Center, Rotterdam), Leo G. Visser (Leiden University Medical Center, Leiden).

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November 2014: Foodborne Infections

Including:

- Blastomycosis Mortality Rates, United States, 1990–2010
- Death Patterns during the 1918 Influenza Pandemic in Chile
- Genomic Definition of Hypervirulent and Multidrug-Resistant Klebsiella pneumoniae Clonal Groups
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- Drug-Resistant Candida glabrata Infection in Cancer Patients
- Novel Chlamydia trachomatis Strains in Heterosexual Sex Partners, Indianapolis, Indiana, USA

Swine Influenza A(H3N2) Virus Infection in Immunocompromised Man, Italy, 2014

Antonio Piralla, Ana Moreno, Maria Ester Orlandi, Elena Percivalle, Chiara Chiapponi, Fausto Vezzoli, Fausto Baldanti, and the Influenza Surveillance Study Group

Because swine influenza virus infection is seldom diagnosed in humans, its frequency might be underestimated. We report a immunocompromised hematologic patient with swine influenza A(H3N2) virus in 2014 in Italy. Local pigs were the source of this human infection.

Pigs are considered the “mixing vessel” in which avian, human, and swine influenza genetic material can be exchanged and result in new influenza viruses (1). Zoonotic influenza A infections in humans caused by swine influenza viruses (SIVs) have been infrequently reported in Europe (1,2), even though at least 19% of occupationally exposed humans, such as pig farmers, slaughterers, and veterinarians, have SIV antibodies (3). However, because the infection is clinically mild in most cases, its frequency might be underdiagnosed in humans (4).

Three influenza A subtypes (H1N1, H1N2, and H3N2) circulate in swine herds in Italy (1). We report a European swine A(H3N2) influenza virus that occurred in an immunocompromised man in Italy in 2014.

The Study

On January 14, 2014, a 67-year-old man with multiple myeloma underwent the eighth cycle of chemotherapy at the Hematology Unit of the Fondazione Istituto Di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo in Pavia, Italy. The patient had mild upper respiratory syndrome (fever, cough, and cold). A nasal swab sample was tested by real-time reverse transcription PCR (RT-PCR) and PCR with a panel for 17 respiratory viruses (5,6). The clinical specimen was positive for influenza A (6 × 10^6 RNA copies/mL). However, attempts to subtype the strain by using real-time RT-PCR specific for human influenza subtypes H1 and H3, as well as avian influenza subtype H7N9, were unsuccessful.

The clinical sample was inoculated onto a mixed-cell (Mv1Lu and A549 cells) monolayer. After 48 h incubation, it scored positive using a monoclonal antibody specific for influenza A/H3 antigen (Millipore, Billerica, MA, USA).

On January 24, 2014, the influenza virus strain A/Pavia/07/2014 was recovered from the supernatant propagated in MDCK cell culture. An RT-PCR that amplifies all 8 segments of the influenza A genome was then conducted (7). The purified amplicons were sequenced by using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). We BLAST searched the sequences obtained for closely related sequences in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Partial nucleotide sequences of polymerase, nucleoprotein, hemagglutinin (HA), neuraminidase (NA), matrix, and nonstructural genes showed swine influenza A(H3N2) virus with an internal gene belonging to the European SIV lineage.

At a second control visit, on January 29, 2014, the patient’s nasal swab sample was negative for respiratory viruses, but cough and cold persisted. No vaccination or antiviral treatment was administered to the patient before or during the influenza episode. During January 2014, he had spent several weeks visiting his relatives on a pig farm in the province of Lodi (northern Italy) and reported contact with pigs, as well as with his grandson. No respiratory symptoms had developed in any of his family members (owners of the pig farm) or in farm co-workers.

On February 6, 2014, the A/Pavia/07/2014 strain was propagated in embryonated specific pathogen–free chicken eggs at the Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna (Brescia, Italy). The sequences of complete genome segments were obtained with the MiSeq platform (Illumina, San Diego, CA, USA) as previously described (8). The data were de novo assembled on BaseSpace Cloud (Illumina) with the DNASTar application and analyzed with the Lasergene package software (version 10.1.2). We conducted phylogenetic analysis online using PhyML v.3.0 (9) and MEGA5 software (10). A phylogenetic tree of the HA and NA genes confirmed that the A/Pavia/07/2014 strain was closely related to European A(H3N2) SIV (Figure 1). In addition, phylogenetic trees constructed with sequences of the polymerase base

1 Members of the Influenza Surveillance Study Group who contributed data are listed at the end of this article.
1, polymerase base 2, polymerase, nucleoprotein, matrix, and nonstructural genes showed that the A/Pavia/07/2014 strain clustered within the European avian-like SIVs, including H1N1, H1N2 and H3N2 subtypes (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/21/7/14-0981-Techapp1.pdf).

The HA gene of the A/Pavia/07/2014 strain is 567 aa long and has antigenic sites identical to those of SIV A(H3N2) strains that circulated in swine in Italy during 2013 (Figure 2, http://wwwnc.cdc.gov/EID/article/21/7/14-0981/F2.htm). In addition, the pattern of A/Pavia/07/2014 glycosylation sites in the HA is identical to that in the A/swine/Italy/282811/2013 HA sequence and different from the human influenza A/Brisbane/10/2007 strain (online Technical Appendix Figure 2).

In December 2013, respiratory symptoms were observed mainly in piglets and weaning pigs on the farm that the patient visited, a farrow-to-finish pig farm where 400 sows were reared. The various production phases (mating, gestation, farrowing, nursery, and growing/finishing) were located in separated buildings. Forty-two nasal swab samples were collected at the end of January 2014 from pigs at all production phases. Considering that clinical signs were observed in piglets and weaning pigs, most samples (30 samples) were collected from weaning pigs. Influenza A real-time RT-PCR yielded negative results, probably because samples were collected ≥2 months after clinical signs appeared. Serologic investigations were performed on 29 serum samples from sows of different ages in mid-May that were collected within a national monitoring plan for Aujeszky disease. Samples were tested by hemagglutination-inhibition test according to standard procedures (12) by using A/Pavia/07/2014 and the reference SIVs A/swine/CA/3633/84 H3N2, A/swine/Italy/1521/98 H1N2, and A/swine/Finistere/2899/82 as antigens. Two-fold serum dilutions were tested starting at 1:20. All animals showed antibodies against H3 (137.16 geometric mean vs. A/Pavia/07/2014 and 84.2 vs. A/swine/CA/3633/84), whereas 15 of 29 serum samples that originated from the oldest animals also yielded positive results for A(H1N1) SIV. No antibodies against A(H1N2) SIV were detected.

**Conclusions**

The swine influenza A(H3N2) viruses present in Europe since 1984 resulted from a genomic reassortment between human-like swine H3N2 viruses and avian-like swine H1N1 viruses (13). Until 2011, only 3 episodes of SIV H3N2 infection had been reported in the Netherlands and Switzerland (1,2). Recently, in 2011 the emergence of a new SIV H3N2 variant was reported in the United States that had limited person-to-person transmission (14). Here we report a case of SIV H3N2 infection in a human host in January 2014 in Italy. In this patient, the presence of an uncommon influenza strain was suspected after the failure of molecular typing in the presence of high influenza load. The SIV strain identified here correlated with strains circulating in pigs during the 2013–14 influenza season in Italy. In addition, serologic results on pig serum collected from a farm close to the patient’s home suggested a recent exposure with an H3N2 strain similar to the A/Pavia/07/2014 isolate. These virologic and serologic data suggest that local pigs were the source of human infection.
In agreement with previous observations (1,2), the European H3N2 swine viruses seem to cause a benign disease with mild influenza-like symptoms in humans. In addition, the SIV strain we identified was found only in an immunocompromised patient. The uncomplicated clinical course might not be uncommon in immunocompromised patients. Indeed, in 2 epidemiologically correlated immunocompromised patients, the emergence of human influenza H3N2-resistant strains was associated with opposite clinical outcomes: 1 patient had mild upper respiratory syndrome; the other died of severe acute respiratory distress syndrome (15). On the other hand, on the basis of the observation that none of the patient’s family members and co-workers showed respiratory infection, we can hypothesize that immune impairment of the patient could have favored the zoonotic transmission of the SIV strain. Surveillance of circulating SIVs and monitoring of occupationaly exposed workers are 2 important tools to prevent spread of potential pandemic viruses.

Additional members of the Influenza Study Group who contributed data: Alessia Griello, Marta Premoli, Francesca Rovida, Bianca Mariani (SS Virologia Molecolare, SC Microbiologia e Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy); Francesca Manola Adella (Dipartimento di Virologia, Istituto Zoonoplogiico Sperimentale della Lombardia ed Emilia Romagna, Brescia, Italy); Anna Maria Belloni (Azienda Sanitaria Locale); Maria Gramegna, Liliana Coppola, Alessandra Piatti, Laura Gemma Brenzoni (DG Sanità, Regione Lombardia, Milan, Italy); Mario Luini (Organizzazione Mondiale per la Salute degli Animali, Laboratorio di riferimento per l’Influenza Suina, Istituto Zoonoplogiico Sperimentale della Lombardia ed Emilia Romagna, Parma, Italy); Emanuela Foni and Laura Baioni (Istituto Zoonoplogiico Sperimentale della Lombardia ed Emilia Romagna, Parma, Italy).

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This study was supported by grants from the Ministero della Salute, Fondazione IRCCS Policlinico San Matteo, Ricerca Corrente (grant no. 80622), Progetto Cariplo 2011-0517, Milan, Italy, and by a grant from the Ministero della Salute, IZSLER PRC2012002. Dr. Piralla is a clinical virologist at the Molecular Virology Unit of the Fondazione IRCCS Policlinico San Matteo in Pavia, Italy. His main research interests include molecular epidemiology of respiratory viruses, the study of virus evolution and interaction with the host, and design of next-generation sequencing protocols to study virus evolution and new pathogen discovery.

References


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Severe Pediatric Adenovirus 7 Disease in Singapore Linked to Recent Outbreaks across Asia

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During November 2012–July 2013, a marked increase of adenovirus type 7 (Ad7) infections associated with severe disease was documented among pediatric patients in Singapore. Phylogenetic analysis revealed close genetic links with severe Ad7 outbreaks in China, Taiwan, and other parts of Asia.

H uman adenoviruses (HAdVs) are classified into >50 types and are associated with clinical manifestations that include respiratory, gastrointestinal, ocular, genitourinary, and neurologic disease (1). HAdV infections have been estimated to cause 5%–10% of acute respiratory illnesses in children <5 years of age. Although most infections are subclinical or result in mild upper respiratory tract illnesses, HAdVs can also cause severe pneumonia. Among the HAdV types, type 7 (Ad7) has most often been associated with severe respiratory disease (2).

Recent reports have noted increased incidence of severe Ad7 disease in Asia: among the general population and pediatric inpatients in Taiwan; among persons in a military training camp in Shaanxi, China; and among those in a police training center in Kuala Lumpur, Malaysia (2–4). During January–June 2013, physicians in Singapore noted an increase in HAdV pediatric inpatients. Here we characterize the clinical and molecular epidemiology of this outbreak by reviewing data from government hospitals, the military, and a nationwide influenza-like illness (ILI) laboratory surveillance network in Singapore.

The Study

We retrospectively reviewed demographic and clinical information of adenovirus infections reported in Singapore during January 2011–July 2013 in 3 populations: 1) pediatric inpatients at KK Women’s and Children’s Hospital (formerly known as Kandang Kerbau Hospital) and National University Hospital, which are the only government hospitals in Singapore that have pediatric departments; 2) military personnel; and 3) outpatients reported to the nationwide ILI surveillance network housed in the National Public Health Laboratories (NPHL). Institutional review boards of the participating hospitals approved this study.

Cases of HAdV infection among military personnel were detected by a sentinel surveillance program in 5 military camps in which occurrences of febrile respiratory illnesses, defined as presence of acute respiratory symptoms (cough, sore throat, or both) and fever (oral temperature ≥37.5°C) (5), are monitored. All male citizens of Singapore undergo 2 years of conscripted military service upon turning 18–19 years of age; new personnel continuously enter the camps. To identify cases among the civilian population, the NPHL ILI laboratory surveillance network processes upper respiratory tract samples from patients with acute onset of fever (oral temperature ≥38°C) and respiratory symptoms referred by physicians at 23 sentinel clinics (6).

HAdV infection cases were defined by the detection of adenovirus by PCR assay, immunofluorescence, viral culture, or antigen detection in clinical samples (respiratory specimens, including nasal wash, bronchoalveolar lavage, endotracheal tube aspirate, oropharyngeal, nasopharyngeal, throat, and nasal swab; or urine or eye swab specimens). HAdV typing was performed by sequencing

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of HAdV hexon gene hypervariable regions 1–6 (HVR1–6) (Ad7 reference Gomem AY594255 hexon gene nt 324–1123) (7). To assess whether Ad7 was associated with severe disease, diagnoses of inpatients were dichotomised as invasive (pneumonia, gastroenteritis, disseminated disease, or hemorrhagic cystitis) and noninvasive (upper respiratory tract infection, acute laryngotracheobronchitis, bronchitis, bronchiolitis, tonsillitis, otitis media, or conjunctivitis) on the basis of clinical syndromes identified by physicians.

During January 2011–July 2013, samples from 421 pediatric inpatients, 752 military personnel, and 85 pediatric outpatients from the NPHL ILI surveillance network were positive for adenovirus. During August 2011–July 2013, a total of 289 (96.0%) pediatric inpatient cases were genotyped. The number of pediatric inpatients increased from 32 during January–July 2012 to 200 cases during January–July 2013 (Figure 1). This increase was predominantly related to Ad7 infections, which were first detected in November 2012 and represented 48.5% (n = 97) of all genotyped adenovirus cases in the first 7 months of 2013. The increase in Ad7 pediatric inpatients was accompanied by a smaller increase in detection of subgroup B HAdV infections (n = 47) among military personnel, from September 2012 to July 2013; all patients recovered with outpatient treatment. Of the samples from military personnel, 35 (74.5%) were genotyped; all were Ad7. During September 2012–July 2013, of 19 HAdV cases among pediatric patients (<16 years of age) detected and genotyped by the community ILI surveillance, none were Ad7; of 17 HAdV cases identified among adults, 3 (17.6%) were Ad7.

Clinical information was available for 188 HAdV-positive pediatric inpatients (<16 years of age) admitted during January–September 2013 (Table 1). A total of 54 patients had invasive infections and 134 had noninvasive infections (Table 2). More patients (n = 21, 38.9%) who had invasive infections had comorbid conditions than did patients who had noninvasive infections (n = 14, 10.5%; p<0.001). Ad7 was more frequently identified among patients who had invasive infection (57.4% vs. 41.0%; p = 0.002). In univariate analysis, invasive infection was significantly associated with presence of comorbid conditions (crude odds ratio [OR] 5.45, 95% CI 2.50–11.88) and Ad7 infection (crude OR 6.95, 95% CI 1.98–24.41; p<0.001). After adjusting for age and gender, presence of comorbid conditions (adjusted OR 6.78, 95% CI 2.59–17.72) and Ad7 infection (adjusted OR 9.00, 95% CI 2.34–34.59) remained significantly associated with invasive infection (p<0.001).

We used the maximum-likelihood method to compare the phylogenetic relationships among representative Ad7 partial hexon gene sequences from pediatric inpatients (n = 9, November 2012–June 2013); the nationwide ILI laboratory surveillance network (n = 1 adult sample, January–June 2013); and military personnel (n = 34, September 2012–May 2013) by using reference Ad7 sequences (GenBank accession nos. KP729815–KP729824) (Figure 2) (8,9). All Singapore Ad7 isolates except KK341 and KK342 had 100% nucleotide identity with strains reported from a 2011 adenovirus community outbreak in Taiwan (JX174430), severe disease in infants in Shaanxi in 2009 (GU230898), and a military training camp outbreak in Shaanxi in 2012 (KC689913) (2,3,10).
Table 1. Demographics and clinical features of 188 hospitalized children with adenovirus, by age group, Singapore

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>3.0 (0.2–15.7)</td>
</tr>
<tr>
<td>Male sex</td>
<td>120 (63.8)</td>
</tr>
<tr>
<td>Positive contact history</td>
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<td>79 (42.0)</td>
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<tr>
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<tr>
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<td>8 (10.1)</td>
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<tr>
<td>Unknown</td>
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</tr>
<tr>
<td>Comorbid conditions†</td>
<td></td>
</tr>
<tr>
<td>Symptom days before care sought, median (range)</td>
<td>35 (18.6)</td>
</tr>
<tr>
<td>Diagnostic method</td>
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</tr>
<tr>
<td>PCR</td>
<td>51 (27.1)</td>
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<tr>
<td>Immunofluorescence</td>
<td>137 (72.9)</td>
</tr>
<tr>
<td>Type</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40 (21.3)</td>
</tr>
<tr>
<td>7</td>
<td>86 (45.7)</td>
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<tr>
<td>Others (1, 2, 3, 5, 11)</td>
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<td>Unknown</td>
<td>29 (15.4)</td>
</tr>
<tr>
<td>Syndrome</td>
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</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>107 (56.9)</td>
</tr>
<tr>
<td>Acute laryngotracheobronchitis</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Bronchiolitis/bronchitis</td>
<td>13 (6.9)</td>
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<td>Tonsillitis</td>
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<tr>
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<td>Conjunctivitis</td>
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<tr>
<td>Pneumonia</td>
<td>35 (18.6)</td>
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<tr>
<td>Gastroenteritis</td>
<td>15 (8.0)</td>
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<tr>
<td>Disseminated adenovirus</td>
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<tr>
<td>Hemorrhagic cystitis</td>
<td>3 (1.6)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>13 (6.9)</td>
</tr>
<tr>
<td>Hospitalization days, median (range)</td>
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</tr>
<tr>
<td>Died</td>
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</tbody>
</table>

Table 2. Number and percentages of 188 hospitalized pediatric adenovirus case-patients with noninvasive and invasive infection, by key characteristics, and risk factors associated with invasive infection, Singapore

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Noninvasive infection, n = 134</th>
<th>Invasive infection, n = 54</th>
<th>Univariate analysis</th>
<th>Multivariate analysis†</th>
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<tbody>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td>p value</td>
<td>Adjusted OR (95% CI)</td>
<td>p value</td>
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<td>1.04 (0.92–1.17)</td>
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<td>0.331</td>
<td>0.53 (0.23–1.20)</td>
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<td>Comorbid conditions†</td>
<td>14 (10.5)</td>
<td>5.45 (2.50–11.88)</td>
<td>&lt;0.001</td>
<td>6.78 (2.59–17.72)</td>
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<td>37 (27.6)</td>
<td>6.95 (1.98–24.41)</td>
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<td>55 (41.0)</td>
<td>4.63 (1.14–18.83)</td>
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<td>18 (13.4)</td>
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Conclusions

An abrupt increase in severe Ad7 disease in pediatric inpatients in Singapore occurred during November 2012–July 2013. A corresponding rise was noted among military personnel during October 2012–April 2013, but no statistically significant increase in Ad7 infections was detected by the NPHL community ILI surveillance program. Partial hexon gene sequences of the Singapore isolates had 100% nucleotide identity with sequences reported from outbreaks in Taiwan and China (2,3,10).

Ad7 has been reported to cause outbreaks in 3 main patterns: 1) severe disease among young children, especially during winter in temperate countries; 2) less severe disease in nonseasonal community outbreaks; and 3) outbreaks among military personnel (11). The outbreak we report was marked by severe disease among young children and mild disease among military personnel. In the 2 government-owned hospitals, 1–2 cases among pediatric patients were identified per month by using community ILI surveillance. Failure to detect an increase in Ad7 remains unexplained but might be related to the low number of samples collected and tested.

Ad7 can be subclassified by restriction enzyme analysis (12). The available Singapore partial hexon gene sequences were most closely related to Ad7d and Ad7d2 genome types, which have been associated with outbreaks of acute respiratory illness in Asia. Ad7d, the predominant Ad7 circulating virus in China since the early 1980s, was the cause of outbreaks in South Korea during 1995–1997; during a community and pediatric outbreak in Taiwan in 2011, Ad7d replaced Ad7b as the main Ad7 strain (2,13). The closely related Ad7d2 was described in Israel in 1992 and has caused outbreaks in the United States and Japan (14,15). Systematic HAdV typing in Singapore was initiated in late 2011, so it remains unknown if Ad7 substrain replacement, specifically the circulating pediatric HAdV strain in early 2011, was a factor in the outbreak we report.

Our findings indicate a need for improved vigilance for detection and surveillance of severe Ad7 disease in Asia, as well as whole-genome sequencing and seroprevalence studies to perform accurate typing of outbreak strains and to identify correlates of pathogenicity. These practices could facilitate effective, early deployment of vaccine prevention and antiviral therapy.

Acknowledgments

We thank the staff of all participating institutions who contributed to patient care and data collection for this analysis.
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Dr. Ng is an Infectious Disease Consultant at Tan Tock Seng Hospital. His primary research interests are pathogen molecular epidemiology, emerging infectious diseases, and HIV.

References


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Hemagglutinin Receptor Binding of a Human Isolate of Influenza A(H10N8) Virus

Irene Ramos, Mena Mansour, Teddy J. Wohlbold, Megan E. Ermler, Ariana Hirsh, Jonathan A. Runstadler, Ana Fernandez-Sesma, Florian Krammer

Three cases of influenza A(H10N8) virus infection in humans have been reported; 2 of these infected persons died. Characterization of the receptor binding pattern of H10 hemagglutinin from avian and human isolates showed that both interact weakly with human-like receptors and maintain strong affinity for avian-like receptors.

Human infections with avian influenza A(H10N8) virus were reported in China during the 2013–14 winter influenza season. The first patient, a 73-year old woman, became ill in November 2013 a few days after visiting a live poultry market in Jiangxi Province (1). Two additional patients, a 55-year-old woman and a 75-year-old man, were admitted to hospitals in the same province in January 2014 (2). Severe pneumonia and subsequent acute respiratory distress syndrome developed in all 3 patients; 2 of the patients died, 5 and 6 days after admission (2).

Epithelial cells of the human upper respiratory tract contain mostly α2,6-linked sialic acids (SAα2,6) and low levels of α2,3-linked sialic acids (SAα2,3) (3). Hemagglutinin (HA) of avian influenza virus strains shows preferential binding to SAα2,3 receptors, which partially accounts for the reduced ability of avian influenza strains to establish infections in humans (3). Interaction with SAα2,6 receptors is one of the requirements for efficient replication in the human upper respiratory tract. In addition, reduced binding to SAα2,3 facilitates respiratory droplet-based transmission in ferrets (4). Therefore, emerging avian influenza viruses with increased binding to SAα2,6 and reduced binding to SAα2,3 pose a major pandemic threat, and active research and surveillance to detect animal viruses with modified receptor binding are warranted.

The Study

We analyzed the amino acid sequence of the receptor binding site of HA from the isolate A/Jiangxi-Donghu/1197-1/2013 (H10-JD346; Global Initiative on Sharing Avian Influenza Data [GISAID, http://www.gisaid.org]) accession no. EP1530526) from the first patient infected by influenza A(H10N8) virus. In addition, several human and avian influenza viruses (sequences from GISAID or the National Center for Biotechnology Information website) and a recent harbor seal isolate (5) were compared with H10-JD346 (Table). We observed that residues involved in receptor binding for H10 subtype influenza viruses suggest avian-like receptor specificity. However, we identified 2 amino acids in avian and human H10, T135 and S186, that are common in circulating human influenza viruses and were associated with changes in receptor binding in other avian influenza A virus subtypes (6,7). In accordance with this finding, Vachieri et al. found substantial levels of binding of an avian H10 HA to SAA2,6 that retained the ability to interact with SAA2,3 (8).

Given the role of receptor binding specificity of emerging influenza viruses, we analyzed the interaction of HA of the human H10-JD346 influenza A(H10N8) virus isolate in comparison with that of an avian H10N7 subtype virus. First, we used a solid-phase binding assay (9,10) and the following biotinylated glycans conjugated with a polyacrylamide (PAA) support (provided by the Consortium of Functional Glycomics [CFG]): Neu5Aca2,6Galβ1–4GlcNAcβ-PAA (6′ SLN-PAA); Neu5Acα ± 2 (GalF1 4GlcNAcF1 3)2β-PAA (6′sDi-LN-PAA); Neu5Aca2,3Galβ1–4GlcNAcβ-PAA (3′ SLN-PAA); Neu5Acα ± 2 (GalF1 4GlcNAcF1 3)2β-PAA (3′sDi-LN-PAA); and Neu5Aca2–3 (Galβ1–4GlcNAcβ)3β-PAA (3′sTri-LN-PAA). We also analyzed recombinant hexahistidine-tagged HAs (11) from H10-JD346, an avian H10N7 subtype strain from North America (A/mallard/Interior Alaska/10BM01929/2010; H10-mallard), a human H3N2 subtype seasonal influenza A virus (A/Panama/2007/1999; H3-P99), and an H5N1 subtype avian influenza virus from a fatal human case (A/Vietnam/1203/2004; H5-Viet).

As expected, H3-P99 bound strongly to the SAα2,6 tested, and H5 showed higher levels of binding to SAα2,3 than to SAα2,6 (Figure 1, panel A). When we analyzed H10-mallard and H10-JD346, we found a similar binding profile, which is consistent with the presence of similar amino acids affecting the receptor binding specificity (Table). Although both H10 proteins had a prevalent avian-like binding profile, low levels of binding to SAα2,6 were also observed.

To confirm this data, we used a flow cytometry–based assay and the same synthetic glycans (9,10). We infected...
per human respiratory tract, which is rich in SA\textalpha2,6 (influenza virus might have the ability to interact with the up
ceptors. Consequently, these data suggest that H10 subtype
receptors and maintains preferential binding to avian-like re
subtype influenza virus interacts slightly with human-like
mallard with 2 independent assays indicated that the H10
than that for the negative control.

JD346 showed similar binding profiles with preferential
in the solid-phase binding assay. H10-mallard and H10-
interacted with respiratory epithelia (Figure 2), which
pected, H3-P99 HA bound to the surface of respiratory
from those of Yang et al. (8) but show some differences
MDCK epithelial cells with H10-JD346 virus (6:2 re-as-
sortant with the backbone of laboratory strain A/Puerto
8/1934 [PR8], which was generated as described)
(9,10); H10-mallard (wild-type); human isolate H3-P99
(wild-type); and H5-Viet 6:2 (low pathogenicity re-
sortant with the backbone of PR8) (9,10) at a multiplicity
infection of 1. Cells were harvested 24-h postinfection
and incubated with antibody against matrix protein 2
(E10), which was detected by using an antibody against
IgG (Alexa 647 antibody; Invitrogen, Carlsbad, CA, USA)
for an avian H10 subtype HA, and binds to cells in the hu-
ected the SA binding profile (Figure 1, panel B).

H3-P99 showed high levels of binding to SA\textalpha2,6 and
H5-Viet bound more efficiently to SA\textalpha2,3 than to SA\textalpha2,6, which is similar to observations with recombinant HAs
in the solid-phase binding assay. H10-mallard and H10-
JD346 showed similar binding profiles with preferential
binding for SA\textalpha2,3 and binding to SA\textalpha2,6 slightly higher than that for the negative control.

Analysis of receptor binding of H10-JD346 and of H10-
mallard with 2 independent assays indicated that the H10
subtype influenza virus interacts slightly with human-like
receptors and maintains preferential binding to avian-like re-
ceptors. Consequently, these data suggest that H10 subtype
influenza virus might have the ability to interact with the up-
ner human respiratory tract, which is rich in SA\textalpha2,6 (3).

To test this hypothesis, we precomplexed H3-P99
and H10-JD346 with primary antibody (mouse anti-His
tag) and secondary fluorescent antibody, then incubated
the complex with 2 human tracheal samples (12). As ex-
pected, H3-P99 HA bound to the surface of respiratory
epithelia (Figure 2). Recombinant H10-JD346 HA also
interacted with respiratory epithelia (Figure 2), which
suggested that the virus might be able to attach and rep-
licate in the human upper respiratory tract. However, the
6:2 reassortant virus H10-JD346 virus showed markedly
decreased replication compared with that of an H3N2
subtype virus (PR8 6:2 reassortant) in a human lung epili-
theil cell line (Figure 3, http://wwwnc.cdc.gov/EID/ article/21/7/14-1755-F3.htm).

Conclusions
HA of novel influenza A(H10N8) virus interacts with
SA\textalpha2,3 and slightly with SA\textalpha2,6, at levels similar to that
for an avian H10 subtype HA, and binds to cells in the hu-
man upper respiratory tract. Our findings are consistent
with those of Vachiery et al. (8) but show some differences
from those of Yang et al. (13) and Wang et al. (14), who
did not detect interaction with SA\textalpha2,6 or human trachea.
Variations in the experimental settings and protocols (e.g.,
concentration of HA or glycans used) might account for
these dissimilarities.

Only 3 cases of human infections with influenza
A(H10N8) viruses have been reported. However, H10N7
subtype viruses have caused conjunctivitis or mild respir-
atory symptoms in humans. An epidemic among seals

Table. Alignment of residues involved receptor binding of hemagglutinin of influenza A viruses*

<table>
<thead>
<tr>
<th>Origin/subtype</th>
<th>Isolate name</th>
<th>Amino acid position (H3 numbering)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human/H3N2</td>
<td>A/Panama/2007/1999</td>
<td>ATSAANSGDSGWRGVS</td>
</tr>
<tr>
<td>Human/H3N2</td>
<td>A/Texas/50/2012</td>
<td>TTSAANNGDFGRNRNP</td>
</tr>
<tr>
<td>Human/H3N2</td>
<td>A/Brisbane/10/2007</td>
<td>TTSAANVNGFRNNIP</td>
</tr>
<tr>
<td>Human/H1N1</td>
<td>A/California/04/2009</td>
<td>DVAAISDSTKRDQEG</td>
</tr>
<tr>
<td>Human/H1N1</td>
<td>A/Texas/36/1991</td>
<td>VVTSLSDAAKRGQE</td>
</tr>
<tr>
<td>Human/H1N1</td>
<td>A/Brisbane/59/2007</td>
<td>TVASLPDAAKEQDRG</td>
</tr>
<tr>
<td>Avian/H1N1</td>
<td>A/duck/Alberta/1976</td>
<td>TVASLPESAEQRGQA</td>
</tr>
<tr>
<td>Avian/H7N1</td>
<td>A/riea/North Carolina/39482/1993</td>
<td>RASSAKGKEKTFSGRD</td>
</tr>
<tr>
<td>Avian/H6N1</td>
<td>A/mallard/Sweden/81/2002</td>
<td>DVKALPETRANQGR</td>
</tr>
<tr>
<td>Avian (human isolate)/H5N1</td>
<td>A/Vietnam/1203/2004</td>
<td>AVSASAVENTKTNGQS</td>
</tr>
<tr>
<td>Avian (human isolate)/H7N9</td>
<td>A/Anhui/1/2013</td>
<td>RASAKVEKKQNGLSG</td>
</tr>
<tr>
<td>Avian/H10N7</td>
<td>A/shorebird/Delaware Bay/10/2004</td>
<td>NTRAKSDEQLNGQSG</td>
</tr>
<tr>
<td>Avian/H10N7</td>
<td>A/mallard/Interior Alaska/10/201/2092</td>
<td>NTRAKSDEQLNGQSG</td>
</tr>
<tr>
<td>Avian (seal isolate)/H10N7</td>
<td>A/harbor/seal/Germany/1/2014</td>
<td>NTRAKSDEQLNGQSG</td>
</tr>
<tr>
<td>Avian (human isolate)/H10N8 Donghu/346-1/2013</td>
<td>NTRAKSDEQLNGQSG</td>
<td></td>
</tr>
</tbody>
</table>

*Residues found in human H1 or H3 and in H10 hemagglutinin but not in other avian hemagglutinin sequences are shown in bold.
caused by this virus subtype is currently ongoing in Europe (5). A study by Beare and Webster showed that 50% of volunteers experimentally infected with influenza A(H10N7) virus shed virus (5), which our data suggests might be caused by initial attachment to the upper respiratory tract. Immune responses were not detected in these volunteers, and mild, if any, symptoms developed, which indicated limited virus replication.

The low incidence of H10 influenza virus indicates a limited pandemic potential of H10N7 and H10N8 viruses. Therefore, further changes in receptor binding, as well as acquisition of genomic segments from other avian influenza virus strains through co-infection, would be required to increase fitness and transmissibility in mammals. Isolate H10-JD346 amino acid sequence had a mixture of E and K in position 627 of basic polymerase protein 2; the K627
mutation is associated with mammal adaptation (1). This finding highlights the need for an efficient surveillance network to track and identify possible changes, as well as extensive research to identify them and understand their functional consequences.

Acknowledgments

We thank CFG for providing reagents. Some of the data will be published on the CFG website (http://www.functionalglycomics.org/). We also thank the Flow Cytometry Shared Facility and the Microscopy Core Facility at Icahn School of Medicine at Mount Sinai for assistance, the Icahn School of Medicine Institutional Biorepository for providing human tissue sections, John Steel and Randy A. Albrecht for providing recombinant influenza viruses, and GISAID for making H10N7 and H10N8 subtype virus sequencing data publicly available.

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References


Figure 2. Interaction of hemagglutinin (HA) of H3-P99 (panels B and E) and H10-JD346 (panels C, F, and G) isolates of influenza A(H10N8) viruses with human trachea. Sections from 2 persons are shown (A–C and D–G). A and D, negative control staining (secondary antibody without HA). Blue indicates nuclei stained with 4′,6-diamidino-2-phenylindole; green indicates HA binding. Scale bars indicate 25 µm.


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Schmallenberg Virus Recurrence, Germany, 2014

Kerstin Wernike, Bernd Hoffmann, Franz J. Conraths, Martin Beer

Schmallenberg virus (SBV) emerged in Germany in 2011, spread rapidly across Europe, and almost disappeared in 2013. However, since late summer 2014, new cases have occurred in adult cattle. Full-genome analysis revealed some amino acid substitution differences from the first SBV sample. Viremia developed in experimentally infected sheep and cattle for 4–6 days.

The Study

Surprisingly, during summer and autumn 2014, SBV reappeared in Germany to a greater extent. Viral genome was repeatedly detected in acutely infected cattle. Several samples from various federal states were submitted to the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (Insel Riems, Germany), to confirm an SBV infection (Table) and to further characterize these reemerging viruses.

To evaluate sequence variations among SBV variants circulating in Germany since 2011, the original SBV isolate (BH80/11) and viruses isolated in 2012 from the blood of viremic sheep (BH619/12) or cattle (D495/12-1 and BH652/12) were compared with 3 genomes obtained from the viruses in acutely infected adult cattle in 2014 (BH119/14-1/2, BH119/14-3/4, BH132/14). RNA was extracted by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s recommendations, and the open reading frames of all 3 genome segments (large [L], medium [M], and small [S] segment; primer sequences available on request) were sequenced as described (7). Sequences generated in the current study were submitted to GenBank (accession nos. KP731865–KP731882). Sequence alignments and translation in amino acids were supported by Geneious version 7.1 (Biomaterts, Auckland, New Zealand). We generated a maximum-likelihood tree (Hasegawa-Kishino-Yano model, 1,000 bootstrap replicates) using MEGA5 (8). For the phylogenetic analysis of the M segment, sequences previously obtained from organ samples from newborns malformed because of SBV infection also were integrated (7).

For the S segment (830-nt long), which encodes the nucleocapsid protein (N) and a nonstructural protein (NSs), sequence analysis revealed very high stability. The samples obtained from acutely infected animals in 2014, BH619/12 and BH652/12, were 100% identical to the original SBV strain BH80/11 from 2011. In the sample D495/12-1 from 2012, a single nucleotide was substituted (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/21/7/15-0180-Techapp1.pdf). The viral RNA-dependent RNA polymerase encoding L segment (6864 nt) also showed high stability, and only a few nucleotide substitutions compared with BH80/11 were found in the latest samples (D495/12-1 and BH652/12: 6 nt; BH619/12: 10 nt; BH119/14-1/2, BH119/14-3/4, and BH132/14: 18 nt). Overall, sequence identity was 99.7%–99.9% (online Technical Appendix Figure).
Nonsynonymous substitutions ranged from 1 aa to 4 aa (online Technical Appendix Figure).

The M segment (4415-nt long) encodes 2 glycoproteins (Gn and Gc) and a nonstructural protein (NSm). It is the most variable genome segment of SBV and related viruses (7,9,10). Despite the identification of a highly variable region within the Gc-coding sequence in viruses in malformed newborns (7,9), there was a high sequence stability of the viruses detected in the blood of acutely infected adult animals; all sequences clustered closely (Figure 1). In contrast to the maximum of 77 nt and 43 aa substitutions or 12 aa deletions or 2 aa insertions found in organ samples of lambs or calves (7), we detected only 6–12 nt and 2 aa (BH619/12), 3 aa (D495/12-1, BH119/14-3/4, BH132/14) or 4 aa (BH652/12, BH119/14-1/2) substitutions (online Technical Appendix Figure). Because Gn and Gc are major immunogens of orthobunyaviruses (10), the mutation hot spot was supposed to be involved in immune evasion mechanisms and/or adaption of the cell tropism within the individual host (7,9). However, insect-transmitted viruses such as SBV have to adapt to 2 hosts and undergo replication cycles in both the arthropod vector and the mammalian host. Thus, the high sequence stability of virus strains detectable in viremic animals might be necessary for transmission to the vector. Notably, in comparison with the original SBV isolate, K→E substitutions at aa 746 and 1340 of the M segment were found in all other samples. Because these mutations have now been consistently present for at least 2 years, they might have occurred during the adaptation to European ruminants or insects and could provide a growth advantage within the individual host or might be beneficial for transmission between host and vector.

To investigate whether the detected sequence variations correlated with any change in pathogenicity, 5 female sheep and 1 female calf were subcutaneously inoculated with pools of up to 5 serum or whole blood samples from 1 of the holdings with new cases confirmed in 2014 (sheep 1 and 3 and the calf with samples from Lower Saxony; sheep 2, 4, and 5 with samples from Saxony [permit no. LALLF-M-V/TSD/7221.3–1.1–004/12]). None of the animals showed fever or any other clinical sign. Blood samples were taken daily for the first 2 weeks after inoculation and analyzed by real-time reverse transcription PCR (11). Thereafter, serum samples were taken in weekly intervals and tested in a microneutralization assay (12) and a commercially available SBV antibody ELISA (ID Screen Schmallenberg Virus Competition; IDvet, Grabels, France). The calf and sheep 2–5 became infected; viral genome was detectable in their blood for 4–6 days (Figure 2), which agrees with the short-lived viremia previously observed after experimental infection of cattle and sheep with the original SBV sample or the first cell culture isolate (1,13). Antibodies were first detected on day 7 after infection (sheep 2, sheep 5) or day 14 after infection (calf, sheep 3, sheep 4) in both tests.

Conclusions

SBV, first detected in 2011, circulated again in Germany in 2014. Virus genome with a high sequence identity to the first SBV sample was repeatedly detected in the blood of acutely infected adult cattle, and in experimentally infected animals, viremia developed that was identical to the original SBV isolate. The renewed virus circulation during the 2014 vector season was observed primarily in an area less affected in the 2 previous years. The missing or markedly reduced virus circulation led to a decline in herd seroprevalence caused by a missing infection of the young stock.
further reasons for the unexpected recurrence of SBV could be persistence within the insect vectors. As a consequence, the infection of naive animals in autumn 2014 resulted in an increasing frequency of the birth of malformed offspring in the following winter.

Acknowledgments
We thank the German local diagnostic laboratories for providing the SBV-positive samples. We are grateful to Kristin Trippler for excellent technical assistance. We gratefully acknowledge Andrea Aebischer’s help with the animal experiment and the dedicated animal care by staff of the isolation unit of the Friedrich-Loeffler-Institut. We also thank Andreas Moss for providing SBV isolate D495/12-1.

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References


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Detection of Circovirus in Foxes with Meningoencephalitis, United Kingdom, 2009–2013

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A fox circovirus was identified in serum samples from foxes with unexplained neurologic signs by using viral metagenomics. Fox circovirus nucleic acid was localized in histological lesions of the cerebrum by in situ hybridization. Viruses from the family Circoviridae may have neurologic tropism more commonly than previously anticipated.

Circoviruses (family Circoviridae) are nonenveloped, single-stranded, circular DNA (∼2 kb) viruses (1). Two genera, Circovirus and Gyrovirus, are recognized, and an additional genus, Cyclovirus, has been proposed (1,2). Circoviruses have an ambisense genome organization with 2 major inversely arranged open reading frames encoding the rolling circle replication initiator protein gene (Rep) and a capsid protein gene (Cap) (1). A conserved stem–loop structure, required for viral replication, is located between the 5′ ends of the 2 main open reading frames. Circoviruses are thought to exhibit host species specificity and have been detected in various species, including birds, pigs, and dogs (1,3,4). These viruses have been associated with a variety of diseases, including respiratory and enteric disease, dermatitis, and reproductive problems (1,3–5). Recently, many small circular DNA genomes have been described from different hosts by using different methods, including high-throughput sequencing (6). Here we describe the identification, characterization, and prevalence of a newly discovered fox circovirus that was present in serum and brain samples from foxes with unexplained meningoencephalitis in the United Kingdom.

The Study

During 2009–2013, a total of 31 adult foxes with signs of a neurologic disorder were brought to the RSPCA Norfolk Wildlife Hospital in East Winch, United Kingdom. The foxes exhibited abnormal behavior, lack of fear, reduced alertness, aimless wandering, circling, facial muscle twitching, hind limb paresis, and visual abnormalities. Cases were only detected when free-living foxes became debilitated and were taken to the wildlife rescue center. Once in captivity, diseased foxes had good appetite and generally survived with no substantial disease progression or death, but they showed no evidence of natural recovery. After a few weeks, the foxes were usually euthanized because they did not respond to (nonspecific) medical treatment. All procedures were performed in compliance with relevant laws and institutional guidelines. Following euthanasia, necropsies were performed according to standard procedures. Samples were stored in 10% neutral buffered formalin and embedded in paraffin, and 4 μm–thick sections were stained with hematoxylin and eosin and evaluated for the presence of histologic lesions.

All foxes had similar histologic findings consisting of chronic multifocal or diffuse lymphoplasmacytic meningoencephalitis oriented on the forebrain with a predilection for cortical gray matter (Figure 1; Table 1; online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/21/7/15-0228-Techapp1.pdf). Characteristic histopathologic features were nonspecific perivascular cuffing, rod cell proliferation, spongiosis, neuronal necrosis, moderate to severe gliosis, neuronal satellitosis, and neurophagia. Substantial pathologic changes were restricted to the central nervous system. Histopathologic changes suggested viral, protozoal, microporidal, immune-mediated, or idiopathic disease. Immunohistochemistry of brain samples was negative for canine distemper virus, canine adenovirus, Borna disease virus, Toxoplasma gondii, and Neospora canium (data not shown). Serologic test results for canine distemper virus, rabies virus, N. canum, and tickborne encephalitis virus were negative, and Ziehl-Neelsen and Giemsa staining results for microporidia were negative. Minor white matter involvement, the duration of animal survival, and the current absence of documented rabies cases in the United Kingdom eliminated rabies virus as the cause of the neurologic disorder.

Serum samples from 6 of the foxes (VS7100001–6) were available for virus discovery studies. To perform the
studies, we used a viral metagenomics approach with the 454 sequence platform (GS Junior; Roche, Basel, Switzerland) as described previously (7–10) (Table 1). More than 22,000 reads were analyzed as described previously (10–12) (online Technical Appendix Figure 2). The complete genome sequences of circoviruses from 3 foxes were obtained; the sequences were 99% identical at the nucleotide level (GenBank accession nos. KP260925–7). The fox circovirus genomes had an ambisense organization characteristic of circovirus (online Technical Appendix Figure 3). Phylogenetic analysis revealed that the genomes were closely related to those of the recently described canine circoviruses (3,13), displaying ≥92% amino acid identity in the Rep protein and ≥89% nt sequence identity across the entire genome (online Technical Appendix Figure 4). On the basis of the suggested criteria demarcating species (1), the fox and canine circoviruses belong to the same species.

A diagnostic real-time fox circovirus PCR was performed targeting the Rep-coding sequence on 32 serum samples from foxes with and without neurologic signs (Table 1). Viral nucleic acid was extracted by using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Indianapolis, IN, USA) and amplified by real-time PCR by using primers VS756 (5′-TCCGAGATAGCC GGGCTGG- GTA-3′), VS757 (5′-CCGGCCACAGATCAAGTACTTA-3′), and VS758 (5′-FAM-ATCCAACTCCGGAGGAGGAGA-TAMRA-3′) and the TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA, USA). In addition to samples VS7100001–6, another 14 fox serum samples were positive for fox circovirus, indicating that the virus had infected foxes in multiple counties in the United Kingdom during past years (Table 1; online Technical Appendix Figure 5). Clinical data indicated that 77% of circovirus-positive foxes had signs of neurologic disease, compared with only 47% of circovirus-negative foxes (Table 2). Fox circovirus was present in male and female foxes and in adults and juveniles (Table 2). In addition, fox circovirus was detected by real-time PCR in brain samples of 2 of 4 foxes with neurologic disease (VS7100017 and 19; cycle threshold value >35) but not in the brain tissues of 2 foxes without disease. The detection of fox circovirus nucleic acid in the cerebrum of foxes with neurologic disease was confirmed by using the RNAscope 2.0 in situ hybridization kit (Advanced Cell Diagnostics, Hayward, CA, USA) and a Rep gene–specific probe according to the manufacturer’s instructions. Negative controls consisted of circovirus-negative foxes without histopathologic disease. Multifocal fox circovirus RNA signal was detected and associated with the aforementioned histologic lesions in the cerebrum (Figure 2). Specifically, RNA signal was detected in mononuclear cells in perivascular cuffs, inflammatory infiltrates in the neuropil, and neuronal somata in cerebral gray matter of circovirus-positive foxes with neurologic disease. No circovirus signal was found in control foxes with lymphocytic cuffs due to other (known) viral infections or in control foxes without neurologic disease (Figure 2).

Conclusions

Our findings indicate that circoviruses commonly cause systemic infections in wild foxes in the United Kingdom and can be detected in the brains of foxes with neurologic disease. It has been suggested that circoviruses are involved in a plethora of diseases in pigs, dogs, and birds (1,3–5). The canine circovirus may be associated with development of vasculitis in dogs (3), and an overall virus prevalence in serum samples of ≥3% has been reported (3,13). However, we found that the prevalence of fox circovirus in serum samples from foxes with and without neurologic disease was much higher and more

Figure 1. Histopathologic features of brain tissue from foxes with possible virus-induced neurologic disease. A) Multifocal, randomly distributed areas of severe encephalitis and meningitis in the cerebrum (original magnification ×40). B) Detail of encephalitis in the cerebrum (original magnification ×200). Gray and, to a lesser extent, white matter of the cerebrum showed randomly dispersed areas of astrocitosis, gliosis, and infiltration with lymphocytes and plasma cells. Blood vessels in affected areas show perivascular cuffing with distention of Virchow-Robin spaces with up to 10 layers of lymphocytes and plasma cells (arrow). C) Detail of white matter in the cerebellum (original magnification ×400). Axons in affected white matter showed degeneration, characterized by formation of spheroids, shrinkage, and fragmentation; axon sheaths containing microglia or macrophages; and presence of gitter cells in surrounding neuropil. Cerebellum was mildly affected, and meninges, especially of the cerebrum, were frequently distended with lymphocytes and plasma cells. D) Detail of gray matter of cerebrum (original magnification ×400). Individual neuronal cell bodies were frequently surrounded by up to 5 glial cells (i.e., satellitosis) and showed margination of Nissl substance, hyperchromasia, degeneration, and necrosis. Tissue sections were subjected to conventional hematoxylin and eosin staining.
Circovirus in Foxes with Meningoencephalitis

Table 1. Overview of testing results for fox serum samples used in a study of the detection of circovirus in foxes with meningoencephalitis, United Kingdom, 2009–2013*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Year</th>
<th>Serum</th>
<th>Age</th>
<th>Sex</th>
<th>County</th>
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<th>Outcome</th>
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<td>VS7100001</td>
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<td>F</td>
<td>Nor</td>
<td>Yes</td>
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<td>Yes</td>
<td>Pos</td>
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<td>VS7100002</td>
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<td>F</td>
<td>Ess</td>
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<td>Euthanized</td>
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<td>Pos</td>
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<td>Pos</td>
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<td>Lin</td>
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<td>M</td>
<td>Nor</td>
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<td>Died</td>
<td>No</td>
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<td>M</td>
<td>Cam</td>
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<td>Died</td>
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<td>Pos</td>
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<td>Lin</td>
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<td>No</td>
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<td>VS7100033</td>
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<td>Juvenile</td>
<td>M</td>
<td>Nor</td>
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<td>Euthanized</td>
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<td>Neg</td>
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<td>No</td>
<td>Pos</td>
<td>39.2</td>
<td>No</td>
</tr>
</tbody>
</table>

*Bed, Bedfordshire; Cam, Cambridgeshire; C, cycle threshold values of real-time PCR; Ess, Essex; FFPE, formalin-fixed paraffin-embedded tissue; Lei, Leicester; Lin, Lincolnshire; ND, not determined; Neg, negative; Nor, Norfolk; Pos, positive; Suf, Suffolk.
†Neurologic signs were abnormal behavior, lack of fear, reduced alertness, aimless wandering, circling, facial muscle twitching, progressive weakness of hind legs, and visual abnormalities.
‡Samples were analyzed by using a viral metagenomics approach with the 454 sequence platform (GS Junior; Roche, Basel, Switzerland).
§TaqMan real-time PCR.

Table 2. Univariate statistical analysis of age, sex, disease signs, and circovirus real-time PCR results for foxes in a study of the detection of circovirus in foxes with meningoencephalitis, United Kingdom, 2009–2013*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCR results for foxes, no. (%)†</th>
<th>p value by χ² test</th>
<th>OR (95% CI)</th>
</tr>
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<tr>
<td>All foxes</td>
<td>Without neurologic signs</td>
<td>With neurologic signs</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>15 (46.9)</td>
<td>17 (53.1)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>9 (60.0)</td>
<td>8 (47.1)</td>
<td>0.502</td>
</tr>
<tr>
<td>F</td>
<td>6 (40.0)</td>
<td>9 (52.9)</td>
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</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile</td>
<td>7 (46.7)</td>
<td>0 (0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Adult</td>
<td>8 (53.3)</td>
<td>17 (100)</td>
<td></td>
</tr>
<tr>
<td>Circovirus positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8 (53.3)</td>
<td>4 (23.5)</td>
<td>0.144</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (46.7)</td>
<td>13 (76.5)</td>
<td></td>
</tr>
</tbody>
</table>

*OR, odds ratio.
†Neurologic signs were abnormal behavior, lack of fear, reduced alertness, aimless wandering, circling, facial muscle twitching, progressive weakness of hind legs, and visual abnormalities.

Circoviruses, which belong to a proposed new genus in the family Circoviridae, were recently found in serum and cerebrospinal fluid of humans with paraplegia and acute infections of the central nervous system (11,13), suggesting that viruses from the family Circoviridae may have comparable to the prevalence of porcine circoviruses among pigs (14). No association of virus infection with vasculitis was apparent. Instead, fox circoviruses may be associated with development of neurologic disease directly or as a contributory complicating cofactor.
neurologic tropism more commonly than previously anticipated. However, a causal link between circovirus infection and disease in humans and animals remains to be proven. Because the prevalence of circoviruses in foxes was relatively high and closely related circovirus species seem pathogenic for both dogs and foxes, additional surveillance is warranted to clarify the epidemiology and pathogenicity of circoviruses in foxes.

This work was partially funded by the European Commission’s COMPARE H2020 project under grant agreement No 643476, the Virgo Consortium, and ZonMW TOP project 91213058.

S.L.S. is employed part time by Viroclinics Biosciences BV.

Mr. Bexton is the senior veterinarian at the RSPCA Norfolk Wildlife Hospital, East Winch, United Kingdom. His main research interests are the epidemiology and pathology of wildlife diseases including novel pathogens in free-living animals.

References


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Figure 2. Detection of fox circovirus–specific transcripts in brain tissue of foxes with neurologic disease showing in situ hybridization of cerebrum with fox circovirus replication initiator protein gene–specific probe (original magnification ×200). A) Negative control fox VS7100012. The serum sample from this fox was negative for circovirus, and the animal did not exhibit signs of neurologic disease. B, C) Affected foxes VS7100005 and VS7100003, respectively. Both animals had neurologic disease, and their serum samples were positive for fox circovirus (see Table 1 for more information regarding these foxes). Black arrows indicate mononuclear cells in perivascular cuffs, blue arrows show inflammatory infiltrates in the neuropil, and red arrows point to staining in neuronal somata in cerebral gray matter of circovirus–positive animals with neurologic disease.
Determination of Predominance of Influenza Virus Strains in the Americas

Eduardo Azziz-Baumgartner, Rebecca J. Garten, Rakhee Palekar, Mauricio Cerpa, Sara Mirza, Alba Maria Ropero, Francisco S. Palomeque, Ann Moen, Joseph Bresee, Michael Shaw, Marc-Alain Widdowson

During 2001–2014, predominant influenza A(H1N1) and A(H3N2) strains in South America predominated in all or most subsequent influenza seasons in Central and North America. Predominant A(H1N1) and A(H3N2) strains in North America predominated in most subsequent seasons in Central and South America. Sharing data between these subregions may improve influenza season preparedness.

During 2002–2008, infection with influenza viruses caused 40,880–160,270 deaths each year throughout the Americas (1). To prevent illness and death, medical staff in 35 countries throughout the Americas administer influenza vaccines (2). However, producing the vaccine takes ≈6 months, and selecting virus strains necessitates assessing which strains are likely to predominate during upcoming epidemics (3).

Surveillance for influenza has improved dramatically, especially in the American tropics (4). Nevertheless, it remains unclear whether virus strains identified in North America subsequently become predominant in South America and vice versa (3). Such information could help public health officials in each hemisphere prepare for upcoming influenza seasons. We describe influenza epidemics in North, Central, and South America and explore whether the virus strains that caused them were similar.

The Study

We obtained the number of respiratory swabs tested throughout each year and the number that were positive for influenza virus from the Global Influenza Surveillance and Response System (5). Data from Canada, Mexico, and the United States (population 458 million) collected during 2002–2013 were aggregated to represent North America; data from Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama (population 42 million) to represent Central America; and data from Argentina, Brazil, Chile, Paraguay, and Uruguay (population 262 million) to represent South America (6). We obtained antigenic characterization data from the Centers for Disease Control and Prevention (Atlanta, GA, USA).

We determined the proportion of respiratory specimens that tested positive for influenza virus each month in North, Central, and South America and then determined the annual median for each subregion; months in which the proportion exceeded the annual median were considered epidemic (7). The timing and length of epidemics in each subregion were also explored, and the proportion of samples testing positive for influenza virus was used as a proxy for epidemic severity. Antigenic virus strains were defined as predominant if they made up the largest proportion of positive samples by type or subtype during an influenza season.

We assessed whether predominant virus strains identified in South America were subsequently identified in Central and North America and whether strains identified in North America were subsequently identified in Central and South America. We also investigated whether predominant strains were represented by components of available Southern or Northern Hemisphere vaccine formulations.

During 2002–2013, South America reported 877,770 influenza-positive respiratory samples (2.8/10,000 persons/y) and North America 4,535,508 results (9.0/10,000 persons/y) to the Global Influenza Surveillance and Response System (5). During 2006–2013, Central America reported 82,163 results (2.4/10,000 persons/y). In each subregion, the number of reports increased during the study period (p = 0.02). During 2006–2013, the Centers for Disease Control and Prevention analyzed 2,971 samples from South America, 1,279 from Central America, and 25,127 from North America for antigenic characterization.

In South America, influenza epidemics started in April, in Central America in June, and in North America in December. With the exception of 2 (25%) of 8 years in Central America and 2 (17%) of 12 years in South America, when there was a southern temperate winter epidemic and a smaller northern temperate winter epidemic, all subregions had 1 annual influenza epidemic that lasted ≈5 months.

The predominant influenza A(H1N1) virus strains in South America predominated in 9 of 9 subsequent seasons
in Central America and 12 (92%, 95% CI 78%–107%) of 13 subsequent seasons in North America (Table 1; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/7/14-0788-Techapp1.xlsx). Similarly, A(H3N2) virus strains in South America predominated in all 11 (92%, 95% CI 76%–107%) of 12 subsequent seasons in Central America and 10 (71%, 95% CI 48%–95%) of 14 subsequent seasons in North America. Predominant influenza B virus strains in South America only predominated in 8 (67%, 95% CI 40%–93%) of 12 subsequent seasons in Central America and 8 (57%, 95% CI 31%–83%) of 14 subsequent seasons in North America. Virus strains in South America during 1 season typically did not predominate in subsequent seasons in South America (54%, 95% CI 38%–70%).

The predominant A(H1N1) virus strains in North America predominated in 7 (78%, 95% CI 51%–105%) of 9 subsequent seasons in Central America and 10 (83%, 95% CI 62%–104%) of 12 subsequent seasons in South America. A(H3N2) virus strains in North America predominated in 8 (67%, 95% CI 40%–93%) of 12 subsequent seasons in Central America and 10 (77%, 95% CI 54%–100%) of 13 subsequent seasons in South America. Influenza B virus strains in North America predominated in 9 (75%, 95% CI 51%–100%) of 12 subsequent seasons in Central America and 7 (54%, 95% CI 27%–81%) of 13 subsequent seasons in South America. Virus strains that predominated in North America during 1 season were less likely to predominate in the subsequent season in North America (62%, 95% CI 46%–77%).

At least 1 component of the Southern Hemisphere vaccine composition recommendations matched a predominant antigenic characterization in South America in 13 (93%, 95% CI 79%–106%) of 14 influenza seasons that occurred during 2001–2014, and at least 1 component of the Northern Hemisphere vaccine composition recommendations matched a predominant antigenic characterization in North America in all 14 influenza seasons that occurred during 2001–2014. Of 33 predominant antigenic virus strains identified in Central America during 2002–2014, 21 (64%, 95% CI 47%–80%) matched the Southern Hemisphere recommendations and 24 (73%, 95% CI 58%–88%) matched the Northern Hemisphere recommendations (Table 2).

Conclusions

Our findings suggest that virus strains identified during influenza epidemics in South America typically became predominant in subsequent epidemics in Central and North America. Almost as frequently, virus strains identified during epidemics in North America became predominant in the subsequent Central and South America epidemics. Although strain selection for 1 hemisphere’s vaccine formulation typically occurs before influenza activity is widespread in the opposite hemisphere, health officials have an opportunity to anticipate which influenza virus strains may predominate by observing activity in other subregions. For example, influenza A(H1N1)pdm09 virus predominated in Brazil during 2013 (8) and became predominant in North America during 2013–2014. Health officials identifying influenza B virus strains in 1 hemisphere would have correctly predicted the predominant influenza B virus strains in the opposite hemisphere only half of the time unless they had also examined other co-circulating influenza B virus strains in the opposite hemisphere.

Table 1. Most commonly identified antigenic characterizations of influenza strains in the Americas during 2001–2014†

<table>
<thead>
<tr>
<th>Year</th>
<th>Influenza A(H1N1) virus</th>
<th>Influenza A(H3N2) virus</th>
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<tr>
<td></td>
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<td>Central</td>
<td>North</td>
</tr>
<tr>
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<td>A</td>
<td>NA</td>
<td>A</td>
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<td>2014</td>
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*Data from Canada, Mexico, and the United States were aggregated to represent North America; data from Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama to represent Central America; and data from Argentina, Brazil, Chile, Paraguay, and Uruguay to represent South America.

†For influenza A(H1N1) virus, A, A/New Caledonia/20/99(H1N1); B, A/Solomon Islands/03/2006(H1N1); C, A/Brisbane/59/2007(H1N1); D, A/California/07/2009(H1N1)pdm09. For influenza A(H3N2) virus, A, A/Panama/2007/99(H3N2); B, A/Fujian/411/2002(H3); C, A/California/07/2004(H3N2); D, A/Wisconsin/07/2005(H3N2); E, A/Brisbane/10/2007(H3N2); F, A/Perth/16/2009(H3N2); G, A/Victoria/361/2011(H3N2); H, A/Texas/50/2012(H3N2). For influenza B virus, A, B/Sichuan/379/99(YAM); B, B/Shandong/7/97(VIC); C, B/Shanghai/361/2002(YAM); D, B/Malaysia/2505/2005(VIC); E, B/Florida/07/2004(YAM); F, B/Florida/04/2006(YAM); G, B/Brisbane/60/2008(VIC); H, B/Wisconsin/01/2010(YAM) I, B/Massachusetts/02/2012(YAM). NA, not applicable.

‡Newly identified strain (B in South America, 2 in Central America, and 7 in North America).
Table 2. Most commonly identified antigenic characterizations of influenza strains in Central America compared with composition of Southern and Northern hemisphere vaccines, 2002–2014*

<table>
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<tr>
<th>Predominant strains in Central America</th>
<th>South America vaccine composition</th>
<th>Matched southern vaccine</th>
<th>North America vaccine composition</th>
<th>Matched northern vaccine</th>
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<td>2002</td>
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Total percentage matching strains 64% 73%

*Values are proportions of occurrences when predominant strains are represented in each vaccine formulation. +, match; –, no match; NA, influenza type not among predominant circulating strains; Solomon Is, Solomon Islands.

strains. Nevertheless, such findings underscore the importance of year-round surveillance, viral characterization, data sharing, and annual influenza vaccination.

Our analyses are based on a convenience sample of respiratory specimens obtained from heterogeneous surveillance systems using different diagnostic assays (e.g.,
PCR and immunofluorescence) and then aggregated by subregion. These samples may not be geographically representative. Additional data will be needed to determine whether the characteristics of 1 subregion reliably predict influenza epidemics in another. New viral strains that appear might be introduced from outside the Americas (3).

In summary, health officials in North and Central America may find clues about which influenza A virus strains are likely to predominate during an upcoming season by observing which were predominant in South America and vice versa. Our findings underscore the need to share timely and representative specimens with World Health Organization Collaborating Centres. In the future, shorter vaccine production times using novel technology might facilitate matching vaccine composition more closely to circulating virus strains.

This paper is dedicated to the memory of Alexander Klimov.

Dr. Azziz-Baumgartner works at the US Centers for Disease Control, Influenza Division, collaborating with the Pan American Health Organization and its member countries. His research interests are surveillance improvements, disease and economic burden studies, and influenza vaccine impact studies.

References

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Arenaviruses are feared as agents that cause viral hemorrhagic fevers. We report the identification, isolation, and genetic characterization of 2 novel arenaviruses from Namaqua rock mice in Namibia. These findings extend knowledge of the distribution and diversity of arenaviruses in Africa.

Arenaviruses are known to cause severe hemorrhagic fevers across the globe with case fatality rates up to 30% (1). The viruses possess a bisegmented, single-stranded RNA genome with ambisense coding strategy consisting of a small segment coding for the nucleoprotein and glycoprotein and a large (L) segment coding for the RNA-dependent RNA polymerase and matrix protein. In Africa, Lassa virus (LASV) and Lujo virus are the only known members of the family Arenaviridae that cause human disease (2,3); however, evidence for lymphocytic choriomeningitis virus, another Arenaviridae sp., was recently reported in Gabon (4). Several other arenaviruses of unknown pathogenic potential have also been found in Africa: Gbagroube, Kodoko, and Menekre viruses from western Africa (5,6); Ippy (IPPYV) and Mobala viruses from central Africa; Mopeia, Morogoro, Luna, and Lunk viruses from eastern Africa; and Merino Walk virus (MWV) from southern Africa (7,8). All of these viruses are carried by rodents of the family Muridae.

Until now, no molecular detection of arenaviruses has been reported from Namibia. A study in 1991 described a low seroprevalence (0.8%) for LASV antibodies in humans in northern Namibia (9). Because of lack of data about arenavirus occurrence and effects in southwestern Africa, we conducted a study of small mammals from Namibia to detect infection with arenaviruses.

The Study
During 2010–2012, animal trapping was performed in 8 areas in central and northern Namibia (Figure 1), and samples from 812 rodents and shrews were obtained (Table 1). The animals were dissected in the field and stored individually in a field freezer at –20°C and later at –80°C. For primary arenavirus screening, lung sections of all animals were homogenized, and RNA was extracted and reversely transcribed by using random hexamer primers. Screening was performed by arenavirus genus-specific reverse transcription PCR (RT-PCR) (10) to detect the L genomic segment. From samples testing positive by

**Figure 1.** Screening for arenaviruses in Namibia. Trapping locations (named according to the nearest urban settlement) of small mammals. Sites where samples positive for new arenaviruses were found are marked by a crossed circle and underlined locality names. Geographic positioning system coordinates of the trapping sites: Ben Hur, 22°87.26′S, 19°21.10′E; Cheetah Conservation Fund (CCF), 16°39.0′E, 20°28.12′S; Mariental, 24°62.08′S, 17°95.93′E; Okahandja, (21°98.33′S, 16°91.32′E); Palmwag, 19°53.23′S, 13°56.35′E; Rundu, 17°56.645′S, 20°05.109′E; Talismanis, 21°84.30′S, 20°73.91′E; Windhoek, 22°49.93′S, 17°34.76′E.
Okahandja specimens were related to MWV, but the sample like origin. Initial phylogenetic analysis showed that the ment of the viral polymerase gene confirmed arenavirus- Sanger sequencing of PCR products from a 338-nt frag

rock mice (Mus, Pa, Palmwag (09/2010), Ta, Talismanis (12/2011), Ma, Mariental (06/2012), Ru, Rundu (01/2011), Wi, Windhoek (09/2010 and 01/2012).

For genome sequencing, pellets from ultracentrifuged supernatant of infected cell cultures were lysed, and total RNA was purified. RNA was then subjected to random-primed RT-PCR as described (11). Next-generation sequencing was performed by using a 454 Genome Se- quencer Junior (Roche, Indianapolis, IN, USA), and results were aligned against the virus database by using blastx algorithms (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequencing results matching arenavirus sequences were verified by Sanger sequencing (Applied Biosystems, Foster City, CA, USA) for virus isolation.

For genome sequencing, pellets from ultracentrifuged supernatant of infected cell cultures were lysed, and total RNA was purified. RNA was then subjected to random primed RT-PCR as described (11). Next-generation sequencing was performed by using a 454 Genome Sequencer Junior (Roche, Indianapolis, IN, USA), and results were aligned against the virus database by using blastx algorithms (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequencing results matching arenavirus sequences were verified by Sanger sequencing (Applied Biosystems, Foster City, CA, USA). Genome segment outermost noncoding termini were sequenced after linkage by T4-RNA-Ligase (New England Biolabs, Ipswich, MA, USA) and RT-PCR amplification.

Of the 812 rodents and shrews tested (Table 1), arenavirus RNA was found in lung tissue samples of 4Namaqua rock mice (Micaelamy s [Aethomys] namaquensis), 3 from Okahandja (N73, N80, N85) and 1 from Mariental (N27).

| Table 1. Small mammals captured in Namibia during 2010–2012 and tested for arenaviruses* |
|-----------------------------------------------|----------------|------------------------|------------------------|
| Mammal species | Common name | Locality of capture† | No. positive/no. tested |
| Aethomys chrysophilus | Red veld rat | Be, CCF, Ok, Pa, Ta | 0/64 |
| Micaelamy s namaquensis |Namaqua rock mouse|CCF, Ma, Ok, Pa, Ru|4/266|
| Crocidura fuscomurina | Bicolored musk shrew | CCF, Pa, Ru | 0/4 |
| Crocidura hirta | Lesser red musk shrew | Ma | 0/5 |
| Dendromys melanotis | Gray climbing mouse | Ta | 0/1 |
| Elephantulus intufl | Bushveld sengi | CCF, Ma, Ok | 0/14 |
| Gerbilliscus spp. | Gerbil | Wi | 0/6 |
| Gerbilliscus leucogaster | Bushveld gerbil | Be, CCF, Ma, Ok, Pa, Ru, Ta | 0/228 |
| Gerbillurus paeba | Hairy-footed gerbil | Be | 0/3 |
| Gerbillurus setzii | Namib brush-tailed gerbil | Be | 0/1 |
| Lemniscomys rosalia | Single-striped grass mouse | Be | 0/2 |
| Mastomys spp. | Multimammate mouse | Be, CCF, Ma, Ok, Pa, Ru, Ta | 0/114 |
| Mus indutus | Desert pygmy mouse | Ma, Pa | 0/5 |
| Petromuscus collaris | Pygmy rock mouse | Pa | 0/3 |
| Rhabdomys pumilo | Four-striped grass mouse | CCF, Ma, Ok, Pa, Wi | 0/73 |
| Saccostomus camerstris | Pouched mouse | Be, CCF, Ok, Pa, Ru | 0/17 |
| Thallomys paediulus | Acacia rat | Pa | 0/4 |
| u.u. Soricidae | Shrew | Wi | 0/2 |

Total 4/812

*Morphologic species identification of the arenavirus positive rodent samples was confirmed by sequencing of partial mitochondrial cytochrome b gene (GenBank accession nos.: N27, KP752173; N73, KP752175; N80, KP752176; N85, KP752174).†Abbreviations and sampling dates for trapping localities: Be, Ben Hur (11/2011); CCF, Cheetah Conservation Fund (02/2011); Ok, Okahandja (06/2012), Pa, Palmwag (09/2010), Ta, Talismans (12/2011), Ma, Mariental (06/2012), Ru, Rundu (01/2011), Wi, Windhoek (09/2010 and 01/2012). from Mariental was a highly divergent member of the genus Arenavirus (Figure 2, panel A). Cell culture isolation was performed with samples N27 and N73 and resulted in 2 novel arenavirus isolates: Mariental virus (MRTV) and Okahandja virus (OKAV), respectively.

The genomes of the 2 arenaviruses were investigated by using next-generation sequencing and RT-PCR Sanger sequencing. The genome data obtained for MRTV and OKAV showed a typical arenavirus nucleotide composition with the L segment (MRTV: 6,840 nt; OKAV: 7,170 nt) coding for RNA polymerase and matrix protein and the S segment (MRTV: 3,360 nt; OKAV: 3,379 nt) coding for glycoprotein and nucleocapsid protein. Table 2 shows the nucleotide and amino acid sequence identities of nucleo- capsid and glycoprotein open reading frame with other Old World (i.e., Eastern Hemisphere locations such as Europe, Asia, Africa) representatives of genus Arenavirus. On the basis of the nucleocapsid amino acid identity, OKAV is most related to MWV (75.7% identity). Furthermore, MRTV has the highest amino acid identity with IPPYV (71.4% identity) and with Gbagroube, Lassa, Luna, and Mobala viruses (≥70% identity). In the nucleocapsid-based phylogenetic tree, OKAV clusters with 100% bootstrap support with MWV detected in Myotomys unisulcatus rodents in South Africa (Figure 2, panel B), and MRTV forms a clade with IPPYV isolated from Praomys spp. in the Central African Republic. The bootstrap support of this monophyletic group of the tree lies at 56%. The analysis of the glycoprotein open reading frame (Figure 2, panel C) leads to a similar result; OKAV shares the most recent common ancestor with MWV, and MRTV clusters with IPPYV but with a weaker bootstrap support.
Novel Arenaviruses, Southern Africa

Conclusions

We detected and isolated 2 novel arenaviruses in Namibia, OKAV and MRTV. OKAV clearly clustered in relationship with the MWV from southern Africa, but MRTV is a more divergent member of the Old World arenavirus clade. According to amino acid identity and phylogenetic analysis, MRTV was most closely related to IPPYV from the Central African Republic; however, the low bootstrap support precluded a stringent estimation of this closest relative.

These new strains comply with the arenavirus species definition (14) on the basis of amino acid differences in

Table 2. Nucleotide and amino acid identities of Mariental (MRTV) and Okahandja (OKAV) viruses compared with Old World representatives of the genus Arenavirus*

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*Identities are shown for glycoprotein and nucleocapsid open reading frames. Highest aa identity values are shown in boldface. S, small; GPC, glycoprotein; NP, nucleocapsid protein; nt, nucleotide; aa, amino acid; LCMV, lymphocytic choriomeningitis virus; L, large segment.
nucleocapsid of ≥12% (>20% for both viruses), presence of specific host species, and existence of laboratory isolates. These properties indicate that MRTV and OKAV represent distinct arenavirus species.

These 2 viruses were found in the same host species located within a radius of 300 km. MRTV was found in only 1 sample (of 266); OKAV was detected in samples from 3 animals. Although more unlikely for OKAV than for MRTV, the possibility of a spillover infection to *M. namaquensis* from a still unknown reservoir host cannot be ruled out for either virus.

The Namaqua rock mouse’s habitat includes the tree and shrub savannahs of Namibia and most parts of southern Africa, including Namibia, South Africa, Botswana, Zimbabwe, and parts of Mozambique (J5). These locations imply the possible occurrence of MRTV or OKAV in larger regions of the continent. Cell culture isolates and genomic sequence data are the first prerequisites for evaluating the public health relevance of these new viruses. Our findings extend the knowledge of geographic distribution and genetic diversity of arenaviruses in Africa.

**Acknowledgments**

We thank C. Chimimba for advice in small mammal systematics, P. Chimwamurombe for advice during preliminary screening for arenaviruses, and C. Priemer for technical support.

Trapping in Namibia was carried out under research permit nos. 1572/2011, 1666/2012 and 1794/2013, granted by Namibia’s Ministry of Environment and Tourism.

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Dr. Witkowski is a postdoctoral researcher at the Institute of Virology of the Charité Medical School in Berlin, Germany. His research interests are viral emerging infectious diseases on the African continent and their clinical impact and evolution.

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Readability of Ebola Information on Websites of Public Health Agencies, United States, United Kingdom, Canada, Australia, and Europe

Enrique Castro-Sánchez, Elpiniki Spanoudakis, Alison H. Holmes

Public involvement in efforts to control the current Ebola virus disease epidemic requires understandable information. We reviewed the readability of Ebola information from public health agencies in non–Ebola-affected areas. A substantial proportion of citizens would have difficulty understanding existing information, which would potentially hinder effective health-seeking behaviors.

The outbreak of Ebola virus disease (EVD) that originated in Guinea in April 2014 has become the largest known epidemic of this pathogen and was declared an international public health emergency (1). In addition, repatriation of health care workers and volunteers to Europe and the United States has resulted in human-to-human transmission in western health care organizations (2), thus bringing Ebola to the fore of public attention in settings far removed from local outbreak areas.

Currently, because there is no antiviral treatment or vaccine, surveillance and strict observation of recommended infection prevention and control measures, aided by public awareness regarding symptoms and prompt health care-seeking behavior, are essential efforts to control Ebola. In Africa, low awareness has led to community misunderstandings and unwillingness to cooperate with medical teams (3). In non–EVD-affected countries, nonrigorous information has resulted in unfounded fear among health care workers and citizens, disrupting the activity of hospitals caring for persons with EVD (4).

For health messages to be followed effectively, they must be tailored to the health literacy of the audience. Health literacy, which refers to “the cognitive and social skills which determine the motivation and ability of individuals to gain access to, understand and use information in ways which promote and maintain good health” (5), has been associated with better self-care (6). However, a substantial proportion of citizens worldwide have insufficient or inadequate health literacy (7).

Several factors, including readability of information provided (8), can help reduce health literacy deficits. Readability refers to “the determination of the reading comprehension level a person must have to understand written materials” (9). It is recommended that health information materials should be written at a level typically understandable by an 11-year-old person (10). Such recommendations for clarity and understandability might be more effective if one considers that anxiety or panic attributed to a highly virulent infection, such as Ebola, might hinder comprehension of related information (11).

We examined readability of EVD public information available from selected public health agencies in non–EVD-affected countries. Countries that have EVD should explore how well this information would serve to reduce panic and anxiety and perform as an effective source of advice for the public.

The Study

Current information on Ebola aimed at the public was downloaded from various websites; a list is provided in online Technical Appendix (http://wwwnc.cdc.gov/EID/article/21/7/14-1829-Techapp1.pdf). Information was retrieved from the European Centre for Disease Control (Ebola factsheet for the general public); the US Centers for Disease Prevention and Control (CDC; Questions and answers on Ebola); Public Health England (PHE) in the United Kingdom (Ebola: public health questions and answers); and the government of Canada (Ebola virus disease) on September 1, 2014 and from the government of Australia (Ebolavirus disease outbreaks in West Africa: important information for travellers, patients and consumers) and the World Health Organization (WHO; Advice for individuals and families. Ebola guidance package) on November 11, 2014.

Any figures, such as maps or pictograms, were removed, and content was then formatted as plain text and uploaded to a free online tool (http://www.readabilityformulas.com/free-readability-formula-tests.php/) from which different readability indicators were obtained (online Technical Appendix). The causes, symptoms, risks, treatment, prevention, and surveillance pages in the Canadian website were individually opened and
Our analyses indicate that the information on EVD provision was written at a higher than recommended reading level. For such a reason, a substantial proportion of citizens with low literacy in the United States, United Kingdom, Canada, Australia, and Europe would have difficulty understanding key EVD messages. These results are of concern because poor readability might prevent or delay adoption of appropriate health-seeking behaviors, prolong ineffective self-care strategies, and perpetuate stigmatizing attitudes toward Ebola.

Providing adequate EVD information for the public might be arduous. Uncertainties remain regarding optimal clinical management for Ebola patients and disagreements in infection prevention and control protocols. The continued modification of procedures also demands constant public engagement efforts to avoid dissemination of conflicting messages and to ensure that information released is up to date and presented at a level that can be adequately understood. Because there have been limited national communication campaigns in non-EVD-affected countries, it is likely that other outlets, including traditional mass media and social media, might have been used by the public to meet their information needs (13), with probable trade-offs between immediacy and accuracy or reliability of information provided. The variation of readability identified in our study suggests that with contributions from health literacy specialists, public health agencies could further adapt the EVD information provided.

We recognize that persons accessing health information online are not representative of the average population because they are more educated and benefit from better information-seeking skills and health literacy (14). Thus,

### Conclusions

Our analyses indicate that the information on EVD provided on websites of different public health agencies is written at a higher than recommended reading level. For such a reason, a substantial proportion of citizens with low literacy in the United States, United Kingdom, Canada, Australia, and Europe would have difficulty understanding key EVD messages. These results are of concern because poor readability might prevent or delay adoption of appropriate health-seeking behaviors, prolong ineffective self-care strategies, and perpetuate stigmatizing attitudes toward Ebola.

### Table. Readability of Ebola public information published by selected public health agencies*

<table>
<thead>
<tr>
<th>Selection website</th>
<th>Readability formula</th>
<th>Gunning Fog Index (hard to read)</th>
<th>Flesch Reading Ease Score (hard to read)</th>
<th>Automated Readability Index (17–18 y old)</th>
<th>Government of Canada (16.38)†</th>
<th>WHO (NA) (10.3)</th>
<th>Government of Australia (12.55)†</th>
<th>Mean ± SD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECDC (20.0)†</td>
<td>13.7</td>
<td>13.9</td>
<td>10.7</td>
<td>11.6</td>
<td>12.9</td>
<td>10.3 (fairly)</td>
<td>14.1</td>
<td>12.6 ± 1.68</td>
</tr>
<tr>
<td>PHE (16.40)†</td>
<td>48.2</td>
<td>45.4</td>
<td>53</td>
<td>7.8</td>
<td>11.8</td>
<td>easy to read</td>
<td>62.3</td>
<td>(10.83–14.36)</td>
</tr>
<tr>
<td>CDC (17.49)†</td>
<td>(fairly difficult to read)</td>
<td>(fairly difficult to read)</td>
<td>(fairly difficult to read)</td>
<td>(18–19 y old)</td>
<td>11.8</td>
<td>(standard/avg)</td>
<td>42</td>
<td>48.85 ± 7.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(12–14 y old)</td>
<td>8.6</td>
<td>(difficult to read)</td>
<td>(difficult to read)</td>
<td>(40.69–57.00)</td>
</tr>
<tr>
<td>Coleman-Liau Index (12th grade)</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>13 (college)</td>
<td>9 (9th grade)</td>
<td>11</td>
<td></td>
<td>11.16 ± 1.47</td>
</tr>
<tr>
<td>SMOG Index (11th grade)</td>
<td>10.7</td>
<td>11</td>
<td>9.4 (9th grade)</td>
<td>11.1</td>
<td>8.4 (8th grade)</td>
<td>(11th grade)</td>
<td>11.5</td>
<td>(9.62–12.71)</td>
</tr>
<tr>
<td>Linsear Write Formula (13 college)</td>
<td>14.1 (college)</td>
<td>8.4 (8th grade)</td>
<td>12.6 (college)</td>
<td>9.5</td>
<td>14.1 (college)</td>
<td>(12th grade)</td>
<td>11.95 ± 2.42</td>
<td>(9.09–11.60)</td>
</tr>
<tr>
<td>Flesch-Kincaid US Grade Level (11th grade)</td>
<td>11.3</td>
<td>12.1</td>
<td>9.2 (9th grade)</td>
<td>11.8</td>
<td>8.8 (9th grade)</td>
<td>(10th grade)</td>
<td>12.4</td>
<td>(9.40–14.49)</td>
</tr>
</tbody>
</table>

*ECDC, European Centre for Disease Control; PHE, Public Health England; CDC, US Centers for Disease Control and Prevention; WHO, World Health Organization; NA, not applicable; avg, average; SMOG, simple measure of gobbledygook. Items in parentheses are general assessments, age levels, or US-equivalent grade levels.

†Percentage of adults 16–65 years of age with literacy proficiency below reading level recommended for health information materials. ECDC percentage refers to a sample of 17 European Union Member States (12).
the online audience might be able to make more effective use of information on websites analyzed. However, such might not be the case for persons whose first language is not English, who might find information provided even more difficult to understand because of linguistic and cultural barriers.

It is accepted that readability measures alone may not reflect the level at which information is written (15). Because the Ebola epidemic has continued since our analysis, it might be possible for currently available information to have been modified and display greater readability. Our analysis was not exhaustive because we assessed selected public health agencies in non–EVD-affected countries and concentrated in English language materials. Therefore, our findings might not be representative of all health pages with EVD information. However, we evaluated key official websites.

Public health agencies in non–EVD-affected countries must improve the readability of EVD information currently provided so that the public could adopt effective self-care strategies, avoid fear, and reduce unnecessary panic and stigma toward persons affected by Ebola. In addition, agencies should consider multimodal Ebola awareness campaigns, including social marketing interventions, to encourage and strengthen public participation in Ebola control efforts.

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E.C.-S. was responsible for the design of the study and collected data. A.H.H. provided technical input during all stages of the project and analysis. All authors were responsible for data analysis, contributed substantially to writing the manuscript, approved its final version, had full access to all data in the study, and take responsibility for the integrity, accuracy, and presentation of data. E.C.-S. is the guarantor.

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References

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Evaluation of Patients under Investigation for MERS-CoV Infection, United States, January 2013–October 2014

Eileen Schneider, Christina Chommanard, Jessica Rudd, Brett Whitaker, Luis Lowe, Susan I. Gerber

Middle East respiratory syndrome (MERS) cases continue to be reported from the Middle East. Evaluation and testing of patients under investigation (PUIs) for MERS are recommended. In 2013–2014, two imported cases were detected among 490 US PUIs. Continued awareness is needed for early case detection and implementation of infection control measures.

Middle East respiratory syndrome coronavirus (MERS-CoV) infection was first reported in September 2012 in a patient with fatal pneumonia in Saudi Arabia (1). Subsequent investigation showed that earlier MERS-CoV infection had occurred in Jordan in April 2012 among a cluster of patients with pneumonia (2,3). As of February 5, 2015, the World Health Organization had reported 971 laboratory-confirmed cases worldwide and at least 356 related deaths (4). All known reported cases have been linked directly or indirectly to the Middle East region; most have been reported by Saudi Arabia and the United Arab Emirates. Typically, the initial symptoms for MERS patients seeking medical care are fever, chills, cough, shortness of breath, and myalgia. These symptoms often progress to severe lower respiratory tract infection, which may require mechanical ventilation and intensive care (3,6). Several asymptomatic or mild MERS cases have been reported (7), particularly in healthy young adults. Little is known about transmission routes, virus shedding, risk factors, and animal reservoirs, although bats and camels have been implicated in transmission and as reservoirs (8,9). Clusters of human-to-human transmission have been associated with household and health care settings (2,3,5).

Using World Health Organization guidelines and definitions (4), CDC developed guidance for evaluating a patient under investigation (PUI) for MERS-CoV infection, collecting specimens, conducting laboratory testing, and managing infection control (http://www.cdc.gov/coronavirus/mers/index.html). The PUI guidance was created to assist health care providers determine which patients should be considered for MERS-CoV evaluation and testing. To inform state and local health departments of the basic demographic and clinical characteristics of PUIs and on assay use, we summarized the descriptive analysis of PUIs in the United States.

The Study
In October 2012, CDC developed real-time reverse transcription PCR (rRT-PCR) assays for detection of MERS-CoV (10). CDC initially performed the testing, but on June 5, 2013, a Food and Drug Administration–issued Emergency Use Authorization allowed for assay deployment in a kit to laboratories through the Laboratory Response Network. As of March 12, 2015, a total of 47 states and the District of Columbia had MERS-CoV testing capability. The assay kit is intended for detection of MERS-CoV RNA in respiratory, serum, and stool samples. CDC also developed serologic tests for detecting MERS-CoV antibodies; these tests have been used by CDC since the summer of 2013. Because MERS-CoV is an emerging pathogen, CDC guidelines and guidance regarding PUI characteristics are periodically updated as new MERS-CoV information and risk factors are identified. CDC recommends evaluating and testing PUIs for MERS-CoV and for other common respiratory pathogens.

On January 1, 2013, CDC began collecting data on PUIs for MERS-CoV infection. Health care providers for persons suspected of having MERS were to contact their state or local health department for consultation and to arrange for MERS-CoV testing, if indicated. PUIs were reported to CDC through state and local health departments by using the single-page PUI short form, which contains no personal identifiers (11). Since its implementation, the short form has been revised 3 times to reflect modifications to the PUI guidance. The short form collects information on basic demographic data, symptoms, disease severity, hospitalization, travel history, risk factors, and laboratory test results at the time of MERS-CoV testing. Follow-up data collection on missing information was not routinely performed. At least 370 (76%) PUIs met the guidance characteristics for PUI for MERS. The remaining 120 (24%) PUIs had incomplete clinical or travel data; the most common missing information was pneumonia data for persons with respiratory symptoms and a recent travel history. The short form was sent electronically to CDC by secure fax or email. Data collected on the short form was entered into a...
Patients under investigation for MERS-CoV

CDC database by using Microsoft Access (Microsoft Corporation, Redmond, WA, USA). SAS version 9.3 (SAS Institute, Cary, NC, USA) was used for data analysis.

During January 1, 2013–October 31, 2014, a total of 490 PUIs were reported to CDC from 45 states and the District of Columbia (Figures 1, 2; Table 1). Of the 490 PUIs, 381 (78%) reported traveling from the Arabian Peninsula or neighboring countries to the United States within 14 days before illness onset (Table 2). A total of 113 (23%) PUIs also reported having close contact with a recently ill traveler from the Arabian Peninsula or neighboring countries within 14 days of symptom onset; the most common contacts were with persons from Saudi Arabia (55/113 [49%]), United Arab Emirates (10/113 [9%]), and Qatar (9/113 [8%]). Non-US residents accounted for 113 (23%) of the PUIs.

The most commonly reported symptoms were cough, fever, and shortness of breath (Table 1). A total of 292 (60%) PUIs were hospitalized, of whom 112 (38%) were admitted to the intensive care unit and 61 (21%) required mechanical ventilation. The most commonly reported underlying conditions among PUIs were immunosuppression and diabetes. Eleven (2%) PUIs died.

In total, 488 PUIs tested negative for MERS-CoV by rRT-PCR, serologic assay, or both. In May 2014, two PUIs tested positive for MERS-CoV by serologic assay and rRT-PCR in serum and respiratory samples, including lower respiratory tract specimens. These 2 patients were health care providers in whom respiratory symptoms had developed within 14 days of travel from Saudi Arabia; both cases were identified as imported MERS (12, 13). Neither patient required mechanical ventilation.

The most commonly detected pathogens among the 490 PUIs were influenza A virus and rhinovirus/enterovirus; however, for 359 PUIs (73%), other pathogen testing was not performed or detected pathogens were not reported (Table 1). Timely reporting of PUIs to CDC may have influenced reporting of pending non–MERS-CoV etiologic pathogen test results.

Conclusions

Currently in the United States, the preferred method for detecting MERS in PUIs with recent symptom onset is to test lower respiratory, naso-oropharyngeal, and serum specimens by using the CDC rRT-PCR assay. For PUIs in whom symptom onset occurred ≥2 weeks before specimen collection, testing using the CDC MERS-CoV
Table 1. Characteristics of 490 PUIs for MERS-CoV, United States, January 1, 2013–October 31, 2014*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>296 (60.4)/186 (38.0)</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>48 (0.3–89)</td>
</tr>
<tr>
<td>Symptom</td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>393 (80.2)</td>
</tr>
<tr>
<td>Fever</td>
<td>388 (79.2)</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>220 (44.9)</td>
</tr>
<tr>
<td>Chills†</td>
<td>153 (35.1)</td>
</tr>
<tr>
<td>Myalgia†</td>
<td>140 (32.1)</td>
</tr>
<tr>
<td>Sore throat†</td>
<td>134 (27.4)</td>
</tr>
<tr>
<td>Headache†</td>
<td>106 (24.3)</td>
</tr>
<tr>
<td>Diarrhea†</td>
<td>58 (13.3)</td>
</tr>
<tr>
<td>Abdominal pain†</td>
<td>34 (7.8)</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>292 (59.8)</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>112 (38.4)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>61 (20.9)</td>
</tr>
<tr>
<td>Clinical finding</td>
<td></td>
</tr>
<tr>
<td>Pneumonia‡</td>
<td>236 (48.2)</td>
</tr>
<tr>
<td>Acute respiratory distress syndrome‡</td>
<td>48 (8.8)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>22 (4.5)</td>
</tr>
<tr>
<td>Died†</td>
<td>11 (2.2)</td>
</tr>
<tr>
<td>Underlying condition†</td>
<td></td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>55 (12.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>40 (9.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>27 (6.2)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>23 (5.3)</td>
</tr>
<tr>
<td>Asthma</td>
<td>20 (4.6)</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>11 (2.5)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>9 (2.1)</td>
</tr>
<tr>
<td>Pregnant</td>
<td>8 (1.8)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>7 (1.6)</td>
</tr>
<tr>
<td>Other†</td>
<td>12 (2.8)</td>
</tr>
<tr>
<td>Specific job classification†</td>
<td></td>
</tr>
<tr>
<td>Health care worker</td>
<td>35 (8.0)</td>
</tr>
<tr>
<td>US military</td>
<td>9 (2.1)</td>
</tr>
<tr>
<td>MERS-CoV rRT-PCR testing</td>
<td></td>
</tr>
<tr>
<td>Specimen type</td>
<td></td>
</tr>
<tr>
<td>Upper respiratory</td>
<td>414 (84.5)</td>
</tr>
<tr>
<td>Lower respiratory</td>
<td>242 (49.4)</td>
</tr>
<tr>
<td>Serum</td>
<td>235 (48)</td>
</tr>
<tr>
<td>Stool</td>
<td>40 (8.2)</td>
</tr>
<tr>
<td>MERS-CoV positive</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Serologic testing for MERS-CoV</td>
<td></td>
</tr>
<tr>
<td>Tested†</td>
<td>67 (13.7)</td>
</tr>
<tr>
<td>MERS-CoV positive</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Other pathogens detected§</td>
<td></td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>41 (8.4)</td>
</tr>
<tr>
<td>Rhinovirus/enterovirus</td>
<td>37 (7.6)</td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>13 (2.7)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>11 (2.2)</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>6 (1.2)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>5 (1.0)</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>Parainfluenzavirus</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Chlamydophila pneumoniae</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Other†</td>
<td>16 (3.3)</td>
</tr>
</tbody>
</table>

*Data are no. (%) unless otherwise specified. MERS-CoV, Middle East respiratory syndrome coronavirus; PUI, patient under investigation; rRT-PCR, real-time reverse transcription PCR.
†Included only in 2 most recent versions of PUI short form (N = 436).
‡Forty-one PUIs had pneumonia and acute respiratory distress syndrome.
§Etiologic pathogen not reported for 359 (73%) PUIs; ≥1 pathogen identified for some PUIs.

Table 2. Countries from which 381 PUIs for MERS-CoV infection had traveled within 14 days of symptom onset, United States January 1, 2013–October 31, 2014*

<table>
<thead>
<tr>
<th>Country</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saudi Arabia</td>
<td>189 (49.6)</td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>60 (15.7)</td>
</tr>
<tr>
<td>Israel</td>
<td>45 (11.8)</td>
</tr>
<tr>
<td>Jordan</td>
<td>34 (8.9)</td>
</tr>
<tr>
<td>Qatar</td>
<td>27 (7.1)</td>
</tr>
<tr>
<td>Kuwait</td>
<td>22 (5.8)</td>
</tr>
<tr>
<td>Egypt</td>
<td>12 (3.1)</td>
</tr>
<tr>
<td>Bahrain</td>
<td>10 (2.6)</td>
</tr>
<tr>
<td>Oman</td>
<td>9 (2.4)</td>
</tr>
<tr>
<td>Iran</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>Iraq</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>Lebanon</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>Turkey</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>Pakistan</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td>Palestinian Territories</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td>Yemen</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td>Other†</td>
<td>13 (3.4)</td>
</tr>
</tbody>
</table>

*Patients may have been in >1 country. PUI, patient under investigation.
†Azerbaijan (1); Afghanistan (2); Bangladesh (1); Greece (1); India (2); Indonesia (2); Kenya (1); Morocco (1); Somalia (1); Syria (1).

The 2 US cases of imported MERS were detected in health care providers from Saudi Arabia. These cases prompted a CDC guidance update recommending evaluation and testing of persons with less severe respiratory illness who had strong epidemiologic risk factors, particularly health care exposure, for MERS-CoV infection. Occupation, recent travel history, recent visit to a health care facility, and contact with ill persons should be determined when evaluating persons with respiratory illness. As testing increases, especially serologic testing, additional MERS cases, including mildly symptomatic cases and cases among younger persons are being identified. These cases highlight the range of severity of MERS-CoV infection and the need to consider testing persons with a compatible travel history who may not match the clinical profile of the initially described case-patients. CDC plans to revise MERS-CoV guidance as needed.
Patients under investigation for MERS-CoV

Acknowledgment
We thank the state and local health departments, healthcare providers, CDC Emergency Operations Center, and CDC MERS Domestic Response Team for all of their hard work on MERS-CoV, especially in evaluating, testing, and reporting PUIs.

Dr. Schneider is a senior medical epidemiologist with the Division of Viral Diseases, CDC. Her current research interests include respiratory viruses.

References
aa=1

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EID Podcast: Carbapenem-Resistant Enterobacteriaceae

Dr. Mike Miller reads an abridged version of the article,
Deaths Attributable to Carbapenem-Resistant Enterobacteriaceae Infections

http://www2c.cdc.gov/podcasts/player.asp?f=8633574
Wildlife Reservoir for Hepatitis E Virus, Southwestern France

Sebastien Lhomme, Sokunthea Top, Stephane Bertagnoli, Martine Dubois, Jean-Luc Guerin, Jacques Izopet

Pigs are a reservoir for hepatitis E virus (HEV). To determine the relative contribution of game to the risk for human HEV infection in southwestern France, we tested wildlife samples. HEV RNA was in 3.3% of wildlife livers, indicating that in this region, eating game meat is as risky as eating pork.

Hepatitis E virus (HEV) is a causative agent of acute hepatitis worldwide. According to the Ninth Report of the International Committee on the Taxonomy of Viruses (http://ictvonline.org/), HEV is the sole member of the genus Hepevirus in the family Hepeviridae. HEV is a nonenveloped, single-stranded, positive-sense RNA virus containing ≈7.2 kb. Its genome contains 3 open reading frames (ORFs)—ORF1, ORF2, and ORF3—which encode nonstructural proteins, the capsid protein, and a small protein involved in virus egress, respectively (1).

Phylogenetic analysis of HEV sequences has led to the identification of 4 major genotypes (1). Genotypes 1 (HEV1) and 2 (HEV2) are pathogenic to humans only. HEV1 is present mainly in Asia and Africa, and HEV2 is in Africa and Mexico. In developing countries, HEV1 and HEV2 transmission is waterborne because of inadequate sanitary conditions. Genotypes 3 (HEV3) and 4 (HEV4) infect not only humans but also pigs, wild boars, deer, and other mammals. HEV3 is widespread, but HEV4 occurs primarily in Asia and was recently introduced into Europe (1). Pigs are a major reservoir of HEV3 and HEV4 (2); however, in recent years, the host range of HEV has expanded substantially (3).

HEV is hyperendemic to the Midi-Pyrénées area of southwestern France; annual incidence of cases among humans is 3.2% (4), and seroprevalence among blood donors has reached 52.5% (5). A multivariate analysis reported that the only factor associated with autochthonous HEV infection in this region was the consumption of game meat (6). However, the prevalence of HEV RNA in wildlife, especially wild boars and deer, has yet to be explored. Identifying the most commonly infected animals (sources of transmission) could help prevent zoonotic foodborne transmission. HEV strains have been recently identified in rabbits (7). Because HEV strains in rats have been recently described (8), we questioned the capacity of coypu to act as an HEV reservoir. Coypu are large, herbivorous, semiaquatic rodents that usually live in fresh or brackish water. In this study, we assessed the prevalence of HEV RNA among wild boars (Sus scrofa), deer (Cervus elaphus), rabbits (Oryctolagus cuniculus), and coypu (Myocastor coypus) and, thus, the potential for these animals to act as sources of HEV infection for persons living in the Midi-Pyrénées area.

The Study

Samples of liver and bile were collected from 86 wild boars, 62 deer, 20 wild rabbits, and 78 coypu in the Midi-Pyrénées area. The wild boars and deer were hunted from February 2010 through January 2011, rabbits were hunted from October 2013 through February 2014, and coypu were hunted in April 2011.

RNA was extracted from 30 mg of liver by using RNAeasy Mini Kits or from 140 μL of bile by using QIAamp Viral RNA Mini Kits as specified by the manufacturer (QIAGEN, Courtaboeuf, France). Real-time PCR based on ORF3 was used to detect and quantify HEV RNA in plasma samples as previously described (9). The limit of detection was 100 copies/mL.

HEV RNA was detected in 5 (5.8%) wild boar livers, 2 (3.2%) deer livers, 1 (5.0%) wild rabbit liver, and no coypu livers (Table). Thus, the overall prevalence of HEV RNA among wildlife, irrespective of species, was 3.3% (8/246) (95% CI 1.1%–5.5%). Because bile samples were available only from animals with negative HEV RNA liver samples, no bile sample was positive for HEV RNA.

Conclusions

The overall prevalence of HEV RNA in game (3.3%) is similar to that among pigs. A recent nationwide study in France reported that HEV RNA was present in the livers of about 4% of farmed pigs of slaughter age (10). These contaminated livers can then enter the food chain and be

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prevalence of HEV RNA in wild boars is similar to that previously
reported for pigs. Consumption of the meat of these wild
animals, and of pig liver sausage, all contribute to the HEV
epidemiology in the Midi-Pyrénées area because of specific
local eating habits. Game meat from this part of France
should be cooked thoroughly to minimize the risk for
HEV infection (15).

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Dr. Lhomme is a researcher in the Virology Department at
Toulouse University Hospital. His main research interest is the
genetic variability of HEV.

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Table. Hepatitis E virus RNA among wildlife, southwestern France*

<table>
<thead>
<tr>
<th>Source, dates collected</th>
<th>No. tested</th>
<th>No. (%) HEV RNA positive</th>
<th>Virus concentration, median log copies/g (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild boars, 2010–2011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>86</td>
<td>5 (6.8)</td>
<td>2.80 (1.57–8.05)</td>
</tr>
<tr>
<td>Bile</td>
<td>29</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Deer liver, 2010–2011</td>
<td>62</td>
<td>2 (3.2)</td>
<td>2.78 (1.11–3.07)</td>
</tr>
<tr>
<td>Wild rabbits, 2013–2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>20</td>
<td>1 (5.0)</td>
<td>8.70</td>
</tr>
<tr>
<td>Bile</td>
<td>13</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Coypu liver, 2011</td>
<td>78</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA, not applicable.


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February 2015: Complicated Datasets

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• Entry Screening for Infectious Diseases in Humans
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• Tickborne Relapsing Fever, Bitterroot Valley, Montana, USA
• Simulation Study of the Effect of Influenza and Influenza Vaccination on Risk of Acquiring Guillain-Barré Syndrome
• Evidence for Elizabethkingia anophelis Transmission from Mother to Infant, Hong Kong
• Microbiota that Affect Risk for Shigellosis in Children in Low-Income Countries
• pH Level as a Marker for Predicting Death among Patients with Vibrio vulnificus Infection, South Korea, 2000–2011

http://wwwnc.cdc.gov/eid/content/21/2/contents.htm
Asymptomatic Malaria and Other Infections in Children Adopted from Ethiopia, United States, 2006–2011

Senait M. Adebo, Judith K. Eckerle, Mary E. Andrews, Cynthia R. Howard, Chandy C. John

We screened 52 children adopted from Ethiopia for malaria because they had previously lived in a disease-endemic region or had past or current hepatomegaly or splenomegaly. Seven (13.5%) children had asymptomatic malaria parasitemia by microscopy (n = 2) or PCR (n = 5). Our findings suggest that adoptees at risk for asymptomatic malaria should be screened, preferably by PCR.

International adoptees are at increased risk for infectious diseases (1). During 2007–2012, Ethiopia was 1 of the top 5 countries of origin for children who were adopted by persons in the United States (2), but few studies have been published on children from Ethiopia who were adopted by persons in the United States (3). Malaria caused by Plasmodium falciparum, P. vivax, and, less frequently, P. ovale is endemic to several regions in Ethiopia (4). Children adopted from Ethiopia are often living in orphanages in Addis Ababa, an area free of malaria, at the time of their adoption, but they may have lived in a malaria-endemic area before their transfer to the orphanage. The prevalence of asymptomatic malaria parasitemia among these children is not known.

The Study
We reviewed medical records of all children adopted from Ethiopia and seen at the University of Minnesota International Adoption Clinic (Minneapolis, MN, USA) during February 2006–June 2011 for results of standard infectious disease screening tests recommended by the American Academy of Pediatrics: tuberculosis (by tuberculin skin test or, in children ≥5 years old, by interferon-γ release assay); intestinal parasites (fecal testing for ova, parasites, and Giardia intestinalis antigen); hepatitis B or C virus; HIV; and syphilis (5). Children were screened for hepatitis A virus at the discretion of the physician seeing the patient and for malaria by blood smear or PCR if they met screening criteria (i.e., history of living in a malaria-endemic region or a history of or current evidence of splenomegaly or hepatomegaly). The study was reviewed and approved by the University of Minnesota Institutional Review Board.

During the period studied, 255 international adoptees from Ethiopia were seen at the clinic. Adoptees’ mean age at medical evaluation was 2.8 years (range 3.4 months–14.9 years); 148 (58%) were female and 107 (41.9%) were male. All 255 children were asymptomatic for malaria, but 52 met malaria screening criteria and were tested by peripheral blood smear (n = 24), PCR (n = 24), or both (n = 4). Of the 52 children, 7 (13.5%) had blood smear (2 children) or PCR (5 children) results positive for Plasmodium species. Table 1 outlines the sensitivity, specificity, and negative and positive predictive values of medical history questions and physical exam signs for asymptomatic malaria. The 2 children with a positive blood smear had low parasite densities (<0.1%), and the species could not be identified. These 2 children were treated before PCR testing was available. Subsequently, PCR became the preferred first-line diagnostic test, and 5 infections were diagnosed on the basis of PCR results: 3 P. vivax, 1 P. falciparum, and 1 mixed P. vivax and P. falciparum. Among the 7 children with parasitemia, 2 had a palpable spleen tip, 2 had a hemoglobin level of <11 g/dL (reference 11–15 g/dL), and none had thrombocytopenia. All children with a positive blood smear or PCR result were treated: atovaquone/proguanil for P. falciparum infections, chloroquine followed by primaquine for P. vivax infections, and atovaquone/proguanil followed by primaquine for the mixed infection and infections with no species identified.

In addition to the malaria results, of 217 children tested for intestinal parasites, 96 (44.2%) had positive results; Giardia intestinalis flagellates were most common (n = 75, 34.6%), followed by Blastocystis hominis protozoa (n = 34, 15.7%) (Table 2). Evidence of tuberculous infection was found in 49 (27.1%) children, hepatitis A virus in 14 (8.7%), hepatitis B virus in 6 (2.6%), and HIV in 1 (0.5%) (Table 2).

Conclusions
In this study, we show that 7 (13.5%) of 52 adoptees from Ethiopia who had lived in a malaria-endemic region or had hepatomegaly or splenomegaly by clinical history or on physical examination had asymptomatic malaria...
parasitemia. We also confirm the findings of previous studies that showed high rates of infection with intestinal parasites (particularly G. intestinalis flagellates) (3), latent tuberculosis (3), and hepatitis A virus (6) in adoptees from Ethiopia.

The rate of asymptomatic malaria parasitemia in international adoptees is not known. As adoptions increase from countries in sub-Saharan Africa and other countries with areas of potential malaria transmission, such as India and Haiti, malaria screening will need to be considered for the adopted children. On the basis of the current data, we believe reasonable first-line criteria for malaria screening in international adoptees are residence in a malaria-endemic country plus either lack of documentation that the child lived for his or her whole life in a region of that country that was malaria free (e.g., Addis Ababa in Ethiopia) or past or current splenomegaly. However, a limitation of our study is that we used essentially these criteria to screen, and the prevalence of asymptomatic malaria might have differed if we used different criteria. For example, we did not screen all children with anemia (hemoglobin level of <11 g/dL); because malaria is a leading cause of anemia in malaria-endemic areas, anemia may be a useful additional screening criterion for malaria.

For over a century, microscopy has been the standard for documentation of malaria infection in persons with clinical malaria, but PCR has greater sensitivity for detection of low-level parasitemia (7) and is now a field standard for detection of asymptomatic parasitemia (8). Multiplex PCR also enables testing and identification of all 5 Plasmodium species that cause disease in humans and can provide species identification at low levels of parasitemia. In our study, microscopy testing on 2 children failed to determine the malaria species, a common difficulty in persons with low-level parasitemia. Without knowing the malaria species, we had to treat the children for both P. falciparum and P. vivax infection, which involved testing for glucose-6-phosphate dehydrogenase deficiency and treatment with multiple antimalarial medications. Knowledge of the prevalent species in immigrants from a specific area can also inform public health efforts and prophylaxis planning for travelers to that area. For these reasons, PCR is likely the test of choice for detection of asymptomatic parasitemia in children adopted from malaria-endemic areas.

For 3 reasons, we treated all adopted children with asymptomatic parasitemia, whether detected by PCR or microscopy. First, a diagnosis of malaria could be missed if these children became febrile. After their adoption, many lived in areas in which malaria is rarely if ever seen, so the diagnosis of malaria might not be considered. Second, malaria can cause severe disease, so a missed diagnosis could have major health consequences for the child. Third, most antimalarial medications have a low toxicity, so treatment is not a danger to the child.

Table 1. Value of certain characteristics or findings for predicting asymptomatic malaria parasitemia in children adopted from Ethiopia who were seen at the University of Minnesota International Adoption Clinic, Minneapolis, Minnesota, USA, 2006–2011*

<table>
<thead>
<tr>
<th>Characteristic or finding</th>
<th>Malaria, no. (%)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of hepatomegaly</td>
<td>Positive, n = 7†</td>
<td>1 (2.2)</td>
<td>0</td>
<td>97.8</td>
<td>0</td>
</tr>
<tr>
<td>History of splenomegaly</td>
<td>Negative, n = 45</td>
<td>1 (2.2)</td>
<td>0</td>
<td>97.8</td>
<td>0</td>
</tr>
<tr>
<td>Presence of splenomegaly</td>
<td>3 (42.8)</td>
<td>2 (4.4)</td>
<td>92.8</td>
<td>88.3</td>
<td>0</td>
</tr>
<tr>
<td>during examination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of hepatomegaly</td>
<td>1 (14.3)</td>
<td>1 (14.3)</td>
<td>84.4</td>
<td>72.0</td>
<td>14.3</td>
</tr>
<tr>
<td>during examination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of splenomegaly</td>
<td>3 (42.8)</td>
<td>4 (8.9)</td>
<td>91.1</td>
<td>91.1</td>
<td>4 (8.9)</td>
</tr>
<tr>
<td>or presence during</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>examination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin level of &lt;11 g/dL</td>
<td>2 (28.6)</td>
<td>6 (13.3)</td>
<td>87.7</td>
<td>25.0</td>
<td>88.7</td>
</tr>
</tbody>
</table>

*NPV, negative predictive value; PPV, positive predictive value.
†Children who were malaria-positive by blood smear or PCR testing.

Table 2. Prevalence of infectious diseases in children adopted from Ethiopia who were seen at the University of Minnesota International Adoption Clinic, Minneapolis, Minnesota, USA, 2006–2011

<table>
<thead>
<tr>
<th>Infection</th>
<th>No. screening results available</th>
<th>No. (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal parasites</td>
<td>217</td>
<td>96 (44.2)*</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>181</td>
<td>49 (27.1)†</td>
</tr>
<tr>
<td>Malaria</td>
<td>52</td>
<td>7 (13.5)</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>161</td>
<td>14 (8.7)</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>233</td>
<td>6 (2.6)</td>
</tr>
<tr>
<td>Syphilis</td>
<td>215</td>
<td>0††</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>219</td>
<td>0††</td>
</tr>
<tr>
<td>HIV</td>
<td>218</td>
<td>1 (0.5)†</td>
</tr>
</tbody>
</table>

*Evidence of infection with ≥1 of the following: Giardia intestinalis flagellates (n = 75, 34.6%), Blastocystis hominis protozoa (n = 34, 15.7%), Hypomneplosis nana tapeworms (n = 2, 0.9%), Dientamoeba fragilis protozoa (n = 2, 0.9%), Ascaris lumbricoides roundworms (n = 2, 0.5%), or Trichuris trichiura roundworms (0.5%).
†By tuberculin skin testing (induration ≥10 mm; n = 46), interferon-γ release assay (n = 1), or both (n = 2). Latent tuberculosis infection was diagnosed in 46 children. Tuberculosis disease was diagnosed initially in 1, but was later reassessed as latent tuberculosis infection; medications for disease were stopped after 4 months of treatment.
‡Initial screening tests results were positive in 2 additional children, but confirmatory tests were negative.
Asymptomatic Malaria in Children from Ethiopia

We now use PCR to screen asymptomatic children from malaria-endemic areas. We recommend this method for centers with rapid access to PCR for all 5 human *Plasmodium* species because increased sensitivity of detection is more important than rapid detection in asymmetrically infected children. However, any symptomatic child (e.g., a child with fever) must have microscopy or rapid diagnostic testing performed immediately, because these results are typically available quickly and can guide immediate decisions regarding treatment.

In summary, this study shows that children adopted from Ethiopia who lived in malaria-endemic regions of Ethiopia or had past or current splenomegaly are at risk for asymptomatic *P. falciparum* and *P. vivax* parasitemia. The study findings support the importance of obtaining a careful history to determine malaria risk and conducting PCR screening for asymptomatic infection in children with the noted risk factors. These findings may also be relevant to children adopted from other malaria-endemic countries.

Dr. Adebo works as a hospitalist pediatrician at the Children’s Hospital of Philadelphia. Her primary research interests are global health and international adoption issues.

References


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February 2014: High-Consequence Pathogens

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- Genomic Variability of Monkeypox Virus among Humans, Democratic Republic of the Congo

http://wwwnc.cdc.gov/eid/content/20/2/contents.htm
Distinct Lineages of Bufavirus in Wild Shrews and Nonhuman Primates

Michihito Sasaki, Yasuko Orba, Paulina D. Anindita, Akihiro Ishii, Keisuke Ueno, Bernard M. Hang’ombe, Aaron S. Mweene, Kimihito Ito, Hirofumi Sawa

Viral metagenomic analysis identified a new parvovirus genome in the intestinal contents of wild shrews in Zambia. Related viruses were detected in spleen tissues from wild shrews and nonhuman primates. Phylogenetic analyses showed that these viruses are related to human bufaviruses, highlighting the presence and genetic diversity of bufaviruses in wildlife.

Bufavirus (BuV), a recently described parvovirus, was initially discovered in the feces of a child with diarrhea in Burkina Faso in 2012 (1). Thereafter, BuV was identified in fecal samples from children and adults with gastroenteritis in Bhutan, Finland, and the Netherlands in 2014 (2–4), respectively. Genome sequences and phylogenetic analyses revealed that BuV comprised at least 3 genotypes and was distinct from all other known members of the Paroviridae family (1,2). The International Committee on Taxonomy of Viruses assigned BuV as a new species of the genus Protoparvovirus in the subfamily Parovirinae (5). Whether BuV is an etiologic agent of human gastroenteritis remains unclear, but knowledge about its distribution and genetic divergence in humans is accumulating. However, whether BuV infection exists in wildlife remains unanswered. Through use of metagenomics, we previously described the enteric virome of wild shrews of the Crocidura genus sampled at Mpulungu, Zambia, in 2012 (6). From this sequence dataset (GenBank/EMBL/DDBJ accession no. DRA002561), we identified sequence reads related to BuV. Here, we describe the genome of this new parvovirus.

The Study
We determined the nearly complete genome sequence of BuV, which we named Mpulungu BuV (MpBuV), by filling genome gaps with primer walking and rapid amplification of cDNA ends (GenBank/EMBL/DDBJ accession no. AB937988). The MpBuV genome comprises 4,613 nt and encodes open reading frames of the nonstructural protein (NS) 1 and the viral capsid protein (VP) 1 and VP2 (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/21/7/14-1969-Techapp1.pdf). blastp (http://blast.ncbi.nlm.nih.gov) searches showed that the MpBuV NS1, VP1, and VP2 proteins were closely related to those of human BuVs and the WUHARV parvovirus (E-value = 0.0). WUHARV parvovirus, identified in rhesus monkeys experimentally infected with simian immunodeficiency virus under laboratory conditions in the United States, was found to be a closely related to human BuVs (7).

In MpBuV, NS1 shares 52.5% aa identity with NS1 of human BuV (GenBank accession no. JX027296). We found that the amino acid sequence identity of VP1 between MpBuV and human BuV is 52.3%, whereas that of VP2 is 51.4%. Similar to human BuV, MpBuV showed potential splicing signals in the VP1 coding region. We also identified the parvovirus-conserved amino acid motifs in NS1, VP1, and VP2 of MpBuV (online Technical Appendix Figure) (8–12). Phylogenetic analysis was performed as described previously (6). A Bayesian phylogenetic tree was generated on the basis of the full-length NS1 proteins of MpBuV, human BuVs, and representative parvoviruses. MpBuV clustered with human BuVs and WUHARV parvovirus (online Technical Appendix Figure). According to the species demarcation criteria of the International Committee on Taxonomy of Viruses, each parvovirus species encodes an NS1 protein sharing <85% aa sequence identity with other known parvovirus species (5). The NS1 protein of MpBuV exhibited <58% sequence identity with that of any known parvovirus species; therefore, we propose that MpBuV is a new species within the Protoparvovirus genus.

Next, we performed PCR screening for MpBuV on shrews captured in Mpulungu. DNA was extracted from intestinal content suspensions and tissue specimens by using a High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany) and QIAamp DNA Mini kit (QIAGEN, Hilden, Germany), respectively. PCR was performed by using Tks Gflex DNA polymerase (TAKARA BIO, Otsu, Japan), forward primer MpBuV-F1 (position 2739–2763 in MpBuV genome, 5′-GAAGTGGTGTTGGTCATTCTCCTACTGGA-3′) and reverse primer MpBuV-R1 (position 3523 3546, 5′-GAAGTGGTGTTGGTCATTCTCCTACTGGA-3′)
Bufavirus in Wild Shrews and Nonhuman Primates

5′-GTTGGAGGTACACATGGATGAGGA-3′).

We detected the MpBuV genome in 5 (22%) of the 23 samples from the intestinal contents of individual shrews captured in Mpulungu. We then tested for the presence of MpBuV in the lung, spleen, liver, and kidney tissues of the shrews that were PCR positive for MpBuV by screening of intestinal contents. The MpBuV genome was detected in 5 spleen and 4 liver samples from the 5 shrews.

This discovery of MpBuV urged us to further investigate BuVs and related paroviruses in wildlife. We designed degenerate primers for nested PCR screening on the basis of a multiple sequence alignment of the NS1 gene from human BuV, WUHARV parvovirus and MpBuV as follows: BuV-F1 (position 190–215 in MpBuV genome, 5′-TCAAWRTMACCTGGAAAGACTACAGA-3′) and BuV-R1 (position 1503 1534, 5′-TCATTGGTTGTCATKAYWACTGGAGTTGGTTC-3′) for the first PCR round, and BuV-F2 (position 980–1006, containing an equimolar mixture of 5′-AGAAAAATGGATGCTCCAAGATCCA-GA-3′ and 5′-AGAAAAATGGATGCTCCAAGATCCA-GA-3′) and BuV-R2 (position 1444 1465, 5′-ATTGCTTG-GCCACTCTAGTAKG-3′) for the second PCR round. PCRs were performed by using Tks Gflex DNA polymerase and the annealing temperatures were set at 50°C and 55°C for the first and second PCR round, respectively.

We screened 536 spleen tissue specimens from wild life. The specimens from 3 nonhuman primate species, 2 shrew species, and 14 rodent genera from 6 locations in Zambia were used for different research projects, as reported previously (13,14). All our sampling activities were conducted with the permission of the Zambia Wildlife Authority (Act No. 12 of 1998). We chose spleen tissue for nested PCR screening because specimens of intestinal contents were unavailable for almost all of the animals we sampled. As we described, MpBuV was detected in spleen samples in addition to samples of intestinal contents from the same animals.

Nested PCR screening for BuVs detected MpBuV from 5 shrews in Mpulungu; these were the same animals that were PCR-positive for MpBuV by using a specific primer set targeting MpBuV. Nested PCR was also positive in 3 primates and 12 shrews (Table). The PCR ampli-...
in Livingstone, respectively. Both baboon-derived BuVs were closely related to the WUHARV parvovirus. These results indicate the presence of BuVs in wild nonhuman primates and in wild shrews.

Conclusions
The nearly complete genome sequence of a new parvovirus, MpBuV, was obtained from a wild shrew in this study. blastp searches indicated that each MpBuV open reading frame shared the highest amino acid identity with other known BuVs. Furthermore, our phylogenetic analysis showed that MpBuV clustered with BuVs but was distinct from any other known parvovirus. Accordingly, we propose that MpBuV should be considered a new species of BuV.

Our nested PCR screening identified 3 additional BuV strains: Solwezi BuV, Mfuwe BuV, and Livingstone BuV. These protoparvoviruses are also phylogenetically related to known BuVs and derived from wildlife (i.e., shrews and nonhuman primates). These results show the presence of human BuV-related genomes in wildlife expanding our knowledge of the distribution and genetic diversity of BuVs.

In summary, we investigated the situation regarding BuVs in Zambian wildlife. Thus far, no evidence exists of BuV transmission between humans and wildlife. Our nested PCR should be helpful for detecting BuVs in mammals and lead to better understanding of the distribution of BuVs.

Acknowledgments
We thank the Zambia Wildlife Authority for its support with this research.

This work was supported by the Global Institution for Collaborative Research and Education (GI-CoRE) and the Japan Initiative for Global Research Network of Infectious Diseases (J-GRID) from the Japan Ministry of Education, Culture, Sports, Science, and Technology and Grants-In-Aid for Scientific Research (KAKENHI, grant no. 24405043) from the Japan Society for the Promotion of Science.

Dr. Sasaki is a veterinary researcher at the Research Center for Zoonosis Control, Hokkaido University, and is certified as a zoonosis control expert by Hokkaido University. His research interests include the molecular basis of viral pathogenesis.

References

Figure. Partial nonstructural protein (NS) 1 gene phylogeny of newly identified bufaviruses, Zambia. The Bayesian phylogenetic tree was generated by using the partial NS1 gene fragments (434–440 bp) of bufaviruses and the corresponding region of known protoparvoviruses and amroviruses. Gray shading indicates bufaviruses identified in this study. GenBank accession numbers of viral sequences are shown in parentheses. Bayesian posterior probabilities are indicated at each tree root. Scale bar indicates nucleotide substitutions per site.
Bufavirus in Wild Shrews and Nonhuman Primates


Address for correspondence: Hirofumi Sawa, Division of Molecular Pathobiology, Hokkaido University, Research Center for Zoonosis Control, West 10 North 20, Kita-ku, Sapporo 001-0020, Japan; email: h-sawa@czc.hokudai.ac.jp

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- Population Structure and Antimicrobial Resistance of Invasive Serotype IV Group B Streptococcus, Toronto, Ontario, Canada
- Norovirus Genotype Profiles Associated with Foodborne Transmission, 1999–2012

Geographic Range Expansion for the Rat Lungworm in North America

Emily M. York, James P. Creecy, Wayne D. Lord, William Caire

Using quantitative PCR analysis and DNA sequencing, we provide evidence for the presence of the rat lungworm (Angiostrongylus cantonensis) in Oklahoma, USA, and identify a potentially novel rat host (Sigmodon hispidus). Our results indicate a geographic range expansion for this medically and ecologically relevant parasite in North America.

Emerging infectious diseases negatively impact humans and wildlife, causing disease outbreaks and deaths and local and global extinctions (1). Zoonotic disease emergence or re-emergence results from numerous factors (e.g., globalization of trade, increased interaction of humans and animals, anthropogenic climate change) that function independently or synergistically (2,3). Consequently, the means by which parasitic zoonoses are studied must be constantly advanced to promote identification, control, and prevention of outbreaks.

The rat lungworm, Angiostrongylus (Parastrongylus) cantonensis, causes eosinophilic meningitis in humans (4) and various disease manifestations (meningoencephalitis, neurologic disorders) in atypical host species, including wildlife and captive animals (5). Transmission of these worms occurs by ingestion of third-stage larvae in raw or undercooked intermediate or paratenic hosts (6). Although variable among geographic regions and within host species, the prevalence of rat lungworms might be high under favorable conditions (7).

The occurrence of A. cantonensis rat lungworms has been documented worldwide, and its distribution has been attributed largely to the spread of intermediate molluscan host species (e.g., Achatina fulica) and definitive rodent host species (e.g., Rattus spp.) (8). Moreover, host specificity of rat lungworms is highly plastic, which contributes to its continuous geographic expansion (4). These factors indicate that the rat lungworm is an emerging zoonotic pathogen of concern to humans and wildlife, and therefore provides an excellent opportunity to evaluate the sensitivity and effectiveness of epidemiologic surveying techniques.

The Study
We evaluated the current distribution and potential spread of the rat lungworm within areas of the Gulf Coast region and midwestern United States by sampling rodent populations in regions of Louisiana and Oklahoma that were predicted by an ecologic niche model to contain suitable and unsuitable habitat (9). We used a quantitative PCR (qPCR) TaqMan assay (Life Technologies, Foster City, CA, USA) (10) to test for the parasite in tissue samples and further evaluated these samples through DNA sequencing analysis.

We trapped animals during the spring, summer, and fall months during 2010–2012. A total of 42 rodents and 3 shrews were collected from McCurtain County in southeastern Oklahoma, and 47 rodents were collected in Louisiana (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/7/14-1980-Techapp.pdf). We also obtained 56 Rattus norvegicus rat brain and lung tissue samples from the City of New Orleans Mosquito, Termite, and Rodent Control Board. Blood, lung, and brain tissue samples were collected from the rodents. Flotation was performed on all 148 lung samples, and all samples were negative for adult A. cantonensis rat lungworms.

Known adult rat lungworms were used as controls for molecular analyses (online Technical Appendix). Cellular DNA was extracted from rodent blood and brain samples. We tested for rat lungworm internal transcribed spacer 1 (ITS1) DNA by using a TaqMan qPCR on an ABI 7500 system (10). A total of 134 blood samples and 137 brain samples contained DNA suitable for analysis. After qPCR, 34 of the 271 total tissue samples were classified as putatively positive for rat lungworm and sequenced, generating a 267-bp fragment of the ITS1 region (online Technical Appendix).

On the basis of DNA sequencing, 3 brain samples were identified as containing A. cantonensis DNA (GenBank accession nos. KP231729, KP231728, and KP231727). These brain tissue samples were obtained from 3 rodents (host catalog nos. 32, 70, and 76), which were identified as 1 Hispid cotton rat (Sigmodon hispidus) and 2 brown rats (Rattus norvegicus), respectively (Table 1). A comparison of the 3 brain samples with those in GenBank by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed a match with rat lungworm (GenBank accession nos. GU587762.1 and GU587759.1) (Table 2).

All sequences were aligned by using MUSCLE in MEGA 5.2 (http://www.megasoftware.net/), manually inspected for consensus, and compared with the 267-bp fragment generated from the known sample of rat lungworm. Maximum-likelihood phylogenetic analysis was performed by using sequence data for the ITS1 region of A. cantonensis (GenBank accession no. GU587759.1), 2 closely related species, A. vasorum...
Because endemic and novel pathogens require different and highly specialized disease management strategies, it is crucial to determine whether a pathogen is novel or endemic (12). Previous work has described the *A. cantonensis* rat lungworm as a novel pathogen in the southeastern United States. However, it is now characterized as endemic to this region, and our results strongly support this notion (11). Such changes in the epidemiologic classification of rat lungworms accentuate the need for techniques that monitor the extent to which parasites infiltrate new geographic areas and potentially pose threats to humans and native wildlife. One such threat includes an increasing prevalence of angiostrongyliasis, which should receive increased scrutiny in patients with eosinophilic meningitis from localities characterized by paratenic and intermediate hosts.

Rat lungworm was found in a previously undocumented mammalian host, *S. hispidus* rats, which strongly suggests that this parasite is an endemic pathogen. Although vegetation is their primary food source, *S. hispidus* rats will eat invertebrates (13). Whether these rats directly (by intentional consumption of host) or indirectly (by consumption of host or free third-stage larvae on vegetation) consume the parasite, we cannot rule out the possibility that acquisition of the parasite could occur in this species and enable further range expansion for rat lungworms. *S. hispidus* rats are a known host for another closely related *Angiostrongylus* species, *A. costaricensis*, which lends additional support for the notion that *S. hispidus* rats might act as a host for rat lungworms. Alternatively, *S. hispidus* rats might simply be an accidental/dead end host for this

### Table 1. DNA sequences generated from 3 rat brain samples positive for *Angiostrongylus cantonensis* rat lungworms, United States*

<table>
<thead>
<tr>
<th>Rat host species</th>
<th>Trapping location</th>
<th>Host catalog no.</th>
<th>Sequence, 5’→3’</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sigmodon hispidus</em></td>
<td>Red Slough WMA, OK</td>
<td>32</td>
<td>TTCATGGATGCGAAGCTAGTACGATCATCGCATATCTACTATACGATGTCATCGCATATCTACTATACGCATATRGTGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTATAATGTTTCAATTGGCGCCACCTATGAAACAGGACACTATTTTTCTACACGTGAAAAATGTGGAACGACGATACGAGGTATATAATATATATATATAACACATATATATGTTGTAATGGAATTGATATACTACGTTCAGCGATGGATCGGTCGATTCGCGTGATCTGAAAGGCATCTA</td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>New Orleans, LA</td>
<td>70</td>
<td>TTCATGGATGCGAAGCTAGTATCGCATATCTACTATACGATGTCATCGCATATCTACTATACGCATATRGTGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTATAATGTTTCATATGGCGCCACCTATGAAACAGGACACTATTTTTCTACACGTGAAAAATGTGGAACGACGATACGAGGTATATAATATATATATATAACACATATATATGTTGTAATGGAATTGATATACTACGTTCAGCGATGGATCGGTCGATTCGCGTGATCTGAAAGGCATCTA</td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>New Orleans, LA</td>
<td>76</td>
<td>TTCATGGATGCGAAGCTAGTATCGCATATCTACTATACGATGTCATCGCATATCTACTATACGCATATRGTGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTATAATGTTTCATATGGCGCCACCTATGAAACAGGACACTATTTTTCTACACGTGAAAAATGTGGAACGACGATACGAGGTATATAATATATATATATAACACATATATATGTTGTAATGGAATTGATATACTACGTTCAGCGATGGATCGGTCGATTCGCGTGATCTGAAAGGCATCTA</td>
</tr>
</tbody>
</table>

*WMA, wildlife management area.

### Table 2. BLAST* results for sequences from 3 rat brain samples, United States†

<table>
<thead>
<tr>
<th>Host catalog no.</th>
<th>Rat host species</th>
<th>Trapping location</th>
<th>Match, %</th>
<th>Coverage, %</th>
<th>e value</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td><em>Sigmodon hispidus</em></td>
<td>Red Slough WMA, OK</td>
<td>92</td>
<td>98</td>
<td>5 × 10⁻¹⁰⁵</td>
</tr>
<tr>
<td>70</td>
<td><em>Rattus norvegicus</em></td>
<td>New Orleans, LA</td>
<td>99</td>
<td>100</td>
<td>3 × 10⁻¹³⁰</td>
</tr>
<tr>
<td>76</td>
<td><em>R. norvegicus</em></td>
<td>New Orleans, LA</td>
<td>99</td>
<td>100</td>
<td>1 × 10⁻¹³³</td>
</tr>
</tbody>
</table>

†WMA, wildlife management area.
The parasite. Although wildlife might become infected with the parasite, not all wildlife are definitive hosts (5,11). Additional field and laboratory studies will clarify the role that S. hispidus rats play in the spread of the rat lungworm.

Because many terrestrial species remain taxonomically undescribed, there is strong potential for continual emergence of unknown pathogens worldwide (14). Global travel, human encroachment into wildlife habitat, and climate change will influence distribution and emergence of disease (2,15). By incorporating field epidemiology with molecular genetic techniques to determine the geographic distribution of pathogens, major advances can be made in preventing the spread of wildlife diseases to human populations. Our results illustrate this point and highlight the need for future work to incorporate and refine these techniques and their application to epidemiology and wildlife disease surveillance.

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This study was supported by the Office of Research and Grants, the W. Roger Webb Forensic Science Institute, and the Department of Biology at the University of Central Oklahoma.

Mrs. York is an integrated pest management and collections specialist at the University of Oklahoma Sam Noble Museum of Natural History. Her research interests include infectious disease surveillance, host–parasite ecology, and animal behavior/interactions.

References


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Plasmodium falciparum resistance to artemisinin derivatives is emerging in Asia. We examined molecular markers of resistance in 78 children in Uganda who had severe malaria and were treated with intravenous artesunate. We observed in the K13-propeller domain, A578S, a low-frequency (3/78), nonsynonymous, single-nucleotide polymorphism associated with prolonged parasite clearance.

Resistance of Plasmodium falciparum parasites to artemisinin derivatives threatens the current first-line treatment for severe malaria. Artemisinin resistance was first reported in 2009 in Pailin, western Cambodia (1), and has since become prevalent in the greater Mekong Delta, Vietnam, where standard 3-day courses of artemisinin combination therapies for uncomplicated P. falciparum malaria are now failing (2–4).

Among several putative genetic determinants of parasite resistance to artesunate (3,5), polymorphisms in the propeller domain of a kelch gene on chromosome 13 (PF3D7_1343700; K13) are now recognized as the major determinant of artemisinin resistance observed in P. falciparum isolates from patients in Southeast Asia (3,4,6,7). Various single amino acid substitutions in the K13 protein are associated with a mean increase of 116% in the parasite clearance half-life (t½) (4). The mechanism of resistance has been illuminated by a recent study of the P. falciparum transcriptomes from >1,000 acute malaria episodes (6). Slow-clearing parasites exhibited increased expression of unfolded protein response pathways (e.g., chaperone complexes); these pathways may mitigate protein damage caused by artemisinin. Slow-clearing parasites also exhibited decreased expression of proteins involved in DNA replication and decelerated development at the young ring stage. Haplotype analysis suggests that K13 mutations emerged independently in multiple geographic locations in Southeast Asia, causing concerns about the ability to contain resistant parasites (7).

With the widespread use of artemisinin treatment, resulting in continued pressure for natural selection of the most resistant parasites, resistance may emerge in regions beyond Asia, including Africa. The possible increase of parasite resistance to treatment highlights an urgent need to map K13 mutations throughout the malaria-endemic world (7). Consequently, recent molecular epidemiologic analyses of K13 in Senegal (8) and Uganda (9) and in a large collection of >1,100 infections from sub-Saharan Africa (10) have been undertaken, revealing the absence of nonsynonymous single-nucleotide polymorphisms (SNPs) associated with artemisinin resistance in Southeast Asia. Other distinct nonsynonymous SNPs have been discovered in parasites of African origin (9,10), but association of these mutations with a resistance phenotype has not been shown.

The Study

We examined parasite clearance kinetics and sequenced the parasite K13 gene in a cohort of 78 children with severe malaria (including 8 children who died) from the placebo group (being treated with artesunate alone) of a randomized, controlled trial conducted at the Jinja Regional Referral Hospital, Uganda, during July 12, 2011–June 14, 2013 (11). Inclusion and exclusion criteria have been described elsewhere (11). The median age of patients was 2.0 years (range 1.0–8.0 years), and 38 (49%) were female. All patients were treated intravenously with artesunate (Guilin Pharmaceutical, Shanghai, China) prequalified by the World Health Organization and according to the Organization’s guidelines (12).

Giemsa-stained peripheral blood smears (thin and thick) were assessed for quantitative malaria parasite density by light microscopy at a quality-controlled central research laboratory, the Makerere University–Johns Hopkins University Research Collaboration Core Laboratory, which is certified by the College of American Pathologists. For each patient, 5 serial parasite densities were measured according to the following sampling schedule: 1) admission; 2) ≈12 hours later; 3) morning of the second day of
admission; 4) morning of the third day of admission; and 5) morning of the fourth day of admission.

To measure parasite clearance kinetics, we used a standardized tool, the parasite clearance estimator, which expresses parasite clearance as the parasite’s half-life ($t_{1/2}$), which was calculated by using the slope of the linear portion of the curve of log-transformed parasite densities over time ($I3$). In addition, we computed the parasite clearance time, defined as the interval between the start of treatment and the first of 2 sequential negative peripheral blood films ($I4$).

To determine molecular markers of resistance, we amplified and sequenced the PF3D7_1343700 gene by using the nested PCR method, as described (3), with some modifications. DNA was extracted from cryopreserved erythrocyte fractions by using QIAGEN columns (QIAGEN, Valencia, CA, USA) according to the manufacturer’s instructions. The K13-propeller domain was amplified by using K13-1 5′-CGGAGGTGACAAATCTGGGA-3′ and 5′-K13-4 GGGAATCTGGTGTAAACGC-3′ for the primary PCR and K13-2 5′-GCCAAGCTGCCATTCTTTG-3′ and K13-3 5′-GCCTTGTTAGAAGAAGCAGA-3′ for the nested PCR. DNA sequencing of the 810-bp nested PCR product was performed to determine the amino acid haplotype of residues. Results were aligned to reference PF3D7 kelch protein, putative (PF13_0238) mRNA, complete coding sequence (National Center for Biotechnology Information reference sequence XM_001350122.1).

We identified limited diversity within the K13 gene. For 16 loci tested (amino acid positions 439, 441, 458, 465, 467, 476, 493, 522, 539, 543, 557, 558, 580, 617, 619, and 637), the wild-type sequence was found in all 78 parasite amplicons. We did not observe any of the most common amino acid substitutions in K13 associated with artemisinin resistance in $P$. falciparum isolates from Cambodia (C580Y, R339T or Y493H) (3), nor the I543T and N458Y mutations most strongly associated with increased clearance $t_{1/2}$ in another recent study (4), nor the M476I mutation selected in vitro under artemisinin pressure (3). Similarly, these point mutations were absent in isolates from Senegal and Uganda and in >1,100 $P$. falciparum parasites from 14 sites across sub-Saharan Africa (8–10). However, a previously reported point mutation, A578S (9,15), was found in 3 (3.8%) of 78 infections.

The Table shows infections with A578S parasites compared with infections caused by wild-type parasites. Parasite clearance time was prolonged in infections with A578S mutant parasites, and a similar trend was observed for clearance $t_{1/2}$. These 2 types of infections showed no differences in prior artemisinin exposure, number of deaths, or recrudescence or reinfection at day 14 from date of admission.

### Conclusions

The role of the A578S amino acid substitution is unclear, but it occurs near the most common K13-propeller mutation (C580Y), which has been associated with delayed parasite clearance in Southeast Asia and with tolerance to artemisinin in vitro (3). Computational modeling suggests that A578S should considerably affect the tertiary structure of the K13 protein, thereby destabilizing the domain scaffold and altering its function (15). Isolates with A578S exhibited a phenotype of prolonged clearance under artesunate treatment in our study. Delayed clearance of A578S parasites was not observed in previous reports (4,9), although the number of isolates in our study and in others was small. Because multiple independent mutations in K13 have arisen in geographic regions engaged in intense treatment of malaria with artemisinin derivatives (7) and because only 2 point mutations were necessary to confer drug tolerance in vitro to a $P$. falciparum isolate from Tanzania (3), we are concerned that parasites with the A578S mutation are already causing severe malaria in children in Uganda, although with low frequency.

The nonsynonymous SNP A578S in the K13-propeller domain may represent another putative marker of delayed response to artesunate, although this indicator occurred infrequently in our cohort of children with severe malaria in Uganda. Our study documents an association between A578S and prolonged parasite clearance time, a finding relevant for future monitoring of $P$. falciparum response to parenteral artesunate in children in Africa.

### Table.

Characteristics of children infected with severe $Plasmodium falciparum$ malaria parasites harboring the Kelch 13 A578S polymorphism compared with children infected with wild-type parasites, Uganda

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A578S mutation, n = 3</th>
<th>Wild-type parasite, n = 75</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite clearance time, median (interquartile range), h</td>
<td>80 (71–200)</td>
<td>45 (40–64)*</td>
<td>0.033</td>
</tr>
<tr>
<td>Clearance half-life, median (interquartile range), h</td>
<td>5.9 (5.6–10.7)</td>
<td>4.5 (3.6–5.8)*</td>
<td>0.074</td>
</tr>
<tr>
<td>Prior artemisinin exposure, no. (%)</td>
<td>1 (33.3)</td>
<td>19 (25.3)</td>
<td>1.000</td>
</tr>
<tr>
<td>Deaths, no. (%)</td>
<td>0</td>
<td>6 (10.7)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*For 7 patients who died, parasite clearance time could not be calculated because of incomplete parasite clearance prior to death. For these same 7 children, the parasite clearance half-time ($t_{1/2}$) could not be calculated because of insufficient data points. For 1 child who died, clearance of parasitemia before death was documented; thus, the parasite clearance time and $t_{1/2}$ could be computed. Estimates of parasite clearance time and clearance $t_{1/2}$ are based on the remaining 68 patients. All fatal cases were associated with wild-type parasites.

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To measure parasite clearance kinetics, we used a standardized tool, the parasite clearance estimator, which expresses parasite clearance as the parasite’s half-life ($t_{1/2}$), which was calculated by using the slope of the linear portion of the curve of log-transformed parasite densities over time ($I3$). In addition, we computed the parasite clearance time, defined as the interval between the start of treatment and the first of 2 sequential negative peripheral blood films ($I4$).

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Trial operational costs were provided by the Sandra Rotman Centre for Global Health. This work was also supported by donations from Kim Kertland, the Tesari Foundation, the Canadian
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Dr. Hawkes is a clinician–scientist (pediatric infectious diseases) at the University of Alberta, Edmonton, Alberta, Canada. His current research includes translational and clinical studies in global pediatric infections (i.e., malaria and pneumonia).

References


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Gastroenteritis Outbreaks Caused by Norovirus GII.17, Guangdong Province, China, 2014–2015

Jing Lu,1 Limei Sun,1 Lin Fang, Feng Yang, Yanling Mo, Jiaqian Lao, Huanying Zheng, Xiaohua Tan, Hualiang Lin, Shannon Rutherford, Lili Guo, Changwen Ke, Li Hui

In the past decade, the most prevalent norovirus genotype causing viral gastroenteritis outbreaks worldwide, including China, has been GII.4. In winter 2014–15, norovirus outbreaks in Guangdong, China, increased. Sequence analysis indicated that 82% of the outbreaks were caused by a norovirus GII.17 variant.

Norovirus infection is a leading cause of nonbacterial gastroenteritis outbreaks in industrialized and developing countries (1,2). On the basis of amino acid identity in viral protein 1, noroviruses can be divided into at least 6 genogroups (GI–GVI). GI and GII infect humans and can be further classified into genotypes; at least 9 genotypes belong to GI and 22 belong to GII (3). During the past decade, most reported norovirus outbreaks were caused by GII.4 norovirus (4,5). New variants of GII.4 have emerged approximately every 2–3 years and have caused norovirus gastroenteritis pandemics globally (6). Since 1999, the major circulating genotype in mainland China has been GII.4, accounting for 64% of all genotypes detected (7). In winter 2014–15, norovirus outbreaks in Guangdong Province, China, increased. Sequence analyses showed that the major cause of continuous gastroenteritis outbreaks in the region was a rarely reported norovirus genotype: GII.17.

The Study

In China, according to the National Public Health Emergency Contingency Plan, an outbreak with a cluster of at least 20 acute gastroenteritis cases (meeting the Kaplan criterion) within 3 days must be reported to Guangdong Provincial Center for Disease Control and Prevention. Samples from each outbreak are first tested for norovirus (Norovirus RT-PCR Kit; Shanghai ZJ Bio-Tech Co., Ltd., Shanghai, China) and for intestinal bacteria at the local Centers for Disease Control and Prevention. The norovirus-positive specimens are delivered to the Guangdong Provincial Center for Disease Control and Prevention for further genotyping.

From each outbreak, 5–10 samples are randomly selected for sequencing. Testing with One-Step RT-PCR (QIAGEN, Valencia, CA, USA) is performed with region C–specific primer, as previously described (8). The positive PCR products are sequenced, and norovirus genotypes are determined by using the Norovirus Automated Typing Tool (http://www.rivm.nl/mpf/norovirus/typingtool) or blastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The major norovirus genotype causing each outbreak is defined as 1 genotype detected in >80% samples from the outbreak.

From January 2013 through January 2015, a total of 52 norovirus outbreaks were reported and were associated with 4,618 clinical cases; of these, 14 outbreaks were associated with ≥100 clinical cases. Of the 52 outbreaks, 44 (85%) occurred in schools and colleges, 5 (9.6%) in factories, and 3 (5.7%) in kindergartens. In Guangdong Province, norovirus outbreaks are highly seasonal; most (96%) outbreaks are reported from November through March (Figure 1). In late 2014, an increase in the number of norovirus outbreaks was noted. From November 2014 through January 2015, a total of 29 identified outbreaks were associated with 2,340 cases compared with 9 outbreaks and 949 cases the previous winter (2013–14).

Samples from 46 (88%) of the 52 outbreaks were successfully genotyped. GI norovirus was detected in samples from 96% of the outbreaks. From January 2013 through October 2014, the most common genotype found was GII.4/Sydney/2012, which was detected in samples from 48% of the outbreaks. Genotype GII.17 was first detected in the city of Guangzhou in November 2014 and thereafter spread rapidly (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/21/7/15-0226-Techapp1.pdf). From November 2014 through January 2015, GII.17 norovirus outbreaks were reported in 10 cities of Guangdong Province and represented 83% (24 of 29) of all outbreaks. In contrast, during 2013 and 2014, norovirus outbreaks caused by GII.4/Sydney/2012 were reported in only 5 cities in Guangdong.

The nucleotide sequences of the norovirus GII.17 strains from Guangdong have been deposited in GenBank.

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Gastroenteritis Caused by Norovirus GII.17, China

For phylogenetic analysis, representative strains from Guangdong were compared with GII.17 reference strains from the GenBank database. On the basis of region C sequences, genotype GII.17 could be divided into 2 major clusters (a and b). GII.17 strains collected from Guangdong during 2014–2015 norovirus outbreaks all clustered together and belonged to cluster b, the cluster to which all strains identified after 2011 belonged. Sequence comparison suggested that the strains most closely related to Guangdong GII.17 were from neighboring regions (e.g., Taiwan, Korea, and Japan) and from groundwater in Kenya (Figure 2).

Conclusions

Outbreaks of nonbacterial gastroenteritis in Guangdong Province, China, during winter 2014–15 were caused by a rare norovirus, genotype GII.17. Previous epidemiologic data suggest that in the past 2 years, GII.4/Sydney/2012 has been the major circulating norovirus genotype worldwide (4,5,9). This GII.4 variant was first detected in Australia in March 2012 and was subsequently detected in France, New Zealand, Japan, the United Kingdom, the United States, and Hong Kong, and led to increased norovirus activity globally (5). In China, the GII.4/Sydney/2012 strain, first reported in October 2012, caused increased sporadic cases in the city of Shanghai (10). In early 2013 in Guangdong, GII.4/Sydney/2012 was the predominant norovirus genotype detected in norovirus outbreaks, while other genotypes including GII.3, GII.6, GI.2, GI.3, and GII.12 were occasionally detected. In winter 2013–14, detection of GII.4/Sydney/2012 decreased while detection of GII.3 and GII.6 increased. Norovirus genotype GII.17 was detected in the outbreak that occurred on November 18, 2014. Compared with GII.4/Sydney/2012, this variant of GII.17 displayed a high epidemic activity; in only 2 months, an increased number of related outbreaks were reported in 10 cities (Figure 1).

Figure 1. Norovirus outbreaks in Guangdong, Province, China, January 2013–January 2015.

Figure 2. Phylogenetic tree of noroviruses based on the 282-bp region of the capsid N terminus/shell gene. Nucleotide sequences were analyzed by using the maximum-likelihood method. Supporting bootstrap values >70 are shown. The subtypes of GII.17 detected in Guangdong Province, China, during 2014–2015 were compressed. GII.21 genotype strains were used as outgroups. Scale bar indicates nucleotide substitutions per site. Sequences of 24 reference norovirus strains are included. Arrowhead represents number of strains from Guangdong, 2014–2015. ETH, Ethiopia; GF, French Guiana; IE, Ireland; JP, Japan; KE, Kenya; KOR, Korea; RSA, The Republic of South Africa; MEX, Mexico.
Sequence comparison with archived GII.17 strains from GenBank suggests that the GII.17 genotype identified in Guangdong is a newly emerged variant, differing from GII.17 strains detected before 2011. The recent detection of this new variant in samples from patients with sporadic cases in several regions of Asia (e.g., Korea, Japan, and Taiwan) and from groundwater in Kenya suggests that this variant of GII.17 has circulated in a wide range of areas in recent years. For GII.17, most (66 [83%] of 80) sequences from the GenBank database are restricted to region C, the short conserved sequences of the N terminus of the capsid gene. This conserved region has been widely used for genotyping strains (12) and phylogenetic studies (13). To include more reference strains and to illustrate the relationship between GII.17 from Guangdong and other regions, we mainly used region C for phylogenetic analyses in this study. Similarly, phylogenetic analysis based on the nearly full length of capsid sequences also showed that the newly emerged GII.17 variant in Guangdong clustered with the strains from Japan and Taiwan in 2013 and 2014 and differed from GII.17 strains detected before 2011 (online Technical Appendix Figure 2).

In conclusion, a norovirus genotype GII.17 variant emerged in winter 2014–15 and caused outbreaks in multiple cities in Guangdong Province, China. The distribution of GII.17 genotype among patients with sporadic cases of gastroenteritis remains unknown. In future studies, epidemiologic and virologic surveillance should be broadened to better clarify virologic, clinical, and epidemiologic patterns of this newly emerged norovirus.

Acknowledgments
We gratefully acknowledge the efforts of local Centers for Disease Control and Prevention in China in the investigation and reporting of these outbreaks.

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References

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Ebola Virus Stability on Surfaces and in Fluids in Simulated Outbreak Environments

Robert Fischer,† Seth Judson,† Kerri Miazgowicz, Trenton Bushmaker, Joseph Prescott, Vincent J. Munster

We evaluated the stability of Ebola virus on surfaces and in fluids under simulated environmental conditions for the climate of West Africa and for climate-controlled hospitals. This virus remains viable for a longer duration on surfaces in hospital conditions than in African conditions and in liquid than in dried blood.

Since March 2014, >22,000 cases of Ebola virus disease (EVD) and ≈9,000 deaths have been reported in West Africa (1). Thousands of health care professionals have been mobilized to West Africa to assist with the ongoing outbreak of EVD (2). More than 800 Ebola virus (EBOV) infections have been reported in health care professionals (1).

Determining the persistence of EBOV on surfaces and under environmental conditions specific to outbreak settings and disease-endemic areas is critical to improving safety practices for these health care workers (3), as well as answering questions about EBOV transmission among the public (4). Researchers have experimentally assessed the stability of other EBOV strains on plastic, glass, and steel within dried media or guinea pig serum (5); in the dark on glass (6); and during exposure to UV light (7). However, the environmental conditions of these studies do not reflect the higher temperatures and relative humidities (RHs) in outbreak regions, or the current outbreak strain. No infectious EBOV could be found during environmental sampling in a ward with EVD patients; however, this result could be more indicative of cleaning measures than actual virus stability (8).

We report stability of EBOV with a current outbreak strain from Guinea (Makona-WPGC07) (9) on 3 clinically relevant surfaces: stainless steel, plastic, and Tyvek (Dupont, Wilmington, DE, USA). We also determined the stability of EBOV in water, spiked human blood, and blood from infected nonhuman primates (NHPs). These experiments were conducted in 2 environmental conditions, 21°C, 40% RH, and 27°C, 80% RH, to simulate a climate-controlled hospital and the environment in West Africa, respectively.

The Study
We tested the stability of EBOV on 3 materials commonly found in an Ebola treatment unit (ETU) in West Africa: 1) utility-grade (308) stainless steel washers (McMaster-Carr, Atlanta, GA, USA); 2) plastic (Teflon [polytetrafluoroethylene]; McMaster-Carr); and 3) Tyvek (from the front of a coverall). For each time point, 3 disks (4-cm diameter) of each material were placed individually into wells of a 6-well plate. Five samples (10 μL/sample) containing a total dose of 10^5 50% tissue culture infectious doses (TCID_{50}) of EBOV in cell-free medium were evenly distributed on the disks. The plates were divided into groups, and each group was placed into a plastic HEPA-filtered box and placed at 21°C, 40% RH, or 27°C, 80% RH. The samples were dried naturally, and virus titers were determined over a 14-day period.

In the surface and fluid stability experiments, all samples were stored at −80°C until titration (1 freeze–thaw cycle of EBOV samples that did not change virus titer). Titrations were performed on Vero E6 cells as described (10,11). The TCID_{50} per milliliter for each sample at each time point was calculated by using the Spearman-Karber method (11).

Because viral decay rates often exhibit first-order kinetics (12), we log_{10} transformed our TCID_{50} calculations to represent virus titer and used a linear regression analysis (Prism version 6.05; GraphPad, San Diego, CA, USA) to determine the log_{10} reduction rate of EBOV on each surface at both environmental conditions (Figure 1; Table 1). We also determined whether linear regression models were significantly different from each other at the p<0.05 level by using an analysis of covariance equivalent test in Prism. Overall, virus remained viable longest in hospital conditions and on Tyvek. Viable EBOV was detectable for 3 days on Tyvek at tropical conditions.

The stability of EBOV in water was assessed by diluting 150 μL virus stock in 2.85 mL of Ambion diethylpyrocarbamide–treated water (Thermo Fisher Scientific, Pittsburgh, PA, USA) and removing residual protein and medium with 1 initial and 2 rinse spins on Amicon Ultra Centrifugal Filters 100K MWCO (Merck, Darmstadt, Germany). EBOV was more stable in water at 21°C and had an ≈1 log_{10} reduction/day in water at 27°C (Table 1; Figure 2, panel A).

The stability of EBOV in human blood was assessed by spiking blood samples from a healthy human volunteer

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to achieve a $10^5$ TCID$_{50}$/mL virus titer. The spiked blood was distributed in 1-mL aliquots into closed screw-top vials to maintain a liquid state, spread in 50-µL aliquots onto the bottom of a 24-well plate, and dried. One group of samples was stored at 21°C, 40% RH, and the other group was stored at 27°C, 80% RH. EBOV stability in drying blood exhibited first-order kinetics and was viable for up to 6 days at tropical conditions (Table 1; Figure 2, panel B).

To approximate the stability of EBOV in naturally infected human blood, we used blood from cynomolgus macaques (Macaca fascicularis) as a proxy. Blood was collected during necropsy from 3 macaques that were previously enrolled in an Animal Care and Use Committee–approved EBOV pathogenesis study and were euthanized because they exhibited signs of EVD and viremia. Blood samples were divided into 2 groups with 2 sets of 150-µL aliquots for each time point; each group was stored at the conditions described above with each set in the liquid or drying state, and virus viability was assessed over a 14-day period. Because of variation in calculated virus titer from each of the individual NHPs, the log$_{10}$ reduction rate could not be approximated and only the initial titer and the duration of viability are shown (Table 2). In general,

![Figure 1. Linear regression model showing the effect of different environmental conditions and surfaces on survival of Ebola virus (EBOV). Virus was dried on 3 surfaces found in outbreak settings at A) 27°C, 80% relative humidity (RH) (West African tropical conditions) and B) 21°C, 40% RH (climate-controlled hospital conditions). Virus concentration was reduced at a significantly slower rate on all surfaces in hospital conditions than in tropical conditions (p<0.0001 for all surfaces). Triplicate samples were taken at each time point. Error bars indicate mean ± SEM virus titer. Dashed line indicates the limit of detection for the assay. An analysis of covariance equivalent test was used to compare linear regression models and determine differences in virus reduction rates. TCID$_{50}$, 50% tissue culture infectious dose.](image)

**Table 1. Linear regression models for survival of Ebola virus on surfaces and in fluids at different environmental conditions**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Temperature, °C</th>
<th>Relative humidity, %</th>
<th>Model†</th>
<th>$r^2$</th>
<th>Virus log reduction time, d‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless steel</td>
<td>27</td>
<td>80</td>
<td>$Y = -2.240X + 6.729$</td>
<td>0.9798</td>
<td>0.45</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>21</td>
<td>40</td>
<td>$Y = -0.7829X + 6.564$</td>
<td>0.8544</td>
<td>1.3</td>
</tr>
<tr>
<td>Plastic</td>
<td>27</td>
<td>80</td>
<td>$Y = -2.205X + 7.008$</td>
<td>0.9745</td>
<td>0.45</td>
</tr>
<tr>
<td>Plastic</td>
<td>21</td>
<td>40</td>
<td>$Y = -0.5445X + 6.188$</td>
<td>0.8303</td>
<td>1.8</td>
</tr>
<tr>
<td>Tyvek</td>
<td>27</td>
<td>80</td>
<td>$Y = -1.599X + 6.939$</td>
<td>0.9713</td>
<td>0.63</td>
</tr>
<tr>
<td>Tyvek</td>
<td>21</td>
<td>40</td>
<td>$Y = -0.4631X + 6.709$</td>
<td>0.8878</td>
<td>2.2</td>
</tr>
<tr>
<td>Drying human blood</td>
<td>27</td>
<td>80</td>
<td>$Y = -0.6806X + 4.951$</td>
<td>0.8724</td>
<td>1.5</td>
</tr>
<tr>
<td>Drying human blood</td>
<td>21</td>
<td>40</td>
<td>$Y = -0.6917X + 4.828$</td>
<td>0.9037</td>
<td>1.5</td>
</tr>
<tr>
<td>Liquid human blood</td>
<td>27</td>
<td>NA</td>
<td>$Y = -0.1148X + 4.651$</td>
<td>0.2892</td>
<td>8.7</td>
</tr>
<tr>
<td>Liquid human blood</td>
<td>21</td>
<td>NA</td>
<td>$Y = -0.05000X + 4.231$</td>
<td>0.05293</td>
<td>20</td>
</tr>
<tr>
<td>Water</td>
<td>27</td>
<td>NA</td>
<td>$Y = -1.13X + 4.483$</td>
<td>0.9607</td>
<td>0.88</td>
</tr>
<tr>
<td>Water</td>
<td>21</td>
<td>NA</td>
<td>$Y = -0.5694X + 4.201$</td>
<td>0.9139</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*NA, not applicable.
†Y, log$_{10}$ 50% tissue culture infectious dose/mL; X, days.
‡In hospital conditions, virus titer on steel was reduced significantly faster than on plastic (p = 0.004) and on Tyvek (p<0.0001), but there was no significant difference in reduction between Tyvek and plastic (p = 0.13). In tropical conditions, there was no significant difference in virus titer reduction on steel and on plastic (p = 0.78). However, virus decayed more slowly on Tyvek than on steel (p<0.0001) and on plastic (p<0.0001). There was no significant difference in reduction rate in virus titer in drying human blood in hospital or tropical conditions (p = 0.92). Stability of virus in liquid blood did not fit a linear regression model. Virus was reduced significantly faster at 27°C than in water at 21°C (p = 0.0001).
EBOV maintained viability for a longer duration in liquid than in drying blood regardless of initial titer or environmental condition.

Conclusions
We found that EBOV can persist on surfaces common in an ETU, highlighting the need for adherence to thorough disinfection and doffing protocols when exiting the ETUs and careful handling of medical waste. In addition, EBOV maintains viability for a longer duration in liquid than in dried blood. EBOV in blood of experimentally infected NHPs persists for a similar duration as EBOV in spiked human blood. A recent study showed that blood in the body cavity of an NHP contained viable EBOV for up to 7 days after death (13). We detected viable EBOV in drying blood for up to 5 days at both environmental conditions in human and NHP blood. Therefore, dried and liquid blood from an infected person in their home or ETU should be treated as potentially infectious. The finding that EBOV remains viable in water for as long as 3 (27°C) or 6 (21°C) days at the experimental concentration warrants further investigation into the persistence of the virus in aqueous environments, such as in wastewater or sewage canals. Viable EBOV has been isolated from urine (14) but not from human stool (8). Therefore, the potential for dissemination of EBOV through wastewater remains unknown.

This study is subject to several limitations. First, because standard volumes for samples were used, different volumes or matrices could influence the stability of EBOV under the tested conditions. Second, blood samples from the NHPs might have different immunologic or biochemical conditions, which can potentially influence virus stability. Third, the experimental conditions in the laboratory are sterile, but in disease-endemic areas and ETUs, bacteria or chemicals could influence EBOV viability.

Overall, we found that different environmental conditions, fluids, and surfaces influence the persistence of EBOV. These findings demonstrate that such factors are crucial in understanding transmission and improving safety practices.

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### Table 2. Stability of Ebola virus in infected nonhuman primate blood under different environmental conditions

<table>
<thead>
<tr>
<th>Blood sample, condition</th>
<th>Initial virus titer, log$<em>{10}$ TCID$</em>{50}$/mL</th>
<th>No. days viable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NHP 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drying 27°C, RH 80%</td>
<td>6.5</td>
<td>5</td>
</tr>
<tr>
<td>Drying 21°C, RH 40%</td>
<td>6.5</td>
<td>1</td>
</tr>
<tr>
<td>Liquid 27°C</td>
<td>7.2</td>
<td>14</td>
</tr>
<tr>
<td>Liquid 21°C</td>
<td>7.2</td>
<td>14</td>
</tr>
<tr>
<td><strong>NHP 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drying 27°C, RH 80%</td>
<td>2.8</td>
<td>5</td>
</tr>
<tr>
<td>Drying 21°C, RH 40%</td>
<td>2.8</td>
<td>1</td>
</tr>
<tr>
<td>Liquid 27°C</td>
<td>4.2</td>
<td>11</td>
</tr>
<tr>
<td>Liquid 21°C</td>
<td>4.2</td>
<td>1</td>
</tr>
<tr>
<td><strong>NHP 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drying 27°C, RH 80%</td>
<td>7.2</td>
<td>4</td>
</tr>
<tr>
<td>Drying 21°C, RH 40%</td>
<td>7.2</td>
<td>4</td>
</tr>
<tr>
<td>Liquid 27°C</td>
<td>6.5</td>
<td>8</td>
</tr>
<tr>
<td>Liquid 21°C</td>
<td>6.5</td>
<td>14</td>
</tr>
</tbody>
</table>

*TCID$_{50}$, 50% tissue culture infectious dose; NHP, nonhuman primate; RH, relative humidity.
Dr. Fischer and Dr. Judson are researchers in the Virus Ecology Unit at Rocky Mountain Laboratories in Hamilton, Montana. Their research interests are the ecology and evolution of emerging infectious diseases and relationships between human and environmental health.

References

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Outbreak of Ciprofloxacin-Resistant *Shigella sonnei* Associated with Travel to Vietnam, Republic of Korea

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We investigated an October 2014 outbreak of illness caused by *Shigella sonnei* in a daycare center in the Republic of Korea (South Korea). The outbreak strain was resistant to extended-spectrum cephalosporins and fluoroquinolones and was traced to a child who had traveled to Vietnam. Improved hygiene and infection control practices are needed for prevention of shigellosis.

*S. sonnei* spp. are etiologic agents of gastrointestinal disease worldwide and are frequently associated with outbreaks because of their low infectious doses and person-to-person transmission (1,2). For the treatment of persons who have severe infections, fluoroquinolones are among the first-line agents for adults; additionally, oral extended-spectrum cephalosporins are used to treat young children. However, the current emergence and spread of drug resistance in *Shigella* strains could hinder empirical antimicrobial therapy, leading to treatment failure. *S. sonnei* is the most frequently isolated species among all cases of *Shigella* infection in industrialized countries (3), and it has become increasingly prevalent across Southeast Asia in recent decades (4). Recently, international travel to areas where the disease is highly endemic has accelerated the global spread of drug-resistant *S. sonnei* to nonendemic regions. Here, we describe a travel-associated outbreak of illness caused by a *S. sonnei* strain that was resistant to extended-spectrum cephalosporins and fluoroquinolones.

The Study

In the beginning of October 2014, six children who were vomiting and experiencing abdominal cramping and diarrheea were admitted to the local hospital in Gyeongsangnam-do, Republic of Korea (South Korea). All patients attended the same daycare center that provided care and food to children from low-income families. Fecal specimens from 6 patients were submitted to the local public health laboratory and were processed according to a standard bacterial culture method. On October 6, Korea Centers for Disease Control and Prevention was notified that *S. sonnei* phase II were identified from all fecal samples. An epidemiologic investigation was conducted to determine the extent of the outbreak and to identify the mode of transmission. A confirmed case was identified by passive and active case-finding on the basis of laboratory-identified *S. sonnei* isolates in the fecal specimens of center attendees and staff members, families of the children, and persons in the community. A probable case was defined as a person with any shigellosis symptoms and an epidemiologic link to infected patients whose cultures were negative. The children’s guardians were interviewed by using a standardized questionnaire that requested information on symptoms, food consumption, recent travel history, and contact persons. This investigation was part of a public health emergency response and was accordingly exempt from institutional review board approval.

The investigation revealed that an 8-year-old boy (the index case-patient in this outbreak) had recently returned after visiting family in Vietnam, where *S. sonnei* infection is highly endemic. He had experienced sustained diarrheal episodes since his return, and after returning to the daycare center, children in the daycare center began having similar symptoms. Cases of shigellosis were also identified among the grandparents of the index case-patient and a person the family visited in a geographically distant location on September 27. No isolates were obtained from the environmental samples collected, including foods, drinking water, and surface swab specimens of the daycare facility.

Eleven laboratory confirmed and 4 probable cases were identified during this outbreak. The median age of the patients in the daycare center was 7.8 (range 4–13) years. Overall, the reported symptoms were diarrhea (≥3 loose stools during 24 hours) and abdominal cramping; 4 patients were asymptomatic but their stool samples were...
culture-positive. Of the 15 persons who became ill (Figure 1), 10 were treated with cefotaxime or ciprofloxacin, after which their stool samples were culture-negative. For 5 patients with continuing positive fecal culture, antibiotic drug treatment was later changed to carbapenems (meropenem or imipenem). According to local infection control guidelines, symptomatic patients were isolated in single-bed rooms until 2 consecutive fecal cultures tested negative for S. sonnei. To prevent the further spread of the disease, public health interventions were encouraged during the outbreak period: enforced handwashing at predetermined times at the daycare facility, strict hygiene measures in affected households, education about shigellosis, and environmental disinfection of the facility.

Laboratory-confirmed strains of S. sonnei were sent to Korea National Institute of Health for further characterization. All 15 isolates had identical or highly similar pulsed-field gel electrophoresis (PFGE) patterns after the XbaI digestion of chromosomal DNA. The main PFGE pattern of this outbreak (SZNX01.183; PFGE pattern number assigned by Korea National Institute of Health) had not been previously reported in domestic cases, and the isolate was genetically indistinguishable from those from Vietnam.

**Figure 1.** Epidemic curve of the outbreak of illness caused by Shigella sonnei infection, by symptom onset date, South Korea, 2014. Black bar sections indicate laboratory-confirmed cases; white bar sections indicate probable cases; stars indicate cases found in daycare center. Arrows indicate dates of the events for an index case-patient with travel history to Vietnam and of public health notification of the outbreak.

**Figure 2.** XbaI pulsed-field gel electrophoresis patterns of Shigella sonnei strains identified during a 2014 outbreak in South Korea and 2 isolated from samples from persons in Vietnam. The dendrogram was constructed by using Dice coefficient and UPGMA clustering, with 1.5% optimization and 1.5% position tolerance. Antibiotic resistance profiles and resistance determinants to extended-spectrum cephalosporins and fluoroquinolones are plotted next to the dendrogram. All strains had QRDR mutations GyrA(S83L,D87G) and ParC(S80I). *Strains 14-5222 and 12-3580 originated in Vietnam. AMP, ampicillin; CIP, ciprofloxacin; CTX, cefotaxime; NAL, nalidixic acid; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; QRDR, quinolone resistance–determining region. Scale bar indicates percentage relatedness.
from a ciprofloxacin-resistant *S. sonnei* strain isolated from a traveler returning from Vietnam during 2012 (Figure 2).

On the basis of MICs of antimicrobial agents determined by using a broth microdilution method (5), the outbreak strains were found to be resistant to both extended-spectrum cephalosporins (cefotaxime, MIC >32 μg/mL) and fluoroquinolones (ciprofloxacin, MIC >8 μg/mL). The strains were also resistant to tetracycline and trimethoprim/sulfamethoxazole but were susceptible to chloramphenicol, gentamicin, amikacin, and carbapenem. For azithromycin, an alternative oral agent for shigellosis, MICs were 1–2 μg/mL (Table). Extended-spectrum β-lactamase (ESBL) typing by using PCR and further sequencing (6) showed that all isolates carried the *bla*<sub>CTX-M-15</sub> and *bla*<sub>TEM-1</sub> genes. ESBL plasmid of *S. sonnei* isolate from the index case-patient were successfully transferred to the recipient *E. coli* J53 Azi<sup>8</sup> strain. PCR-based *inc/rep* typing and pMLST analysis of a transconjugant strain (7,8) showed that this ESBL plasmid was of the ST16/IncI1 type, which was previously identified in strain pKHSB1 from Vietnam (9). The genetic environment of the *bla*<sub>CTX-M-15</sub> gene was analyzed by PCR and sequencing with specific primers for the insertion sequences IS*Ecp1* and orf477 (6). An intact IS*Ecp1* and truncated orf477 were identified at 48 bp upstream and downstream of the *bla* gene, which has also been found in CTX-M-15-encoding plasmids from *Enterobacteriaceae* (6,9,10).

The outbreak strains had 2 mutations in the quinolone resistance-determining region of *gyrA* (Ser83Leu and Asp87Gly) and 1 mutation in *parC* (Ser80Ile [Figure 2]), which have been reported to be responsible for ciprofloxacin resistance in *S. sonnei* (11). However, *gyrB* and *parE* mutations and plasmid-mediated quinolone resistance genes were not detected (12).

**Conclusions**

We describe a shigellosis outbreak affecting children attending a daycare center, their family members, and residents of the surrounding community. To limit the extent of the outbreak, laboratory investigations of outbreak strains and infection-control measures including contact isolation and hand hygiene were immediately implemented, which may have contributed to preventing the further spread of this multidrug-resistant *S. sonnei* strain.

The outbreak strain was resistant to extended-spectrum cephalosporins and fluoroquinolones and was introduced by a daycare center attendee who had returned from travel to Vietnam. The *bla*<sub>CTX-M-15</sub> gene in *S. sonnei* was first described in 2005 (13) and since then has been reported worldwide; we described an outbreak of CTX-M-15–producing *S. sonnei* in Korea in 2008 (6). The PFGE pattern of the 2008 outbreak strain (SZN0X1.176) showed only 82.8% genetic similarity with that of the outbreak strains of the current study but was observed in several traveler-associated cases originating from China. These findings suggest that, despite the lack of direct evidence, various antimicrobial drug–resistant *S. sonnei* clones have been imported across geographic regions and may eventually spread globally and lead to increased illness and death rates.

In summary, we report a shigellosis outbreak in South Korea caused by a ciprofloxacin-resistant CTX-M-15–producing *S. sonnei* strain that originated from Vietnam.

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**Table.** Susceptibility profiles of outbreak *Shigella sonnei* isolate from index case-patient and *Escherichia coli* transconjugant strain used for testing, South Korea, 2014

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th><em>Shigella sonnei</em> 14-5222</th>
<th><em>E. coli</em> J53</th>
<th><em>E. coli</em> J53, TC-14-5222</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid</td>
<td>&gt;128</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
<td>&lt;0.12</td>
<td>&lt;0.12</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Amoxicillin/sulbactam</td>
<td>16</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>16</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Cefazedimide</td>
<td>8</td>
<td>&lt;0.25</td>
<td>8</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>64</td>
<td>&lt;0.25</td>
<td>64</td>
</tr>
<tr>
<td>Cefotaxime/clavulanate</td>
<td>&lt;0.12</td>
<td>&lt;0.12</td>
<td>&lt;0.12</td>
</tr>
<tr>
<td>Cefepime</td>
<td>4</td>
<td>&lt;1</td>
<td>4</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>128</td>
<td>&lt;0.12</td>
<td>128</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>&gt;32</td>
<td>0.5</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>&gt;64</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>&gt;128</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>128</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>&gt;16</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Amikacin</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Because international travel can contribute to the spread of multidrug-resistant pathogens, enhanced surveillance is necessary to control the dissemination of antimicrobial drug resistance. Improved hygiene, infection control plans, and better education for travelers are also required.

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References


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Rapidly Expanding Range of Highly Pathogenic Avian Influenza Viruses

Jeffrey S. Hall, Robert J. Dusek, Erica Spackman

The movement of highly pathogenic avian influenza (H5N8) virus across Eurasia and into North America and the virus’ propensity to reassort with co-circulating low pathogenicity viruses raise concerns among poultry producers, wildlife biologists, aviculturists, and public health personnel worldwide. Surveillance, modeling, and experimental research will provide the knowledge required for intelligent policy and management decisions.

The recent introduction of highly pathogenic avian influenza (HPAI) subtype H5N8 virus into Europe and North America poses major risks to poultry industries, zoologic collections, and wildlife populations; thus, this introduction warrants continued and heightened vigilance. First discovered in early 2014 in poultry and wild birds in South Korea, HPAI H5N8 virus apparently arose in China from reassortment events between HPAI subtype H5N1 virus (clade 2.3.4.4) and several low pathogenicity viruses (LPAIVs) (1–3). The H5N8 virus was subsequently detected in waterfowl in Russia in September 2014, and since then, H5N8 virus and reassortants have been detected in poultry and wild birds in Europe (Netherlands, Germany, Italy, the United Kingdom, Hungary, and Sweden), Taiwan, Japan, Canada (British Columbia), and the western and central United States (Washington, Oregon, California, Idaho, Utah, Minnesota, Missouri, Arkansas, Kansas, Iowa, Wyoming, and Montana).

Wild waterfowl are a primary natural host for LPAIVs, and infection rates in these populations peak at autumn migratory staging locations, where large numbers of immunologically naive juvenile birds congregate (4). The HPAI H5N8 virus has apparently adapted to wild waterfowl hosts: few or no clinical signs or adverse effects are apparent in these hosts when infected with the virus. Thus, it seems probable that the virus was disseminated out of Russia into Europe, East Asia, and North America by migrating waterfowl during autumn 2014 (5).

The HPAI H5N8 virus has encountered, interacted with, and reassorted with co-circulating LPAIVs in migratory and overwintering waterfowl populations, creating new HPAI viruses (HPAIVs). In Taiwan, new Eurasian lineage reassortant HPAIVs (i.e., H5N2 and H5N3 subtypes) and the parental H5N8 subtype virus have been detected in poultry and wild birds (6). In North America, HPAI H5N8 virus continues to circulate among waterfowl and commercial and backyard poultry flocks. In addition, new HPAIV reassortants (i.e., H5N2 and H5N1 subtypes) that are combinations of HPAI H5N8 virus and genetic elements from Eurasian and North American viruses are also circulating in these populations (7,8) (Figure).

Persistence of the original HPAI H5N8 virus for >1 year, the creation of multiple reassortant viruses that have maintained high pathogenicity in poultry, and adaptation of the virus to migrating waterfowl all indicate that these viruses could persist and spread in Northern Hemisphere waterfowl populations for an extended period. This dynamic of HPAIVs being transported by wild birds to new populations raises critical issues and poses a series of questions that researchers and modelers should examine in more detail. The risks are significant that these HPAIVs will continue to circulate and that new genetic combinations will arise in concentrations of overwintering waterfowl and then spill over into poultry operations and aviculture. The spillover risk is particularly high for operations with rudimentary biosafety practices (e.g., backyard flocks) and that trend toward outdoor access for organic poultry. Such events have already occurred in commercial poultry operations in Canada and some US states (California, Minnesota, Missouri, Iowa, and Arkansas), and subsequent culling operations and trade restrictions have caused substantial local economic losses. As wild birds begin their spring migrations and disperse into their breeding ranges, will they be transporting these viruses to new regions, including the rest of North America? Is this an inevitable outcome of HPAI H5N8 transmission in wild bird populations? Can these viruses be transported from Europe to eastern North America by migratory birds via North Atlantic routes (9)? Are there risks of these viruses reassorting with viruses from other species, such as swine, particularly feral swine whose populations are rapidly expanding, and will these reassortant viruses present greater risk of zoonotic disease?

These HPAIVs do not appear to pose substantial risks to waterfowl populations, but they may have detrimental effects on other, perhaps more sensitive, wildlife populations. Birds of prey seem to be particularly susceptible to HPAIV infection (10), including the HPAIV H5N8 virus that killed captive gyrfalcons (Falco rusticolus) that...
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were fed infected duck carcasses (7). In North America, other raptor species have been found infected with H5N8 or H5N2 virus: Cooper’s hawk (Accipiter cooperii), great horned owl (Bubo virginianus), red-tailed hawk (Buteo jamaicensis), peregrine falcon (Falco peregrinus), and bald eagle (Haliaeetus leucocephalus). It is not known what effect these viruses will have on small, at-risk wild bird populations, such as California condors (Gymnogyps californianus), that may prey on or scavenge infected birds, but the possible effects should be considered in conservation management decisions.

As HPAIVs continue spreading and evolving, the questions posed here, along with many more questions, will need to be answered to understand the risks to agriculture, zoologic collections, wildlife, and, potentially, human populations. As other researchers have recently pointed out, robust, targeted surveillance programs among wild birds (11) and poultry, modeling of the movements of HPAIV-infected wild birds, and experimental research studies will provide the knowledge required for intelligent policy and management decisions regarding agriculture, wildlife, and public health.

Figure. Genealogy of subtype H5N8 HPAIV, its spread from China to other countries, and its evolution in wild birds. Stars represent probable spread of virus and/or reassortment in wild birds; question marks indicate unknown mode. GER, Germany; HPAIV, highly pathogenic avian influenza virus; ITA, Italy; LPAIVs, low pathogenicity avian influenza viruses; Neth, the Netherlands; UK, United Kingdom; US, United States.

References


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Cluster of Ebola Virus Disease, Bong and Montserrado Counties, Liberia


Lack of trust in government-supported services after the death of a health care worker with symptoms of Ebola resulted in ongoing Ebola transmission in 2 Liberia counties. Ebola transmission was facilitated by attempts to avoid cremation of the deceased patient and delays in identifying and monitoring contacts.

Reports of what has become the largest and longest epidemic of Ebola virus disease (EVD) began in March 2014 in West Africa (1). To interrupt Ebola transmission, health care authorities must promptly isolate and treat persons with EVD and identify and monitor exposed persons before symptoms develop (2). Effective contact tracing can limit the number of new cases; however, a single missed contact can result in many new cases (3). Gaps in contact tracing have been reported as challenges for infectious diseases such as sexually transmitted infections and tuberculosis (4–6). Because contact tracing requires patients to reveal names of persons with whom they have had contact and whom they may have exposed to illness, public health officials must quickly establish trust with sick persons and those at risk for disease (3, 7).

We describe a cluster of EVD cases involving transmission across 2 jurisdictions in Liberia. Data for this report were derived from interviews, case reporting forms, treatment records, and laboratory results. This EVD cluster highlights the challenges associated with public health measures to interrupt transmission of Ebola.

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The Investigation

On December 8, 2014, a 78-year-old man (patient 1) from Gbarnga (Bong County), Liberia, was admitted to the Bong County Ebola Treatment Unit (ETU) where test results were positive for Ebola by reverse transcription PCR. He reported recent travel to Monrovia (Montserrado County), where he cared for his 32-year-old son, a health care worker who died from an acute illness.

On December 9, another son of patient 1 (patient 2, 39 years of age), who lived in Monrovia, had fever, headache, and malaise and sought care at hospital A in Bong County. He did not report contact with patient 1, nor did he report that he provided care for his sick brother in Monrovia. On December 10, hematemesis developed, and the patient was transferred to the Bong County ETU and treated for laboratory-confirmed EVD. Contact tracing identified 20 contacts living in Gbarnga. All contacts were initially symptom free and were quarantined at a local holding center for 21-day monitoring. No contacts in Monrovia were reported by patients 1 or 2.

On December 16, Bong County health officials were notified that a 15-year-old girl (patient 3) with fever, subconjunctival hemorrhage, and thrush was at hospital A. She had traveled 4 hours by taxi from Monrovia to be near her ill grandfather and father (patients 1 and 2) and did not report exposure to EVD patients or contacts in Monrovia. She was admitted to the ETU, and EVD was confirmed.

The next day, 4 additional family members who traveled by taxi from Monrovia were stopped at a roadside monitoring station in Gbarnga. All had fever and nonhemorrhagic symptoms and were transferred by ambulance to the ETU for evaluation; 2 family members (patients 4 and 5) had positive test results for EVD. The 2 family members whose results were negative for EVD, along with the taxi driver and a nonfamilial passenger, were transferred to a local holding center for 21-day monitoring. Contact investigations for patients 4 and 5 revealed no new contacts in Monrovia, but the patients reported that they resided in the same house in Monrovia with patients 2 and 3, who were receiving treatment in Bong County. Because family members with EVD had recently arrived from Monrovia and were being treated in Bong County, yet sources of infection and additional contacts were uncertain, Bong County requested that Montserrado County health officials conduct an investigation to identify patients and contacts at the Monrovia address so that potential EVD patients could be isolated and monitored.
The Monrovia investigation revealed that patients 1–3 had contact with patient 1’s ill son, who was designated the putative source-patient (patient 0). Patient 0 was a nurse’s aide at a community clinic. Fever, headache, joint pain, and abdominal pain developed in patient 0 on November 14, 2014, and he was cared for at home by his family for 7 days while his symptoms worsened. Although the patient and family members were aware of the EVD epidemic, they did not think patient 0 had EVD because he had no vomiting, diarrhea, and hemorrhagic symptoms; they believed he had a spiritual illness. On November 21, he was taken to a church with the hope that he would be healed through prayer. He died there on November 24, and his body was carried to his residence for mourning and burial preparation. Because all unexplained deaths were presumed to be Ebola related, an EVD burial crew retrieved his body for cremation the following day, despite resistance from the family and after being provided with new mattresses and a small ration of food. At this time, they revealed 2 previously unreported symptomatic family members (patients 7 and 8). As of January 11, 2015, a total of 10 cases were included in this cluster. Eight (80%) patients in this cluster were not identified as contacts before their EVD diagnosis, and 4 (40%) sought care outside the county where they resided (Table; Figure).

**Conclusions**

Identifying sources of infection for index patients and tracing contacts are major components of EVD prevention and control efforts (3), yet carrying out these policies is challenging when those ill with EVD do not reveal the names of possible sources or contacts who could have been exposed to disease. Detection delays and ineffective contact tracing occurred in this cluster in part because the family believed that the mandatory cremation and property destruction taken as public health actions in Monrovia harmed more than helped. Consequently, some family members sought care in Bong County, riding 4 hours in a taxi from their home in Monrovia, a distance of \( \approx 197 \) kilometers. Furthermore, family members were reluctant to reveal contact names in Monrovia and initially concealed knowledge of symptomatic persons.

This cluster may have been prevented if patient 0, presumably infected at the clinic where he worked, had been trained in infection control procedures and had access to personal protective equipment. Additional exposures and subsequent infections could have been prevented.
if he had been identified earlier as a suspected EVD patient, if testing had been performed on his body, if the results had been reported to the family, and if the Monrovia contacts had been followed daily to identify, isolate, and treat symptomatic persons. Had contact tracing identified patients 1–3 as patient 0’s contacts and isolated them immediately after symptoms developed, 6 cases of EVD (in patients 4–9) and 4 deaths (patients 4, 5, 7, and 8) might have been prevented.

Rapid implementation of contact tracing to prevent disease transmission and increased coordination and communication between jurisdictions are critical to control of EVD. These efforts can identify case-patients who may have entered the community from another jurisdiction (to better understand importation and transmission patterns) and improve case finding and contact tracing to ensure that no cases are missed (8,9). The effectiveness of these efforts depends on trust between public health officials and the communities they serve.

Figure. Timeline (A) and transmission diagram (B) of Ebola virus disease cluster, Bong and Montserrado Counties, Liberia, November–December 2014.

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Mr. Nyenswah is the Head Minister of Health for the Ministry of Health and Social Welfare, Monrovia, Liberia. He has led the Ebola response in Liberia since 2014.

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EMERGING INFECTIOUS DISEASES
A Peer-Reviewed Journal Tracking and Analyzing Disease Trends

Instructions for Emerging Infectious Diseases Authors

Types of Articles

**Perspectives.** Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

**Synopses.** Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

**Research Studies.** Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. Report laboratory and epidemiologic results within a public health perspective. Although these reports may be written in the style of traditional research articles, they should 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 be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief 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 1,000–1,500 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 two); and a brief biographical sketch of first author—both authors if only two. Dispatches are updates on infectious disease trends and research. The articles 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.

**Commentary.** Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but should not include figures or tables.

**Another Dimension.** Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the anticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader’s literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

**Letters.** This section includes letters that present preliminary data or comment on published articles. Letters (500–1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

**Book Reviews.** Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

**News and Notes.** We welcome brief announcements (50–150 words) of timely events of interest to our readers. Announcements may be posted on the journal’s Web page only, depending on the event date. In this section, we also include summaries (500–1,000 words) of emerging infectious disease conferences. Summaries may provide references to a full report of conference activities and should focus on the meeting’s content.

See our website for more information: http://wwwnc.cdc.gov/eid/pages/author-resource-center.htm
The recent measles epidemic in the United States has aroused public disbelief that a disease well-controlled for decades is reemerging to threaten children in the United States. Controversy surrounds measles vaccination in the United States; some parents have even avoided vaccinating their healthy children by exposing them to measles-infected children. However, measles has repeatedly reemerged in the United States during the past 3 centuries or longer (1,2), and its emergence patterns and means of preventing and controlling it are well understood. Until measles is globally eradicated—a goal within reach—it will continue to reappear, sicken, and kill almost anywhere, and we must energetically control each outbreak.

When we consider modern measles prevention, it is worth recalling what epidemics were like before vaccines and organized public health systems. One vivid account of measles describes the disease’s deadly spread through a prominent Boston household >300 years ago. In 1713, America’s first important medical figure (3), Puritan minister Cotton Mather (1663–1728), called by one authority “the Dr. Spock of the colonial New England” (4), wrote about a measles epidemic in the American colonies, describing not only its epidemiology and devastation but also the fear it elicited. Mather’s account reminds us of the need for such modern medical and public health tools as vaccination, patient isolation, and prevention policies in saving families from the once-unpreventable diseases that compelled us to develop effective medical advances in the first place.

The following account, condensed from Cotton Mather’s personal diary (5), focuses on illnesses in his own household, including those of his wife, 9 children, and a maidservant, over the course of 6 weeks during October–November, 1713.

**Diary Excerpts**

[18 October] …The Measles coming into the Town, it is likely to be a Time of Sickness…

[19 October] [I must]… lay hold on the Occasion to awaken Piety, and Preparation for Death, in the Souls of the children.

[24 October]… [on ≈18 October] my Son Increase fell sick…

[26 October] I must quicken the preparation of my Domesticks…

[27 October] My desirable Daughter Nibby, is now lying very sick of the Measles…

[28 October] … a very sensible Calamity is begun upon the Town… [with] some Degree of Mortality.

[30 October] The Spreading of the Measles… [is much worse in] Families, where they conflict with Poverty…

This day, my Consort [wife], for whom I was in much Distress, lest she should be arrested with the Measles which have proved fatal to Women that were with child, after too diligent an Attendance on her sick Family, was surprized with her Travail [went into labor]… [and] graciously delivered her, of both a Son and a Daughter… wherein I receive numberless Favors of God. My dear Katy, is now also down with the Measles…

[1 November] Lord’s Day. This Day, I baptized my new-born twins… So I called them, ELEAZAR and MARTHA….

[4 November] In my poor Family, now, first, my Wife has the Measles appearing on her…

My Daughter Nancy is also full of them…

My Daughter Lizzy, is likewise full of them…

My Daughter Jerusha, droops and seems to have them appearing.

My Servant-maid, lies very full and ill of them.

Help Lord; and look mercifully on my poor, sad, sinful Family…

[5 November] My little son Samuel is now full of the Measles….

[7 November]… my Consort is in a dangerous Condition, and can gett no rest… Death… is much feared for her… So, I humbled myself before the Lord, for my own Sins… that His wrath may be turned away…

[8 November] …For these many Months… I have often, often express’d my Fear unto my Friends concerning [the measles]. And now, the Thing that I greatly feared is coming upon me!
...this Day we are astonished, at the surprising Symptoms of death upon [my wife]... Oh! The sad Cup, which my Father has appointed me!... God made her willing to Dy. God extinguished in her the Fear of Death... God enabled her to Committ herself into the Hands of a great and good Savior; yea, and to cast her Orphans there too...

I pray’d with her many Times, and left nothing undone...

[9 November] On Munday... [9 November], between three and four in the Afternoon, my dear, dear, dear Friend expired.... [I] cried to Heaven...

[10 November] ...I am grievously tried, with the threatening Sickness of my discreet, pious, lovely Daughter Katharin.

And a Favour which gives a violent Shock to the very Life of my dear pretty Jerusha.

[11 November] This day, I interr’d the earthly part of my dear consort...

[12 November] ...The epidemical Malady began upon this Town, is like to pass thro’ the Country... it [might] be a service unto the public, to insert in the News-paper, a brief Direction for the managing of the sick. I will advise with a Physician or two.

[13 November] ...I hear of some aged and bedrid people, which I design speedily to visit...

[14 November] This Morning... the death of my Maid-servant, whose Measles passed into a malignant Feaver...

Oh! The trial, which I am this Day called unto in... the dying Circumstances of my dear little Jerusha!

The two Newborns, are languishing in the Arms of Death...

[15 November] ... my little Jerusha. The dear little Creature lies in dying Circumstances. Tho’ I pray and cry to the Lord... Lord she is thine! Thy will be done!...

[18 November] ...About Midnight, little Eleazar died.

[20 November] ...The distressed Families of the Poor to which I dispense... are now so many...

Little Martha died, about ten a clock, A.M.

I begg’d, I begg’d, that such a bitter Cup, as the Death of that lovely [Jerusha], might pass from me...

[21 November] ...Betwixt 9 h. and 10 h. at night, my lovely Jerusha Expired. She was 2 years, and about 7 months old. Just before she died, she asked me to pray with her; which I did... and I gave her up unto the Lord. [Just as she died] she said, That she would go to Jesus Christ...

Lord I am oppressed; undertake for me!

[23 November] ...My poor Family is now left without any Infant in it, or any under seven Years of Age...

This day, I followed my dear Jerusha to the Grave... with Resolutions... especially what I may do for my own and other Children.

[25 November] ... several Things may I do for the Service of the Town in its Adversity... [including] Charitable Distributions among the Poor... I will procure, and I will dispense, as many of these, as I can...

[28 November] Breathing in the midst of so many Deaths, what can there be so needful and so proper for me, as for me to Die Daily, and become a man dead unto this World...

[17 December] This day was kept as a Day of Prayer in the several Churches of Boston, because of the heavy Calamity on the Town. And a liberal Collection was made, for the Relief of the Poor, under the Calamity of Sickness, and growing Scarcity. It was a most bitter season...

[23 December] ...I have given to the Printer, a Letter about the Right Management of the Sick under the Distemper of the Measles [note: the actual publication bears a different title; see Mather, 6]; which is now spreading and raging in the Country. I propose to scatter it into all parts... to save many lives....

Discussion

Mather’s chronicle of explosive measles in his own family documents a shocking case-fatality rate (5 of 11 infected household members, or 45%). It also reminds us that emerging and reemerging diseases such as measles once appeared suddenly to kill almost anyone (7), a reality with which most persons lived until modern times (and with which many in the developing world who lack access to good nutrition and modern medical care still live today).

The fear that measles engendered in the citizens of Boston, expressed in Mather’s many diary entries of tenderness, hope, and despair, should bring to mind another disease of similar case-fatality that arouses comparable fear and despair: Ebola virus disease. The ravages of this disease in West Africa have recently been broadcast on television screens in the United States. Like the stunned looks in the eyes of grieving West Africans, the account of a father watching his family suffer and succumb to death elicits sympathy for those struggling against fatal diseases that even modern parents may not fully understand, and that even aggressive public health measures may prevent only with great difficulty. The writer of these passages was not just America’s preeminent theologian and medical authority, but a husband and father...
whose grief can be universally understood. One of Mather’s most noteworthy sermons, preached 24 years earlier, observed that: “Yet few outward Earthly Anguishes are equal unto these. The Dying of a Child is like the Tearing off [of] a limb…” (8).

Several medical aspects of Mather’s entries are noteworthy. From Mather’s brief notations, we cannot be certain of the exact dates of onset of most of the illnesses he described. However, he clearly chronicles 2 serial generations of measles within his family (one of no more than 17 days, the other of no more than 15). These intervals correspond to what textbooks began to describe, more than a century later, about the patterns of measles spread. Measles seems to have been brought into the household by Mather’s son Increase around October 18, 1713. Increase apparently infected his mother, Elizabeth Clark-Hubbard Mather, and his 4 youngest siblings; these 5 became ill on or shortly before November 4. A second generation of measles involved twins, born on October 30 and said to be ill on November 14, consistent with infection at or shortly after birth (or, much less likely, given the apparent date of onset of disease in their mother, infected in utero). It is curious that Mather correctly understood measles to be especially dangerous for parturient women. He may have concluded this on the basis of reports circulating among physicians or because he knew that the same phenomenon had long been documented for influenza; an influenza pandemic complicated by pleuroneumonia had begun, and swept through the Americas, during 1697–1698.

Also noteworthy, and mentioned in his referenced letter (6) but not specifically in the diaries, is Mather’s realization that pleuritick fever, probably corresponding to pneumonia, was a serious complication of measles. Viral pneumonia and secondary postmeasles bacterial pneumonia are now considered to be among the most fatal complications of this disease (9,10).

Today, high case-fatality rates for measles are seen only in ill and immunosuppressed persons or in those who are malnourished. However, in the premodern world measles was, confusingly, sometimes benign, sometimes deadly. The reasons for this documented pattern remain obscure: differences in virulence among the various extant measles clades have not been found. The deadliest historical measles outbreaks seemed to occur disproportionately in the poor and disadvantaged, especially including young children in orphanages or environments of desperate poverty, or in indigenous persons living in areas of potential relative nutritional deficiency (9). These findings suggest a key role for host and environmental factors in measles severity. Even a modest deficiency in vitamin A is now known to exacerbate measles severity, and postmeasles bacterial pneumonias appear to be much more common in situations of poverty, crowding, and high bacterial circulation.

The Letter Mather promised was “published for the benefit of the poor” (6) in December 1713. It informed those unfortunate citizens without access to a physician’s care about the typical clinical appearance and course of measles, and about simple treatments for it. In recommending generic remedies for unbalanced “humours,” it broke no new ground, suggesting (italics and capitalization preserved): Syrup of Saffron and Treacle Water, Syrup(s) of Maiden-hair or Hyssop, Tea of Sage or Rosemary, Sugar-Candied, or Buttered Pills, Hot Beer and Rum, Hot Cyder, Hot Honey, Water with Roasted Apples in it, Shavings of Castile Soap in a Glass of Wine or Beer, or Tea made of Rhubarb, and sweetened with a Syrup of Marshmallow (Althaea officinalis). These were all ingredients that the poor could afford, and that might at least be comforting, if not life-saving.

The 1713 Boston measles epidemic occurred 21 years after the Salem Witch Trials, in which historians still debate Mather’s role as instigator or mitigator; 7 years after Mather discovered that inoculation might be able to prevent smallpox; and 8 years before Mather passionately advocated inoculations in response to a deadly smallpox epidemic. Because Mather died 30 years before preventative measles inoculation is known to have been attempted (11) and 225 years before the first effective measles vaccine was developed, we have no way of knowing what he would have thought about measles immunogens, their use in public health programs, or policies to ensure universal vaccination of children.

In this writer’s opinion, however, there is little doubt that Mather—were he alive today—would strongly support all reasonable measles control efforts, including universal and publicly enforced vaccination. After all, he was a proponent of smallpox inoculation, and he fought energetically in public forums against all who tried to prevent inoculations on the grounds that it was inherently risky and might theoretically prolong or even start epidemics. He also had lived through the most devastating tragedy of his life: the loss of his own wife and children from epidemic measles. Moreover, as the first person in the New World to espouse an “animalcular” theory (germ theory) of disease (3), Mather would surely have been predisposed to accept the scientific basis of immunization, and he surely would have been impressed that aggressive global measles vaccination has, in little more than a decade, reduced the death rate by a factor of at least 5-fold and saved ≈1 million lives each year. It seems highly likely that Mather would not only advocate measles prevention and control, on the basis of the most up-to-date medical tools and public health information, but also measles elimination and eradication.

Mather’s grief and despair, expressed in line after line of his diary, should remind us not only that the risks of infectious diseases like measles are real and ongoing but
Also why previous generations of physicians and scientists, supported by a public desperate for medical advances to save their children, worked so long and so well to develop and deploy the very vaccines that some people now avoid and decry. As we debate today how best to deal with yet another measles epidemic in the United States, we should look closely at the lessons Cotton Mather and his contemporaries learned 3 centuries ago. Emerging infectious diseases like measles keep reminding us that “the past is never dead. It isn’t even past” (12).

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Influenza A(H5N6) Virus Reassortant, Southern China, 2014

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To the Editor: Avian influenza A viruses generally do not cause disease in aquatic birds, the natural reservoir of these viruses (1). Influenza A(H5N6) was first isolated from mallards by García et al. in 1975 (2). Influenza viruses continue to evolve and reassort to generate novel, highly pathogenic viruses. Novel H5 highly pathogenic avian influenza virus subtypes, such as H5N2, H5N5, and H5N8, have been reported (3,4). Highly pathogenic influenza A viruses are endemic to many countries (http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2015/), cause tremendous economic losses to the poultry industry, and represent a serious threat to public health.

In March 2014, an influenza A(H5N6) outbreak caused the death of 457 birds in Laos (http://www.oie.int/wahis_2/public%5C..%5Ctemp%5Creports/en_imm_000015052_20140507_182757.pdf). During the same month, a flock of ducks in Guangdong Province in southern China exhibited typical respiratory signs of influenza A virus infection. This flock also had 70% decreased egg production and a slightly increased mortality rate. Throat swab specimens were taken from the symptomatic and dead ducks, and the samples were used to inoculate chicken embryos for virus isolation. Hemagglutination (HA) and neuraminidase (NA) inhibition assays were performed to identify the subtype of the isolated virus, which was designated A/duck/Guangdong/GD01/2014 (H5N6) (GD01/2014). The complete RNA genome was amplified by reverse transcription PCR and cloned into the pMD-19T vector for sequencing (5). The complete genome sequence of the GD01/2014 virus was submitted to GenBank (accession nos. KJ754142–KJ754149).

Multiple-sequence alignments showed that the HA gene of GD01/2014 shared 97.5% nt identity with A/wild duck/Shandong/628/2011 (H5N1) and NA genes shared 96.6% and 98.3% nt identity with A/swine/Guangdong/K6/2010 (H6N6) and A/duck/Shantou/1984/2007 (H6N6), respectively. All internal genes shared high levels of nucleotide identity (97.6%–98.5%) with A/wild duck/Fujian/2/2011(H5N1). The whole genes of GD01/2014 and the H5N6 viruses in Laos (LAO/2014) shared 98.2%–99.7% nt identity, indicating the same genotype. Phylogenetic analysis of the HA gene revealed that the isolated virus belonged to clade 2.3.4.6 (online Technical Appendix Figure, panel A, http://wwwnc.cdc.gov/EID/article/21/7/14-0838-Techapp.pdf) (6). The NA gene of GD01/2014 was clustered with some H6N6 viruses circulating in China (online Technical Appendix Figure, panel B). The 6 internal genes of GD01/2014 were closely related with A/wild duck/Fujian/2/2011(H5N1) and A/wild duck/Fujian/1/2011(H5N1) (online Technical Appendix Figure, panels C–H). Phylogenetic analysis showed that all 8 genes of GD01/2014 and LAO/2014 were closely related although genetically distant from the earlier isolated H5N6 viruses (online Technical Appendix Figure). These findings suggest that GD01/2014 and LAO/2014 are reassortants of wild duck H5N1 and H6N6 viruses, both of which have pathogenic and potential pandemic capacity in southern China. A previous report that H5N1 and H6N6 co-infected a duck suggests that GD01/2014 might be generated from the co-infection of H5N1 and H6N6 in the same host (7).

The intravenous pathogenicity index of GD01/2014 was 3.0, which indicates that the isolate is highly pathogenic for chickens. GD01/2014 had multiple basic amino acids (LRERRRRK/GLF) at the cleavage site between HA1 and HA2; this characteristic is typical of highly virulent influenza viruses (8). The HA protein contained E190, R220, G225, Q226, and G228 (H3 numbering) residues at the receptor-binding pockets, indicating that the virus preferentially binds to the sialic acid-2,3-NeuAcGal of the avian-like receptor (9). The HA protein has 7 potential N-glycosylation sites (PGSs); the HA1 protein has 5 PGSs; the HA2 protein has 2 PGSs. The NA protein of GD01/2014 and LAO/2014 had a deletion of 11 aa residues at positions 59–69 (N6 numbering) in the NA stalk region. Moreover, a deletion of 5 aa residues from positions 80–84 in the nonstructural 1 protein was found in GD01/2014 and LAO/2014. The position 627 and 701 of the polymerase basic 2 protein were E and D, respectively, characteristics of the avian influenza virus (10).

was the same genome type as and was highly homologous with the H5N6 virus in Laos. The findings in this study are also supported by the previous genetic characterization of these viruses by Wong et al. (11). However, the adaptation, host range, and virulence of this reassortant H5N6 virus are still unclear and should be further investigated. Furthermore, the potential for infection, outbreaks, and pandemic in other poultry and mammals should be carefully monitored.

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Characterization of 3 Megabase-Sized Circular Replicons from Vibrio cholerae

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To the Editor: Prokaryotes typically have a single circular chromosome. However, some bacteria have >1 chromosome. *Vibrio* bacteria, for example, have 2 circular chromosomes: 1 (Ch1) and 2 (Ch2) (1–3). Most recognizable genes responsible for essential cell functions and pathogenicity are located on Ch1. Ch2 is also thought to encode some genes essential for normal cell function and those associated with virulence. Both chromosomes are controlled coordinately in their replication and segregation (4). Evidence suggests that Ch2 was originally a mega-plasmid captured by an ancestral *Vibrio* species (2,5). We report the characterization of recent isolates of *V. cholerae* O1 from Thailand that carry a novel gigantic replicon (Rep.3) in addition to Ch1 and Ch2.

Cholera outbreaks occurred in Tak Province, Thailand, during March–December 2010. We obtained 118 isolates of *V. cholerae* O1 and subjected their NotI digestos to pulsed-field gel electrophoresis (PFGE), which differentiated the isolates into 8 different patterns (6). The profile of PFGE type A6 was identical to that of PFGE type A4, except that a large DNA band existed in type A6. The PFGE profile of the intact (undigested) DNA of the type A6 isolates exhibited a unique genome structure consisting of 3 large replicons (Figure, http://wwwnc.cdc.gov/EID/article/21/7/14-1055-F1.htm).

Three isolates of PFGE type A6 (TSY216, TSY241, and TSY421) were obtained during June 3–July 5, 2010, from 3 unrelated residents of a village near the Thailand–Myanmar border. The isolates were classified as multilocus variable-number tandem-repeat analysis type 16, suggesting that they are of clonal origin (6). Next, we performed whole-genome sequencing of TSY216, as a representative of PFGE type A6 isolates, by using the GS FLX Titanium
system (8 kb–span paired-end library; Roche, Indianapolis, IN, USA). Using Newbler version 2.6, the Roche 454 GS De Novo Assembler software (454 Life Sciences, Branford, CT, USA), we assembled 424,273 reads into 3 large scaffolds comprising 119 contigs at 18.3-fold coverage. The gaps between contigs were closed by PCR, and the PCR products were then sequenced. Illumina sequence data (Illumina, Inc., San Diego, CA, USA) were used to improve low-quality regions. The whole-genome sequence of TSY216 was completed and deposited in GenBank (accession nos. CP007653–55).

Full-genome sequencing revealed that V. cholerae O1 El Tor TSY216 consists of 3 circular replicons, Ch1 (3,053,204 bp), Ch2 (1,051,284 bp), and Rep.3 (896,006 bp), with an average G+C content of 47.7%, 47.0%, and 37.3%, respectively. In total, 4,579 coding sequences were detected and annotated by using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The whole-genome comparison between 2010EL-1786 (an outbreak isolate from Haiti) (7) and TSY216 revealed that Ch1 and Ch2 shared nearly identical gene content and showed conserved synteny, but integrative and conjugative elements were distinguishable. Strain TSY216 carries CTX-3, whereas strain 2010EL-1786 possesses CTX-3b. These CTXs represent wave 3 of the seventh cholera pandemic (8). Rep.3 of TSY216 did not share a conserved region with Ch1 and Ch2. Thus, this replicon may have been gained fairly recently through horizontal gene transfer from unknown organisms.

Rep.3 encodes 999 coding sequences and 66 transfer RNAs, among which 39 have been assigned putative functions and 960 encode hypothetical proteins and proteins of unknown function. The origin of the replicon could not be traced from the coding sequences in the public databases. Of note, Rep.3 encodes a specific transfer RNA for each amino acid, for a total of 20 amino acids. In addition, Rep.3 carries 2 genes encoding the histone-like nucleoid-structuring protein. In this regard, a 165-kb plasmid, pSf-R27, in Shigella flexneri encodes a histone-like nucleoid-structuring protein that was claimed to be a transcriptional repressor of the plasmid (9). Rep.3 may have a stealth strategy similar to that of pSf-R27.

We assessed the stability of the Rep.3 of the 3 A6 isolates. In total, 96 colonies for the 3 isolates were subcultured each day for 30 consecutive days. Then, using PCR and PFGE, we determined whether Rep.3 remained in the 96 subcultures. The Rep.3-specific primer set (Rep3hns-F: 5′-TTCAATGGCTCCAGCTTGC-3′ and Rep3hns-R: 5′-TCGCCACTCTATACAGGCC-3′) for PCR was designed for detection of the histone-like nucleoid-structuring protein gene encoded on the third replicon. All subcultures maintained Rep.3 in an unchanged state. However, when the organisms were cultured at 42°C, ≥70% of the subcultures lost Rep.3. The growth rates of the organisms with and without Rep.3 showed no substantial difference when the organisms were cultured in Luria-Bertani medium at 37°C.

The appearance of V. cholerae O1 variants with additional circular replicons may contribute to evolution of the bacteria in unexpected manners. Clones from the seventh cholera pandemic, which began in 1961, share nearly identical gene content (8,10). However, some clones, such as TSY216, can gain a replicon of megabase class and maintain it stably. Eventually, epidemic V. cholerae O1 may gain the ability to incorporate genes that change properties such as antigenicity or pathogenicity. The function of Rep.3 remains under investigation.

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Severe Malaria Not Responsive to Artemisinin Derivatives in Man Returning from Angola to Vietnam

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To the Editor: Partial artemisinin-resistant Plasmodium falciparum malaria, characterized by delayed parasite clearance after treatment with artesunate or artemisinin-based combination therapy, was first detected in western Cambodia and has now spread to or emerged de novo in 5 countries of the Greater Mekong Subregion (GMS) (1). However, most reported cases of malaria have been in Africa, and detecting artemisinin and multidrug resistance in Africa will have consequences for policy and containment plans (2).

Thus, vigilant monitoring is pivotal, and it is therefore with great interest that we read the case report on a patient in Vietnam with severe P. falciparum malaria, acquired in Angola in 2013, that was not responsive to artesunate or several other antimalarial combinations (3). We believe that there are several issues that challenge the conclusion that artemisinin resistance has reached Angola: 1) the phenotypic and genotypic characteristics of the infecting strain in this patient were very different from artemisinin-resistant strains in the GMS; 2) pharmacokinetic issues cannot be ruled out; and 3) perhaps of most relevance, the study documents severely delayed clearance of multiple strains in this polyclonal P. falciparum infection, suggesting splenic hypofunction as an important contributor.

The parasite clearance half-life calculated with the World Health Organization (WHO) online slope analyzer from the log linear segment of the clearance curve after start of artesunate therapy was 102.5 hours, which is ≈10 times longer than observed in the most artemisinin-resistant parasites in Cambodia. Postpublication genotyping of the infecting strain provided by the authors to WHO showed a wild-type Kelch (K13) gene, which is a recently discovered molecular marker for artemisinin resistance strongly correlated to the resistant phenotype in the GMS (1).

No pharmacokinetic assessment was made, and subtherapeutic artesunate and dihydroartemisinin (as well as clindamycin, piperaquine, quinine, and doxycycline) blood concentrations cannot be excluded. The intravenous artesunate regimen used differed from the WHO guideline of 2.4 mg/kg on admission, after 12 h, then daily. Pharmacokinetic modeling of the split doses used in the described case indicate that this dosing schedule results in ≤20% artesunate and dihydroartemisinin blood concentrations. In addition, quality issues in the artesunate batch might have played a major role. Batch no. 511002 used for this patient (not 511004 as mentioned in the article) was manufactured by Pharbaco (Hanoi, Vietnam) in April 2011 and had a shelf-life of 3 years; it was quality controlled and passed the quantitative testing by high pressure liquid chromatography in January 2014 (National Institute of Drug Quality Control, Vietnam). However, according to information shared with WHO, a test for clarity after reconstitution was not performed, whereas other samples from the same batch had failed this specific test, which led the Drug Administration of Vietnam to withdraw this batch from the market. The patient was subsequently treated with nasogastric-administered dihydroartemisinin/piperaquine and quinine plus doxycycline. Reduced intestinal absorption in this severely ill patient, related to reduced splanchnic blood flow, could have resulted in reduced bioavailability (4).

Host factors can affect parasite clearance. In this case, the parasitological response to artesunate and clindamycin, dihydroartemisinin/piperaquine, quinine, and doxycycline were all unusually slow. Functional asplenia results in very slow parasite clearance after artesunate treatment, resembling the clearance characteristics in the described case (5).

This interpretation is supported by finding genotypes representing ≥2 clones of parasites persisting >1 week after treatment with multiple antimalarial drugs. It seems very unlikely that this patient harbored multiple highly artemisinin-resistant parasite strains. Dead circulating intraerythrocytic parasites in patients who have hyposplenia can be recognized morphologically, but the article does not provide details on this.

Circulation of multidrug resistant malarial strains in sub-Saharan Africa can have disastrous consequences, and it is critical to detect its arrival at an early stage. The case report by Van Hong et al. implies the unlikely event of independent emergence of multidrug resistant strains in a traveler from Vietnam in Angola, without evidence of local declining artemisinin-based combination therapy efficacy. WHO and partners are investigating the phenotype and genotype of parasite strains from the same geographic area in Angola to address the concerns raised above. We believe that this single case report is insufficient to raise the alarm.

References
The discussion triggered by the publication of our case report raises the question of what should be reported to the attention of the scientific community and public health authorities. Besides being an obligation for clinical physicians, reporting unusual treatment failures such as our case is also an essential component of anti-malarial resistance surveillance. As mentioned by Ringwald and Dondorp, “vigilant monitoring is pivotal” for the detection of possible foci of resistance. For early detection of artemisinin resistance, we would rather have a more sensitive than specific system, because the latter would probably miss the first emerging cases of resistance. Reporting cases similar to the one we published should be encouraged.

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Diversity of *Bartonella* spp. in Bats, Southern Vietnam

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To the Editor: To investigate bats as potential reservoirs for *Bartonella* spp. in Vietnam, we screened a range of bat species to determine the prevalence and genetic diversity of *Bartonella* spp. in bat populations in southern Vietnam. In a study of bat biodiversity in southern Vietnam, 60 bats were trapped at 6 sites in Dong Nai Nature Reserve and Cat Tien National Park, Vietnam, in May 2013. Bats were trapped by using mist nets and harp traps set at ground level, and were euthanized by using isoflurane (http://www.avma.org/KB/Policies/Documents/euthanasia.pdf) for cataloguing at the Vietnam Academy of Sciences and Technology, Hanoi. Blood specimens were collected by cardiac puncture, and external measurements were recorded. Bats were speciated according to morphological and ecological relationships with other bats, which would be expected if persistent infection were caused by persistent infection of bats with *Bartonella* spp. did not differ between sampling locations (Table) or by estimated age of the bat (determined by deviation above or below the median tibial length of each species); prevalence was 33.3% (9/27) in younger bats and 36.4% (12/33) in older bats.

DNA sequences from the 21 PCR citrate synthase A gene amplicons (GenBank accession nos. KP100340–KP100360) were subjected to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to assess sequence similarity. Potentially novel *Bartonella* phylogroups were identified as having <96% sequence similarity with all publicly available sequences in GenBank (4). The sequences were then manually aligned with those of a representative sample of *Bartonella* spp. and trimmed to the 327-nt region (positions 801–1127) commonly used for taxonomic classification (4). A neighbor-joining tree was constructed by using the Hasegawa–Kishino–Yano plus gamma model of nucleotide substitution in Geneious version 7.1.7 with 1,000 bootstrap replications (5).

Sequence analysis identified 10 distinct *Bartonella* phylogroups (I–X) among 21 *Bartonella*-positive blood samples from bats in Vietnam (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/7/14-1760-Techapp1.pdf). Nine of these phylogroups showed <96% sequence similarity to all previously identified *Bartonella* sequences, suggesting they might belong to new *Bartonella* species. *Bartonella* spp. in *Rhinolophus* spp. bats were classified into phylogroups I, III, VIII, IX, and X. Phylogroups II and VII were detected in samples from *Hipposideros* spp. bats, and phylogroups IV and V were detected in *Megaderma* spp. bats. Phylogroup VI was detected only in a *Megaerops* spp. bat.

Although 9 lineages (I, III–X) were novel, phylogroup II was identified in 4 *Hipposideros* spp. bats and showed 96.3%–97.2% similarity to *Bartonella* spp. isolated from a bat fly found on a *Hipposideros* spp. host in Malaysia (GenBank accession no. JX416238). This similarity might suggest widespread distribution of this *Bartonella* spp. lineage in *Hipposideros* spp. bats or their ectoparasites in Southeast Asia. Additional genetic characterization of strains is needed to determine whether any of these novel phylogroups represent new species and to investigate their evolutionary and ecological relationships with other *Bartonella* spp. identified in Vietnam and elsewhere.

The primary observation in this study was detection of *Bartonella* spp. (by DNA amplification) in bats in southern Vietnam at a prevalence of 35.0%, which is comparable with that reported in Kenya (30.2%) and Guatemala (33.0%) (Table) (6,7). However, the use of conventional PCR in this study might underestimate the true prevalence.

Although high prevalences have been proposed to be caused by persistent infection of bats with *Bartonella* spp., our findings indicate no increase in prevalence by age of bat, which would be expected if persistent infection were common. This finding, and detection of multiple lineages infecting individual bat species, may instead reflect high levels of transmission within and between bat species.
caused by crowded roosting areas and sharing of roosts by multiple species. This behavior provides opportunities for transmission of *Bartonella* bacteria or exchange of infected ectoparasites, such as *Cyclopodia* spp. (8), although the precise roles of these 2 processes are unknown.

Although no human cases of *Bartonella* ssp. infection have been reported in Vietnam, *Bartonella* spp. have been identified in beehive humans elsewhere in Southeast Asia (9) and are also common in rats in southern Vietnam (10). Because close contact with bats (i.e., through manure farming and consumption of bat meat) and potential arthropod vectors (i.e., through handling and consumption of fruit) is common in parts of Vietnam, targeted screening of bats and their human contacts might improve our understanding of the zoonotic potential of these bacteria and their potential effect on public health.

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Ms. Pham is a research assistant at the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam. Her primary research interests focus on characterizing the diversity and spread of potential agents of zoonotic disease in domestic and wild animal populations across Vietnam.

### References


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### Seropositivity for Avian Influenza H6 Virus among Humans, China

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### Table. Prevalence of *Bartonella* ssp. in bats from 2 sites in Dong Nai, Vietnam, 2013

<table>
<thead>
<tr>
<th>Bat species</th>
<th>No. <em>Bartonella</em> ssp.–positive bats/total bats trapped (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cat Tien National Park</td>
</tr>
<tr>
<td><em>Cynopterus sphinx</em></td>
<td>0/0</td>
</tr>
<tr>
<td><em>Hipposideros armiger</em></td>
<td>2/6</td>
</tr>
<tr>
<td><em>Hipposideros larvatus</em></td>
<td>3/5</td>
</tr>
<tr>
<td><em>Megaerops niphanae</em></td>
<td>0/0</td>
</tr>
<tr>
<td><em>Megaderma spasma</em></td>
<td>0/0</td>
</tr>
<tr>
<td><em>Megaderma lyra</em></td>
<td>1/1</td>
</tr>
<tr>
<td><em>Rhinolophus acuminatus</em></td>
<td>0/0</td>
</tr>
<tr>
<td><em>Rhinolophus cheselii</em></td>
<td>2/5</td>
</tr>
<tr>
<td><em>Rhinolophus sinicus</em></td>
<td>0/3</td>
</tr>
<tr>
<td><em>Rhinolophus lucius</em></td>
<td>0/1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8/21 (38.1)</strong></td>
</tr>
</tbody>
</table>

*Fruit-eating.
†Insectivorous.
‡Carnivorous.*

LETTERS
To the Editor: Influenza virus subtype H6 was first isolated from a turkey in 1965 in the United States (1) and was subsequently found in other parts of the world (2). Over the past several decades, the prevalence of H6 virus has dramatically increased in wild and domestic birds (2–4). In China, highly pathogenic influenza A(H5N1), low pathogenicity influenza (H9N2), and H6 are the most prevalent avian influenza viruses among poultry (5). Although only 1 case of H6 virus infection in a human has been reported worldwide (6), several biological characteristics of H6 viruses indicate that they are highly infectious to mammals. Approximately 34% of H6 viruses circulating in China have enhanced affinity to human-like receptors (α2,6 NeuAcGal) (2). H6 viruses can also infect mice without prior adaptation (2,7), and some H6 viruses can be transmitted efficiently among guinea pigs (2). To evaluate the potential threat of H6 viruses to human health, we conducted a systematic serologic study in populations occupationally exposed to H6 viruses.

During 2009–2011, a total of 15,689 serum samples were collected from live poultry market workers, backyard poultry farmers, large-scale poultry farmers, poultry-slaughter factory workers, and wild bird habitat workers in 22 provinces in mainland China. A/chicken/Y94/Guangdong/2011 (H6N2), a representative isolate of predominant H6 viruses in mainland China, was used for the serologic testing (online Technical Appendix Table 1, Figures 1, 2, http://wwwnc.cdc.gov/EID/article/21/7/15-0135-Techapp1.pdf). Hemagglutination inhibition (HI) assay was performed for all serum samples, and samples with an HI titer ≥20 were verified by a microneutralization (MN) assay, as indicated by World Health Organization guidelines (8). An MN result of ≥20 was considered positive.

The HI result was ≥20 for H6N2 virus in 298 of the 15,689 specimens, and the MN result was positive in 63 of the 298 specimens (overall seropositivity range 20–320, mean 32.7, 0.4%) (online Technical Appendix Table 2). The proportion of group members who were seropositive differed significantly according to occupational exposure (p = 0.0125). Seropositivity was highest among workers in live poultry markets, backyard poultry farmers, and workers in wild bird habitats (0.66%, 0.42%, and 0.51%, respectively) (Table). According to χ² test results, seropositivity among workers in live poultry markets was significantly higher than that among large-scale poultry farmers (p = 0.0015, adjusted α = 0.005). Analysis by unconditional logistic regression model showed that exposure to live poultry markets was a risk factor for human infection with avian influenza H6 virus (odds ratio 2.1, 95% CI 1.27–3.47).

Seropositivity did not differ significantly among male and female persons tested (p = 0.08) (Table). No children were positive for the H6N2 virus. For other age groups, seropositivity ranged from 0.25% to 0.45%, but differences were not significant (p>0.05) (Table).

Of the 22 provinces from which serum specimens were collected, 11 were northern provinces and 11 were southern provinces. Positive specimens were detected in all southern provinces. In northern China, no seropositive results were detected in Henan, Liaoning, or Jilin Provinces. According to χ² test results, seropositivity in southern China was significantly higher than seropositivity in northern China (p = 0.0375) (Table).

Human infection with influenza H6 virus in mainland China has not been reported, but 63 serum specimens tested in our study were positive for the H6 virus. This level of seropositivity is much higher than that for highly pathogenic

**Table. Seropositivity of occupationally exposed populations for the influenza (H6N2) virus, China, 2009–2011†**

<table>
<thead>
<tr>
<th>Population</th>
<th>Total no. serum samples</th>
<th>Mean titer for MN ≥20</th>
<th>No. serum samples with MN ≥20</th>
<th>Seropositivity (95% CI)</th>
<th>Odds ratio† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>15,689</td>
<td>32.7</td>
<td>63</td>
<td>0.40 (0.40–0.41)</td>
<td></td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live poultry market</td>
<td>3,950</td>
<td>43.08</td>
<td>26</td>
<td>0.66 (0.64–0.68)</td>
<td>2.10 (1.27–3.47)</td>
</tr>
<tr>
<td>Poultry farm</td>
<td>3,762</td>
<td>25.71</td>
<td>7</td>
<td>0.19 (0.18–0.19)</td>
<td>0.40 (0.18–0.87)</td>
</tr>
<tr>
<td>Backyard poultry farm</td>
<td>4,324</td>
<td>26.67</td>
<td>18</td>
<td>0.42 (0.40–0.43)</td>
<td>1.05 (0.61–1.82)</td>
</tr>
<tr>
<td>Poultry slaughter factory</td>
<td>1,235</td>
<td>30.00</td>
<td>2</td>
<td>0.16 (0.15–0.17)</td>
<td>0.38 (0.09–1.57)</td>
</tr>
<tr>
<td>Wild bird habitat</td>
<td>788</td>
<td>20.00</td>
<td>4</td>
<td>0.51 (0.47–0.54)</td>
<td>1.28 (0.47–3.54)</td>
</tr>
<tr>
<td>Other</td>
<td>1,630</td>
<td>23.33</td>
<td>6</td>
<td>0.37 (0.35–0.39)</td>
<td>0.91 (0.39–2.11)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>7,620</td>
<td>24.29</td>
<td>28</td>
<td>0.37 (0.36–0.38)</td>
<td>Reference</td>
</tr>
<tr>
<td>M</td>
<td>8,069</td>
<td>39.39</td>
<td>35</td>
<td>0.43 (0.42–0.44)</td>
<td>1.18 (0.72–1.94)</td>
</tr>
<tr>
<td><strong>Age group, y</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children, &lt;14</td>
<td>74</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Youth, 15–24</td>
<td>1,168</td>
<td>20.00</td>
<td>3</td>
<td>0.26 (0.24–0.27)</td>
<td>0.75 (0.19–3.00)</td>
</tr>
<tr>
<td>Adult, 25–59</td>
<td>1,2450</td>
<td>34.07</td>
<td>54</td>
<td>0.43 (0.43–0.44)</td>
<td>1.27 (0.54–2.94)</td>
</tr>
<tr>
<td>Elderly, ≥60</td>
<td>1,748</td>
<td>13.33</td>
<td>6</td>
<td>0.34 (0.33–0.36)</td>
<td>Reference</td>
</tr>
<tr>
<td>No age record</td>
<td>249</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td><strong>Geographic distribution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South</td>
<td>10,522</td>
<td>32.00</td>
<td>50</td>
<td>0.48 (0.47–0.48)</td>
<td>Reference</td>
</tr>
<tr>
<td>North</td>
<td>5,167</td>
<td>35.38</td>
<td>13</td>
<td>0.25 (0.24–0.26)</td>
<td>0.59 (0.30–1.15)</td>
</tr>
</tbody>
</table>

*MN, microneutralization; † not applicable.
†Odds ratios were calculated by using unconditional logistic regression model (SPSS 17.0, Armonk, NY, USA).
avian influenza A(H5N1) virus, for which only 2 of the serum specimens we tested were positive (data not shown), but much lower than the seropositivity level for low pathogenicity avian influenza A(H9N2) virus; 3.4% of the samples tested were positive for A/Chicken/Hong Kong/G9/1997(H9N2)–like virus (data not shown). A previous US study has reported H6N2-positive antibodies in veterinarians (9). Our results and the veterinarian study indicate that the H6N2 virus could infect humans.

In our study, positive samples were detected in 19 of 22 provinces and in all tested worker populations, suggesting that the H6 virus has been broadly circulating in birds in China. Live poultry market exposure is the major risk factor for human infection with avian influenza H6 virus. The limitation of this study is that antigen selection may not accurately detect neutralization antibodies for different subtypes of H6 viruses. Surveillance of the H6 virus in birds and occupationally exposed populations should be strengthened for pandemic preparedness.

Acknowledgments
This study was performed under the serology surveillance system of occupationally exposed populations in China. We are deeply thankful for the contributions of all National Influenza Surveillance Network members, including the China Centers for Disease Control and Prevention in the provinces and in the prefects, all of which collected samples for years. We also thank Ms. Qiao-hong Liao for providing consultation on statistical analysis.

This study was supported in part by the China–United States cooperation project “Developing sustainable influenza surveillance networks and response to avian and pandemic influenza in China” and by the China National Mega-projects for Infectious Diseases (2014ZX10004002).

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Absence of MERS-Coronavirus in Bactrian Camels, Southern Mongolia, November 2014

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To the Editor: Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified among humans in 2012 in Saudi Arabia (1). As of February 5, 2015, a total of 971 MERS cases and 356 associated deaths had been confirmed (2). Because MERS is a zoonotic disease, it is essential that the animal reservoirs and hosts that sustain virus circulation in nature be identified.

Seroepidemiologic and virologic studies have demonstrated evidence of MERS-CoV infection in dromedary camels (Camelus dromedarius) in the Arabian Peninsula (3), and viruses isolated from dromedaries appear capable of infecting the human respiratory tract (4). In some instances, MERS-CoV infection in dromedaries has preceded infection in humans (5), indicating that dromedaries are a natural host for MERS-CoV and a possible source of human infection. Thus, it is important to define the geographic range of MERS-CoV infection in camels and the species of camelids that are infected by MERS-CoV in nature.

Two species of camels exist: 1-hump dromedaries (C. dromedarius) and 2-hump Bactrian camels (C. bactrianus).

1These authors contributed equally to the article.
Dromedaries are common in hot desert terrains of the Arabian Peninsula, the Middle East, Afghanistan, central Asia, India, and parts of Africa. Bactrian camels are found in colder steppes of Mongolia, Central Asia, Pakistan, and Iran. Studies have demonstrated a high seroprevalence (>90%) of MERS-CoV in adult (≥5 years of age) dromedaries from the Middle East and from northern, eastern, and parts of central Africa (6), but whether MERS-CoV circulates among Bactrian camels is unknown.

To determine whether MERS-CoV is circulating among both species of camels, we studied apparently healthy Bactrian camel herds in southern Mongolia during November 24–30, 2014. We investigated 11 herds in Umnugovi Province (170 sampled animals) and 1 herd in the adjacent Dundgovi Province (30 sampled animals) (Table). A convenience sample was collected from each herd; younger animals were oversampled. Serum and nasal swab samples were collected from each animal. The nasal swab samples were placed in virus transport medium and later tested by real-time PCR targeting open-reading frame 1a and upstream of envelope protein gene, as previously described (7); all samples were negative for MERS-CoV RNA. The serum samples were tested for the presence of MERS-CoV antibody by using a validated MERS-CoV (strain EMC) spike pseudoparticle neutralization test (8); no samples were positive, indicating a lack of recent or past MERS-CoV infection. A random sample of 5 serum samples each from camels in Umnugovi and Dundgovi Provinces was tested by using a microneutralization test against bovine coronavirus (BCoV) as previously described (8); all 10 samples were positive (titer range 1:20–1:640).

The sampled animals included 127 camels ≥5 years of age from 12 herds across 2 provinces in southern Mongolia. Thus, the negative test results indicate that MERS-CoV is not circulating among Bactrian camels in southern Mongolia. The seroprevalence of MERS-CoV among adult dromedaries in the Middle East and Africa is typically >90%, so the lack of any serologic reactivity in camels from Mongolia implies that MERS-CoV infection does not infect Bactrian camels or that the geographic range of the virus does not extend to northeastern Asia. In contrast, infection with a BCoV-like coronavirus seems ubiquitous in Bactrian camels, as it is in dromedaries (7).

Dipeptidyl peptidase-4 (DPP4; cluster of differentiation 26) is the receptor for MERS-CoV. As deduced from the human DPP4–MERS-CoV spike protein structural model, the differences in the amino acids in DPP4 molecules of dromedary and Bactrian camel were found in 2 small regions far from the binding interface of DDP4 and MERS spike protein (9). The 15 aa of DPP4 critical for binding with MERS-CoV spike protein are conserved between dromedaries and Bactrian camels. Definitive evidence of susceptibility, or lack thereof, of Bactrian camels to MERS-CoV can be established only by experimental infection of these animals.

Even if Bactrian camels are susceptible to MERS-CoV infection, geographic separation may be an alternative explanation for the absence of MERS-CoV among camels in Mongolia. So far, Australia is the only country where dromedaries appear to be free of MERS-CoV; however, as with dromedaries elsewhere, dromedaries in Australia are infected by a BCoV-like virus (8). Dromedaries in Australia originated from Afghanistan; these camels were shipped to Australia in the early part of the twentieth century to work on railroad construction projects. There are 2 plausible explanations for the lack of MERS-CoV in Australia: the small numbers of adult animals that were transported from Afghanistan to Australia might not have been sufficient to introduce the virus into Australia or the virus might have been absent from dromedaries in Afghanistan.

Our study was limited by sample size and by the breadth of the study area. Mongolia has 21 provinces and ≈349,300 Bactrian camels, but we studied just 2 southern

<table>
<thead>
<tr>
<th>Herd no.</th>
<th>Province, district</th>
<th>Age, y</th>
<th>No. sampled/no. total in herd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Umnugovi, Khankhongor</td>
<td>&lt;1</td>
<td>23/36</td>
</tr>
<tr>
<td>2</td>
<td>Umnugovi, Khankhongor</td>
<td>1–5</td>
<td>18/31</td>
</tr>
<tr>
<td>3</td>
<td>Umnugovi, Khankhongor</td>
<td>&gt;5</td>
<td>13/28</td>
</tr>
<tr>
<td>4</td>
<td>Umnugovi, Khankhongor</td>
<td>0–1</td>
<td>26/65</td>
</tr>
<tr>
<td>5</td>
<td>Umnugovi, Bayan-Ovoo</td>
<td>2–4</td>
<td>17/70</td>
</tr>
<tr>
<td>6</td>
<td>Umnugovi, Bayan-Ovoo</td>
<td>&gt;5</td>
<td>4/9</td>
</tr>
<tr>
<td>7</td>
<td>Umnugovi, Bayan-Ovoo</td>
<td>2–4</td>
<td>11/33</td>
</tr>
<tr>
<td>8</td>
<td>Umnugovi, Bayan-Ovoo</td>
<td>&gt;5</td>
<td>10/54</td>
</tr>
<tr>
<td>9</td>
<td>Umnugovi, Bayan-Ovoo</td>
<td>2–4</td>
<td>13/36</td>
</tr>
<tr>
<td>10</td>
<td>Umnugovi, Bayan-Ovoo</td>
<td>&gt;5</td>
<td>20/24</td>
</tr>
<tr>
<td>11</td>
<td>Umnugovi, Bayan-Ovoo</td>
<td>2–4</td>
<td>30/58</td>
</tr>
<tr>
<td>12</td>
<td>Dundgovi, Khuld</td>
<td>&gt;5</td>
<td>200/491</td>
</tr>
</tbody>
</table>
Oligella ureolytica
Bacteremia in Elderly Woman, United States

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To the Editor: Oligella ureolytica is an aerobic gram-negative coccobacillus found as a commensal organism in human urinary tracts (1). Previously referred to as CDC Group IVe, this bacterium is not commonly encountered as a source of infection and is difficult to isolate by using conventional laboratory procedures (2). The few cases of pathogenic infection with O. ureolytica described in the literature have occurred in patients ranging in age from newborn to 89 years and from the varied locations of India, Turkey, Canada, and the United States (3–7). We report a case of O. ureolytica bacteremia in a patient in whom septis was diagnosed and review the current literature on this emerging pathogen.

A 66-year-old woman sought treatment in our emergency department for a fever of 100.7°F, femur fracture, and a right buttock stage III decubitus ulcer. She reported having fallen 4 days earlier, after which she was unable to walk and spent 4 days laying in her own urine and feces. Blood tests revealed an elevated leukocyte count of 24.4 × 10^9 cells/L (76% neutrophils, 2% bands), and urinalysis showed trace leukocyte esterase, +3 bacteria, and 5–10 leukocytes. Chest radiograph and head computed tomography images were unremarkable. Her electrocardiogram showed nonspecific ST wave changes. Samples from the patient’s blood, urine, and wounds were collected while the patient was in the emergency department and were sent for culture.

Wound cultures showed growth of Proteus mirabilis and Enterococcus spp. The urine culture grew >100,000 CFU Escherichia coli. The first set of blood cultures grew O. ureolytica in aerobic and anaerobic bottles, but another set drawn 30 min later showed no growth. The blood cultures were processed by using the Bact/Alert 3D (bioMérieux, Marcy l’Étoile, France) and Gram stained. Identification was from the Vitek 2 compact system (bioMérieux). The O. ureolytica sample was sensitive to amikacin, ampicillin/sulbactam, ceftazidime, ceftriaxone, gentamicin, imipenem, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, and chloramphenicol. No resistance was found.

Because of the unique bacteremia, further diagnostics were conducted. The results of chest, abdomen, and pelvic computed tomography scans were unremarkable. HIV

provinces and a total of 200 camels. Umnojvo Province has the largest, and Dundgoji Province the fifth largest, camel population in the country (113,000 and 28,000 animals, respectively). Further studies on the epidemiology of MERS-CoV infection in dromedaries and Bactrian camels from central Asia, China, and Mongolia are warranted.

The field work for this study was supported by a research grant from The University of Hong Kong; the laboratory testing was supported by the National Institutes of Health, National Institute of Allergy and Infectious Diseases (contract N27220140006C).

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The incubation period is long (4 days), and not all laboratories incubate cultures for that long, as occurred in the 2013 urinary tract infection case (1,3,5). Also, the identification of less commonly encountered bacteria is not always pursued to the genus and species level (2). Furthermore, it is believed that Oligella spp. can be misidentified as phenotypically similar organisms, such as Bordetella bronchiseptica and Achromobacter spp. (4,10).

We believe that many cases of O. ureolytica infection have gone unrecognized or were incorrectly identified. Some cases may also have been dismissed as contamination because of laboratorians’ and clinicians’ lack of familiarity with this bacterium. Our review suggests that advancing laboratory techniques will lead to more recognized cases and that further studies are necessary to understand this bacterium’s clinical significance.

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**References**

By December 31, 2014, the Ebola epidemic in West Africa had resulted in treatment of 10 Ebola case-patients in the United States; a maximum of 4 patients received treatment at any one time (1). Four of these 10 persons became clinically ill in the United States (2 infected outside the United States and 2 infected in the United States), and 6 were clinically ill persons medically evacuated from West Africa (online Technical Appendix 1, Table 6). To plan for possible future cases in the United States, we produced a tool to estimate future numbers of Ebola case-patients needing treatment at any one time in the United States (2). Another study considered the overall risk for exportation of Ebola from West Africa but did not estimate the number of potential cases in the United States at any one time (3).

We provide for practicing public health officials a spreadsheet-based tool, Beds for Ebola Disease (BED) (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/21/7/15-0286-Techapp2.xlsx) that can be used to estimate the number of Ebola patients expected to be treated simultaneously in the United States at any point in time. Users of BED can update estimates for changing conditions and improved quality of input data, such as incidence of disease. The BED tool extends the work of prior studies by dividing persons arriving from Liberia, Sierra Leone, and Guinea into the following 3 categories: 1) travelers who are not health care workers (HCWs), 2) HCWs, and 3) medical evacuees. This categorization helps public health officials assess the potential risk for Ebola virus infection in individual travelers and the subsequent need for post-arrival monitoring (4).

We used the BED tool to calculate the estimated number of Ebola cases at any one time in the United States by multiplying the rate of new infections in the United States by length of stay (LOS) in hospital (Table). The rate of new infections is the sum of the rate of infected persons in the 3 listed categories who enter the United States from Liberia, Sierra Leone, or Guinea. For the first 2 categories of travelers, low and high estimates of Ebola-infected persons arriving in the United States are calculated by using low and high estimates of both the incidence of disease in the 3 countries and the number of arrivals per month (Table). Calculating the incidence among arriving HCWs required estimating the number of HCWs treating Ebola patients in West Africa (online Technical Appendix 1, Tables 2–4). For medical evacuations of persons already ill from Ebola, we calculated low and high estimates using unpublished data of such evacuations through the end of December 2014.

Although only 1 Ebola case has caused additional cases in the United States (7), we included the possibility that each Ebola case-patient who traveled into the United States would cause either 0 secondary cases (low estimate) or 2 secondary cases (high estimate) (Table). Such transmission might occur before a clinically ill traveler is hospitalized or between a patient and HCWs treating the patient (7). To account for the possibility that infected travelers may arrive in clusters, we assumed that persons requiring treatment would be distributed according to a Poisson probability distribution. Using this distribution enables us to calculate, using the BED tool, 95% CIs...
around the average estimate of arriving case-patients. The treatment length used in both the low and high estimate calculations was 14.8 days, calculated as a weighted average of the LOS of hospitalized case-patients treated in West Africa through September 2014 (online Technical Appendix 1 Table 5) (8). We conducted a sensitivity analysis using LOS and reduced case-fatality rate of patients treated in the United States (online Technical Appendix 1 Table 6).

For late 2014, the low estimate of the average number of beds needed to treat patients with Ebola at any point in time was 1 (95% CI 0–3). The high estimate was 7 (95% CI 2–13).

In late 2014, the United States had to plan and prepare to treat additional Ebola case-patients. By mid-January 2015, the capacity of Ebola treatment centers in the United States (49 hospitals with 71 total beds [9]) was sufficient to care for our highest estimated number of Ebola patients. Policymakers already have used the BED model to evaluate responses to the risk for arrival of Ebola virus–infected travelers, and it can be used in future infectious disease outbreaks of international origin to plan for persons requiring treatment within the United States.

### Acknowledgments

We thank Careese Campbell and Bishwa Adhikari for compiling various data and the Centers for Disease Control and Prevention’s Ebola Response Global Migration Task Force for data on HCW arrivals.

### References


Highly Pathogenic Avian Influenza A(H5N1) Virus in Poultry, Nigeria, 2015

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To the Editor: In Nigeria, from February 2006 through July 2008, outbreaks of highly pathogenic avian influenza (HPAI) subtype H5N1 virus infection in poultry negatively affected animal and public health as well as the agricultural sector and trade. These outbreaks were caused by viruses belonging to genetic clades 2.2 and 2.2.1 (1). In January 2015, seven years after disappearance of the virus, clinical signs of HPAI (swollen head and wattles, hemorrhagic shank and feet) and increased mortality rates were observed among backyard poultry in Kano and in a live bird market in Lagos State, Nigeria. The virus was isolated from 2 samples independently collected from the poultry farm (parenchymatous tissues) and the market (tracheal swab), and H5 subtype virus was identified by reverse transcription PCR. The samples were adsorbed onto 2 Flinders Technology Associates cards (GE Healthcare Life Sciences, Little Chalfont, UK), which were sent to the World Organisation for Animal Health/FAO and the United Nations Organisation for Animal Health/Food and Agriculture Organization of the United Nations Reference Laboratory for Avian Influenza in Italy for subtype confirmation and genetic characterization. Influenza A(H5N1) virus was detected in both samples, and sequencing of the hemagglutinin (HA) gene showed that the viruses possessed the molecular markers for HPAI viruses with a multibasic amino acid cleavage site motif (PQRERRRRKR*G).

The complete genome of the virus from backyard poultry was successfully sequenced from the genetic material extracted from the Flinders Technology Associates cards by using an Illumina MiSeq platform (2) and was submitted to the Global Initiative on Sharing All Influenza Data database (http://platform.gisaid.org) under accession nos. EPI556504 and EPI567299–EPI567305. Maximum-likelihood trees were estimated for all 8 gene segments by using the best-fit general time reversible plus invariant sites plus gamma 4 model of nucleotide substitution with PhyML (3). The topology of the phylogenetic tree of the HA gene demonstrated that the H5N1 virus from Nigeria (A/chicken/Nigeria/15VIR39-2/2015) falls within genetic clade 2.3.2.1c (Figure, panel A). In particular, the HA gene sequence clustered with H5 viruses collected in China in 2013 and with an H5N1 virus (A/Alberta/01/2014) isolated from a Canada resident who had returned from China (similarity 99.3%–99.5%) (4).

The remaining 7 genes were closely related to genes of A/Alberta/01/2014(H5N1), although the 2 viruses differed by 32 aa (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/7/15-0421-Techapp1.pdf). Just as for the virus from Canada (4), 7 of 8 gene segments of the virus from Nigeria clustered with HPAI A(H5N1) virus circulating in Vietnam and China, while the polymerase basic 2 gene segment (Figure, panel B) resulted from reassortment with viruses circulating in the same Asian countries but belonged to the H9N2 subtype. Differing from the strain from Canada (only 2 aa mutations compared with the 2.3.2.1c candidate vaccine strain; 5), the strain from Nigeria possesses 6 aa differences: 3 in HA1 and 3 in HA2 (online Technical Appendix). The effect of these mutations on the antigenic relatedness of these strains should be further explored.

Molecular characterization demonstrated that the polymerase basic 2 sequence contains glutamic acid at position 627, establishing the lack of a well-known mammalian adaptation motif (6). Mutations associated with increased virulence in mice have been observed in the nonstructural protein 1 (P42S, D87E, L98F, I101M, and the 80–84 deletion) and in the matrix 1 proteins (N30D, T215A). In addition, the substitutions D94N, S133A, S155N (H5 numbering) associated with increased binding to α-2,6 sialic acid have been identified in the HA protein. However, most of these substitutions are present in the H5N1 virus sequences from Asia included in our phylogenetic analyses, suggesting that they may be common among the HPAI H5 virus subtype. Mutations associated with resistance to antiviral drugs have not been detected (7).

The results obtained from whole-genome analysis provide evidence that a novel clade of the A(H5N1) virus, specifically clade 2.3.2.1c, has reached Nigeria. Although ascertaining how and exactly when this has happened is difficult, it seems most likely that the virus entered the country in December 2014, as evidenced by unverified
accounts of increased poultry deaths in some live bird markets in Lagos, after the birds had been moved from the north (Kano) to the south during the festive season. The identification of genetic clustering between the strains from Nigeria analyzed here and the HPAI A(H5N1) viruses originally identified in Asia suggests an unknown epidemiologic link between these regions, probably associated with human activities, migratory bird movements, or both.

Considering that this virus is an intersubtype reassortant and has already caused infection in humans, we believe that complete characterization of the strain in terms of virulence and host range is of high priority. Furthermore, because the reemergence of subtype H5N1 virus was followed by epidemiologic amplification (≈265 outbreaks in 18 states as of February 2015; T. Joannis, pers. comm., 2015) for which virus genetic characterization is not yet available, local veterinary and public health services and international organizations should take necessary measures to identify critical control points and stop circulation of this virus.

Acknowledgments

We gratefully acknowledge the contributing authors and the originating and submitting laboratories for the sequences from the Global Initiative on Sharing All Influenza Data EpiFlu database on which this research is based. We also acknowledge Olorunsola Bankole and Idris Ibrahim for sample collection and the Federal Department of Veterinary and Pest Control Services, Federal Ministry of Agriculture, Abuja, Nigeria, for technical support. We thank Silvia Ormelli, Alessia Schivo, and Francesca Ellero for their excellent technical assistance.

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Pulmonary Complications of HIV

Charles Feldman, Eva Polverino, and Julio A. Ramirez, editors

European Respiratory Society, Lausanne, Switzerland, 2014

Pages: 265; Price: US $75.00 (paperback)

Pulmonary Complications of HIV summarizes current practices for diagnosing and treating common HIV-related pulmonary complications. It is a well-written, educational work that will interest anyone managing the care of HIV-infected persons. The content and amount of information packed into this easy-to-read textbook is impressive. Each chapter is well organized and well referenced, and important concepts and definitions are laid out clearly.

Since HIV/AIDS was first described, clinicians have found that the lung is the site most frequently affected and that pulmonary complications are a major cause of illness and death for HIV-infected persons. However, over the years, the discovery and use of antiretroviral therapy has increased life expectancy for HIV-infected persons, and the spectrum of infectious and noninfectious pulmonary complications has changed. For example, the incidence of opportunistic pneumonias has declined dramatically, whereas the incidence of bacterial pneumonia has not decreased proportionately. Furthermore, noninfectious complications, such as chronic obstructive pulmonary disease and lung cancer, are increasing. It is critical for anyone managing the care of HIV-infected persons to be aware of these lung complications and understand their diagnoses, possible treatments, and prevention. Pulmonary Complications of HIV does an excellent job discussing these aspects.

The authors are well-respected researchers and clinicians from throughout the world who work in the fields of pulmonary medicine and HIV-related lung diseases. The literature on HIV-related pulmonary complications is still lacking in certain areas, which most likely led to some chapters (e.g., Bronchiectasis) to be shorter and less comprehensive than others. Of the book’s 19 chapters, the first 2 discuss the global epidemiology of HIV and current antiretroviral therapy guidelines, which will be useful for clinicians who might not regularly manage the care of HIV-infected patients. The third chapter discusses pulmonary immunity, a complicated topic but one the authors explain simply by emphasizing essential concepts. The next several chapters highlight various diseases and strategies for preventing them in the field, including vaccine guidelines. In addition, the authors cover a number of other key aspects to HIV care, such as pregnancy, pediatrics, and infectious and noninfectious complications, completing a thorough review of the literature. Although infectious disease specialists and others who care for HIV-infected patients might consider the first few chapters too simplistic, the latter chapters on pulmonary complications will be relevant and instructive. Pulmonologists will find that the first few chapters discuss aspects of HIV care to which they are not readily exposed and the latter chapters provide information on the epidemiology, clinical manifestations, diagnosis, and management of common complications seen in HIV-infected persons.

Pulmonary Complications of HIV does an exceptional job summarizing the major pulmonary manifestations of HIV/AIDS and discussing the progress in overall HIV treatment. Because the book itself is fairly short (265 pages), it appears to be more of a simple paperback rather than a reference textbook. Regardless, it is worthy of a spot on your bookshelf. As a clinician, educator, and researcher in the field of HIV-related lung disease, I found the book to be informative, easy to read, and a quick and simple reference to have on hand. It would be valuable to any medical trainee or clinician who manages the care of HIV-infected patients.

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The Emergence of Tropical Medicine in France

Michael A. Osborne

University of Chicago Press, Chicago, Illinois, USA
ISBN: 978-0-22611452-1 (print); 978-0-22611466-8 (ebook)
Pages: 312; Price: US $50.00 (print); US $7.00–$44.00 (ebook)

The idea of naval medicine as a specific and discrete art is richly illustrated in The Emergence of Tropical Medicine in France, Michael Osborne’s historical account of French colonial medicine. For expanding European empires, the nineteenth century was a time when theories of tropical disease evolved as responses to distinct challenges on ships, in colonies, and in home ports. In France, a system of provincial medical schools was built by the navy
in the port cities of Brest, Rochefort-sur-Mer, Toulon, and Bordeaux. Each faculty held to and taught their own system of medical knowledge retained within the regional boundaries. As described in this book, beliefs on causality and therapeutic options remained divided among the discrete spheres among institutions. The lack of accepted curricula seems a distant reminder of the many gains made before evidence-based medicine. To enrich the perspective, most of the book’s content is set in advance of the study of medical geography, which settled some longstanding misconceptions about ethnicity, location, and disease. Confusion reigned in colonial settings because of the similarity of causes implied during outbreaks of yellow fever, cholera, plague, typhus, and typhoid fever.

This book does not address the scientific advancements on infectious etiologies; rather, it provides the context for French innovation within colonial functionaries, clashing ideologies, and commercial considerations. Medical training played a pivotal role in French colonial activity, as in Madagascar in 1895, when expeditionary forces were decimated by the thousands from malaria. While the prevailing belief was that tilled and swampy land caused the illness, that belief was overturned, by persons with medical training, in favor of insect bites. Success in Madagascar, as well as other overseas colonies, depended upon knowing disease cycles and managing interaction in the human populations.

This book is a worthwhile investment for those interested in historical narratives on tropical medicine previously unavailable in the English language. Naval physicians like Charles-Adolphe Maher did remarkable studies while touring the tropics. In 1823, after having studied at Rochefort, he spent 2 years voyaging and encountered yellow fever outbreaks in Havana and Veracruz. Within the confines of his ship, Maher carefully compared the spectrum of symptoms. His conclusion on intermittent fever being a variety of malaria was far from correct, yet Mahler did initiate brave comparisons of therapies among patients, albeit with bloodletting and dietary privations. Maher’s lifelong findings on medical statistics, Statistique Medicale de Rochefort, first published in 1874 and recently reprinted, recount the lively experiences of Mahler and many other persons investigating médecine exotique. Osborne’s book provides key insight regarding influential persons who revolutionized notions of disease, recognizing their contributions as harbingers for the vast developments to follow in the twentieth century.

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Correction: Vol. 21, No. 3

The number of invasive pneumococcal disease reports was listed incorrectly in Risk Factors for Death from Invasive Pneumococcal Disease, Europe, 2010 (A. Navarro-Torné et al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/21/3/14-0634_article).
English Victorian botanical artist Marianne North is celebrated for her meticulous attention to detail, form, and color. Her collection of 833 paintings, which portray more than 900 species of plants, comprises her life’s work and is on permanent display in the Marianne North gallery at the Royal Botanic Gardens, Kew, United Kingdom. Because North painted with oils, rather than with watercolors, and because she predates color photography, her body of work offers an enduring visual record of these plant species, some of which are quite rare or extinct.

North did not take lessons in oil painting until 1867 or begin her odyssey to paint flora from around the world until 3 years later when she was 40 years of age, which makes the quality and volume of her work more remarkable. After exhibiting her paintings in a London gallery in 1879, North wrote to Kew director Sir Joseph Hooker, offering to build a gallery if he would agree to display her life’s work there. He consented, and North then devoted a year arranging her paintings inside her eponymous gallery before its public opening in 1882. Her paintings still hang in this gallery, which was faithfully restored to its original character in 2009.

Earlier in her life, North had traveled broadly with her father Frederick North, who had been a member of parliament for Hastings. Following his death in 1869, her substantial inheritance and many political connections allowed her free rein to travel and pursue her passion for painting. During the next 13 years, she visited 16 countries on 6 continents, including Brazil, Japan, Singapore, Sri Lanka, and South Africa. Following a recommendation of Charles Darwin, a friend both of her late father and Hooker, North also explored Australia, New Zealand, and Tasmania.
North was not interested in mingling with political leaders or ambassadors or splurging on indulgences. Strange, even unwelcoming terrain teeming with botanical specimens beckoned her. She preferred to paint images of her specimens where they naturally grew. This free spirit favored solitary travel—she was known to elude travel companions or guides assigned to her—and did not mind simple, primitive accommodations that enabled her to be close to local flora. She did, however, consider a supply of paper and oil paints to be indispensable. In her autobiography, she notes that painting for her was “a vice like dram-drinking, almost impossible to leave off once it gets possession of one.”

North painted this month’s cover image, “Foliage, Flowers, and Seed-vessels of a Peruvian Bark Tree,” while traveling in South America during the early 1870s. North’s graceful painting captures many key facets of her specimen. In the center, a small branch is clustered with white flowers thrusting about a number of examples of this specimen’s oval leaves. Her palette of greens, reds, and browns amply juxtaposes the leaves in various stages from shiny new growth to older leaves. Many of the coveted cinchona seeds cling to twigs to the right of the flowers. A small branch in the upper right corner provides our only close look at the much-valued and bitter tasting bark. A tree-covered ridge juts above the lower forest, and clouds and mist swirl across the sky and down the ridge, showing the lushness of this tree’s natural habitat.

The Peruvian bark tree, also known as the Jesuit tree or the fever tree, is a cinchona1 of the family Rubiaceae, native to the western forests of the South American Andes. Its bark produces several alkaloids, including quinine, which has potent antimalarial properties, and quindine, which has antiarhythmic properties. The medicinal properties of the cinchona tree are thought to have been discovered by the Quechua, indigenous people from Peru and Bolivia. After the Jesuits learned about cinchona and brought it to Europe, its bark was widely used there to treat fevers starting in the 17th century. Not long after French scientists Pierre Joseph Pelletier and Joseph Bienaimé Caventou isolated quinine from cinchona bark in 1820, the governments of Bolivia, Columbia, Ecuador, and Peru unsuccessfully attempted to embargo the exportation of cinchona seeds, seedlings, or trees.

Smuggled seeds enabled Europeans to establish cinchona plantations in Southeast Asia, and the Dutch soon held a monopoly on supplies. In 1942, Japan gained control of the cinchona trees cultivated for quinine in parts of Asia, and Germany captured the quinine reserves in Amsterdam. Also in 1942, confronted by advancing Japanese troops, Colonel Arthur Fischer boarded the last plane to leave Mindanao, the second largest island in the Philippines, with a tin can filled with cinchona seeds. These seeds were used to establish plantations in Costa Rica and Ecuador, but those plantations were too late to benefit the war effort. The scarcity of quinine during the war led to the development of alternate antimalarial drugs, some of which are still in use today. During the 1960s, several strains of the malarial parasite Plasmodium falciparum developed resistance to some synthetic drugs, particularly chloroquine. The parasite remained sensitive, however, to quinine, leading to a resurgence of its use, despite potential side effects from large doses.

The World Health Organization (WHO) documents that 198 million cases of malaria occurred globally in 2013 (uncertainty range 124–283 million) and that malaria was responsible for 584,000 deaths (uncertainty range 367,000–755,000). WHO further reported that in 2014, malaria transmission was ongoing in 97 countries and territories and ≈3.3 billion persons remained at risk for malaria. However, recent increases in resources, political will, and commitment have led to great improvements in malaria control in many parts of the malaria-endemic world. These efforts must be sustained to ensure progress toward malaria elimination and ultimately eradication.

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August 10–21, 2015

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McLean, VA, USA
http://smhs.gwu.edu/cehp/activities/courses/idbr

September 17–21, 2015

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Article Title

Disseminated Infections with Talaromyces marneffei in Non-AIDS Patients Given Monoclonal Antibodies against CD20 and Kinase Inhibitors

CME Questions

1. According to the case series report by Chan and colleagues, which of the following statements about the clinical and epidemiologic characteristics of Talaromyces marneffei infection is correct?
   A. T. marneffei infection is the most important pathogenic thermal dimorphic fungus causing systemic mycosis in Southeast Asia
   B. T. marneffei infection is typically limited to the gastrointestinal and/or urinary tract
   C. T. marneffei infection is usually self-limited with a good prognosis for recovery
   D. T. marneffei infection is common among non-AIDS hematology patients

2. Your patient is a 57-year-old Chinese man with acute myeloid leukemia and fever. According to the case series report by Chan and colleagues, which of the following statements about the recent emergence of disseminated T. marneffei infection in non-AIDS patients with hematologic malignant neoplasms treated with targeted therapies is correct?
   A. The appearance of 4 cases in the past 2 years is the result of a change in the methodologies of a laboratory diagnosis of T. marneffei infection
   B. The appearance of 4 cases in the past 2 years is the result of a dramatic increase in the number of hematology patients at the investigators' hospital
   C. Recent emergence of disseminated T. marneffei infection is most likely because of overall immunosuppression
   D. Recent emergence of disseminated T. marneffei infection is most likely because of targeted therapies, such as anti-CD20 monoclonal antibodies and kinase inhibitors

3. According to the case series report by Chan and colleagues, which of the following statements about possible mechanisms of action underlying disseminated T. marneffei infection in non-AIDS patients with hematologic malignant neoplasms treated with targeted therapies would most likely be accurate?
   A. Rituximab and obinutuzumab used in 2 cases are anti-CD20 monoclonal antibodies that predominantly target T cells
   B. T. marneffei infection is usually seen at CD4+ counts of more than 300/µL
   C. Patients with B-cell dysfunction may have impaired production of neutralizing antibodies against key virulence factors of T. marneffei
   D. The investigators do not suggest any role of cytokine-producing B-cells

Activity Evaluation

1. The activity supported the learning objectives.
   | Strongly Disagree | 1 | 2 | 3 | 4 | 5 | Strongly Agree |
2. The material was organized clearly for learning to occur.
   | Strongly Disagree | 1 | 2 | 3 | 4 | 5 | Strongly Agree |
3. The content learned from this activity will impact my practice.
   | Strongly Disagree | 1 | 2 | 3 | 4 | 5 | Strongly Agree |
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   | Strongly Disagree | 1 | 2 | 3 | 4 | 5 | Strongly Agree |
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Article Title
Parechovirus Genotype 3 Outbreak among Infants, New South Wales, Australia, 2013–2014

CME Questions

1. Your patient is an 18-month-old Australian boy presenting with fever and presumed sepsis. According to the surveillance report by Cumming and colleagues, which of the following statements about the clinical and epidemiologic features of a human parechovirus genotype 3 (HPeV3) outbreak among Australian infants is correct?
   A. Between October 2013 and February 2014, a total of 183 cases of HPeV3 were identified in NSW infants
   B. Three-quarters of affected infants were girls
   C. Half of affected infants required hospitalization
   D. Common symptoms were diarrhea, sleepiness, and cough

2. According to the surveillance report by Cumming and colleagues, which of the following statements about the presentation of the HPeV3 outbreak in Australia compared with that in the northern hemisphere is correct?
   A. The Australian outbreak affected slightly younger infants
   B. The Australian outbreak had a more even gender split
   C. Frequency of skin rash was lower in the Australian outbreak
   D. Peak number of cases was later than documented in the northern hemisphere

3. According to the surveillance report by Cumming and colleagues, which of the following statements about the efficacy of active surveillance in detecting and monitoring the HPeV3 outbreak among Australian infants would most likely be accurate?
   A. Syndromic surveillance was not useful for outbreak monitoring
   B. Public health response had no apparent effect on infant length of stay
   C. Awareness-raising communication strategies were ineffective
   D. Active surveillance is resource intensive but helped to define the infection and link it with a syndromic surveillance indicator

Activity Evaluation

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<th>1. The activity supported the learning objectives.</th>
<th>2. The material was organized clearly for learning to occur.</th>
<th>3. The content learned from this activity will impact my practice.</th>
<th>4. The activity was presented objectively and free of commercial bias.</th>
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<td>Strongly Disagree</td>
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Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

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Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., “Here is what we found, and here is what the findings mean”).

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., “The Study” and “Conclusions.” Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

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 epidemiologic 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 and table and should not be divided into sections. No biographical sketch is needed.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references (not to exceed 15) but no abstract, figures, or tables. Include biographical sketch.

Books, Other Media. Reviews of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person’s identity, and five possible answers, followed by an essay describing the person’s life and his or her significance to public health, science, and infectious disease.

Etymology. Etymology (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eeditor@cdc.gov.
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